PHARMACEUTICAL COMPOSITIONS OF WNT PATHWAY INHIBITORS FOR WOUND TREATMENT

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] [RESERVED]

FIELD

[0002] One or more embodiments of the present disclosure described herein relates to pharmaceutical compositions and methods for treatment of disease using the same.

BACKGROUND

[0003] All publications, patents and patent applications cited within this application are herein incorporated by reference in their entirety to the same extent as if the disclosure of each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

[0004] The Wnt pathway has been shown to play a role in dermal fibrosis and scarring. The Wnt pathway is an evolutionary conserved pathway that regulates crucial aspects of cell fate determination, cell polarity, cell migration, neural patterning, and organogenesis during embryonic development. This pathway plays a role in ensuring proper tissue development in embryos and tissue maintenance in adults. Wnt signaling is involved at the beginning stages of skin development, where following gastrulation, embryonic cells of the ectoderm and the mesoderm differentiate to form the epidermis and dermis, respectively.

[0005] Although there are at least three distinct Wnt signaling pathways involved in the signal transduction process, the canonical (or β-catenin dependent) Wnt pathway is well understood. β-Catenin is an effector molecule resulting from the signaling of the canonical Wnt pathway, and its protein levels are regulated through a "destruction complex". In the absence of a Wnt signal, the transcriptional activator β-catenin is actively degraded in the cell by the actions of a protein complex, designated the "destruction complex". Within this complex, Axin-1 and -2 with adenomatous polypsis coli form a scaffold that facilitates β-catenin phosphorylation by casein-kinase 19a and glycogen synthase kinase 3β. Phosphorylated β-catenin is recognized and ubiquitinylated, resulting in its proteosomal degradation. Tankryase I and II (TK1 and TK2) are poly(ADP-ribose) polymerases (PARPs) that function to parsylate and destabilize Axin-1 and -2 proteins, thus destabilizing the β-catenin destruction complex. Once the destruction complex is destabilized, this allows β-catenin to be dephosphorylated, and subsequently stabilized and allowed to accumulate in the cytoplasm and

enter the cell nucleus, where it interacts with members of the Tcf/Lef family. β-catenin converts the Tcf proteins into potent transcriptional activators by recruiting co-activator proteins, thus ensuring efficient activation of Wnt target genes. The Wnt pathway, once activated by the Wnt family of natural ligands, upregulates TNK1 and TNK2 to help destabilize the destruction complex. Studies have shown that TNK1 and TNK2 are regulators of canonical Wnt signaling.

[0006] XAV939 is a small molecule that selectively inhibits Wnt/β-catenin-mediated transcription through TK 1 and TK2 inhibition with an IC_{50} of 11 nM/4nM in cell-free assays, regulates axin levels, and does not affect CRE, F-κB, or TGF-β. Recently, topical application of XAV939 in a mouse ear punch assay demonstrated that XAV939 significantly increased rate of wound closure with reduced fibrosis (scarring). However, XAV939 was dissolved in dimethyl sulfoxide (DMSO) and used as a "research tool" compound due to its low aqueous solubility (<1 μg/mL). The problem with this approach is that humans cannot tolerate the use of DMSO.

[0007] The prior art has shown the utility of a matrix component comprising graphene oxide (GO) and hyaluronic acid (HA) as being effective in both providing a supportive matrix for XAV939 and allowing the use of XAV939 as a therapeutic for wound healing in humans and animals, see for example US20210000959, where XAV939 in a GO-HA matrix provides substantial improvement to the quality of wound healing; specifically causing the tissues to limit scarring following a fibrotic wound healing pathway. Further, the prior art has reported the observation of increased cartilage regeneration and healing following acute injury, for example by way of a 2mm biopsy punch wound made in the center of the cartilaginous region of a C57Bl/CJ mouse, by way of administration of XAV939 dissolved in DMSO (Bastakoty, D. et al. 2015, 29(12): 4881–4892).

[0008] While the utility of XAV939 to improve the quality of regeneration of tissue following injury, including the regeneration of cartilage, and while reducing the healing by way of fibrotic mechanisms which results in scarring has been demonstrated, XAV939 has poor solubility. While XAV939 inhibits the Wnt pathway, there is a need for improved Wnt inhibitors with improved characteristics such as increased solubility in water, increased Wnt inhibition, increased bioavailability or modified biological stability once administered to a patient in need.

BRIEF DESCRIPTION OF DRAWINGS

[0009] **FIG. 1** is a bar graph showing results (scores) of a pharmaceutical composition according to one or more embodiments, Formulation 1, vs vehicle in re-epithelization, neovascularization, rete ridge, scabbing, and granulation (Biological Example 1A).

[0010] **FIG. 2** is a plot showing the average area size of the wounds of the vehicle group and the Formulation 1 group during the course of a 21-day treatment. The results indicate that topical administration of Formulation 1 increased the rate of healing of a diabetic rat impaired healing animal model compared to vehicle (Biological Example 1A).

[0011] **FIG. 3** shows the distance between opposing cartilage endpoints in mice from a 30-day treatment of 2 millimeter (mm) biopsy punch wounds with: dimethylsulfoxide (DMSO) control; saline and XAV939 in DMSO (XAV939); and a 15-day treatment with GO-HA, and a 30-day treatment of pharmaceutical composition according to one or more embodiments, Formulation 1 (Biological Example 1B).

[0012] **FIG. 4** shows the distance between opposing cartilage endpoints in mice as between 30-day treatment of 2 mm biopsy punch wounds with XAV939 in DMSO and 15-day treatment with Formulation 1 (Biological Example 1B).

[0013] **FIG. 5** shows the distance between opposing cartilage endpoints in mice from a 15-day treatment of 2 mm biopsy punch wounds with GO-HA and a 15-day treatment with Formulation 1 (Biological Example 1B).

[0014] **FIG. 6** shows the distance between opposing cartilage endpoints in rabbits from a 21-day treatment of 6 mm biopsy punch wounds with saline and a 21-day treatment with Formulation 2 (Biological Example 2).

[0015] **FIG. 7** shows inhibition of the Wnt transcription signaling pathway activity by a Wnt inhibitor of Formula (I) (**Compound 1** in DMSO) compared to a pharmaceutical composition (Formulation 3, comprising GO-HA + **Compound 1**) (Biological Example 3).

[0016] **FIG. 8** shows inhibition of the Wnt transcription signaling pathway activity by a Wnt inhibitor of Formula (I) (**Compound 7** in DMSO) compared to a pharmaceutical composition (Formulation 4, comprising GO-HA + **Compound 7**) (Biological Example 3).

[0017] **FIG. 9** shows inhibition of the Wnt transcription signaling pathway activity by a Wnt inhibitor of Formula (I) (**Compound 8** in DMSO) compared to a pharmaceutical composition (Formulation 5, comprising GO-HA + **Compound 8**) (Biological Example 3).

[0018] **FIG. 10** illustrates the results of a rabbit ear study (Biological Example 5), comparing eight injury sites (L1, L2, L3, L4, R5, R6, R7, and R8) on three specimens at day 0

(top) and at day 23 (bottom). The specimens were treated with saline as a control, and a pharmaceutical composition (Formulation 14 with **Compound 8** or Formulation 15 with **Compound 18**). Saline was applied via a spray, Formulation 14 (with **Compound 8**, 1 mg/mL) was applied via a phospholipid spray, and Formulation 15 (with **Compound 18**, 1 mg/mL) was applied via a phospholipid spray (Biological Example 5).

[0019] **FIG. 11A** shows rate of ear closure from days 1-21 (test 1). Saline control is compared to pharmaceutical compositions (Formulation 14 with **Compound 8** and Formulation 15 with **Compound 18**) (Biological Example 5).

[0020] **FIG. 11B** shows rate of ear closure from days 14-39 (test 1). Saline control is compared to pharmaceutical compositions (Formulation 14 with **Compound 8** and Formulation 15 with **Compound 18**) (Biological Example 5).

[0021] **FIG. 12** shows rate of ear closure from days 14-39 (test 2). Saline control is compared to pharmaceutical compositions (Formulation 14 with **Compound 8** and Formulation 15 with **Compound 18**) (Biological Example 5).

[0022] **FIG. 13** (top) shows the location of an embed cut across the center point of a healed wound for purposes of processing tissue after cartilage regeneration tests (rabbit ear study, Biological Example 5). **FIG. 13** (bottom) shows a slide with the cross section of sectioned tissue at the center of the wound. Resultant tissue sections placed on the slide are shown in images in **FIGS. 15-17**.

[0023] **FIG. 14** shows the average distance between opposing cartilage endplates (mm) after 45 days in cartilage regeneration tests (rabbit ear studies). The measurements were taken from Safranin O stained cross sections of healed 8 mm ear punch wounds 45 days after treating with saline (control) or a pharmaceutical composition (Formulation 14 with **Compound 8** or Formulation 15 with **Compound 18**).

[0024] **FIG. 15** shows a representative sample of a cross section from the cartilage regeneration test (Biological Example 5) after treatment with saline (control), collected at day 45. Analysis is shown 9-10 mm from the wound margin. The top image includes the wound margin. Top image is at 0.3X magnification, middle image is at 2.5X magnification, and bottom image is at 10X magnification. The gray square at the middle image represents the image shown in the bottom image. Gray arrows in the bottom image show an area with regenerating cartilage.

[0025] **FIG. 16** shows a representative sample of a cross section from the cartilage regeneration test (Biological Example 5) after treatment with a pharmaceutical composition (Formulation 14 with **Compound 8**), collected at day 45. Analysis is shown 9-10 mm from the wound margin. The top image includes the wound margin. Top image is at 0.3X magnification,

middle image is at 2.5X magnification, and bottom image is at 10X magnification. The gray square at the middle image represents the image shown in the bottom image. Gray arrows in the bottom image show an area with regenerating cartilage.

[0026] **FIG. 17** shows a representative sample of a cross section from the cartilage regeneration test (Biological Example 5) after treatment with a pharmaceutical composition (Formulation 15 with **Compound 18**), collected at day 45. Analysis is shown 9-10 mm from the wound margin. The top image includes the wound margin. Top image is at 0.3X magnification, middle image is at 2.5X magnification, and bottom image is at 10X magnification. The gray square at the middle image represents the image shown in the bottom image. Gray arrows in the bottom image show an area with regenerating cartilage.

[0027] **FIG. 18** shows a schematic diagram of wound site locations on a mammal (swine) in a full thickness excisional wound healing study.

[0028] **FIG. 19** (top) illustrates suture formation with a simple interrupted closure used in the full thickness excisional wound healing study (Biological Example 6), with the first throw **3401,** the second throw **3403**, and a portion of the wound closed by an interrupted suture **3405** shown. **FIG. 19** (bottom) shows a cross section view of the portion of the wound closed by the interrupted suture **3405**.

[0029] **FIG. 20A** shows results of the full thickness open excisional wound healing, rete ridge formation, with a pharmaceutical composition (Formulation 19 with **Compound 8** + GO-HA), a saline control, and uninjured skin.

[0030] **FIG. 20B** shows results of the full thickness open excisional wound healing rete ridge formation (outliers removed), with pharmaceutical compositions (Formulation 16 with **Compound 7**, Formulation 17 with **Compound 8**, Formulation 18 with **Compound 7** + GO-HA, and Formulation 19 with **Compound 8** + GO-HA), a saline control, and GO-HA alone ($p^{*}>0.05$; $p^{**}>0.01$; and $p^{***}>0.001$).

[0031] **FIG. 21** depicts results of the 3rd degree burn wound healing study (Biological Example 6) comparing a pharmaceutical composition (Formulation 22 with **Compound 8**) to a saline control. Wound area (cm^2) was measured at 2-6 day intervals. Results from day 16 to day 30 are shown.

[0032] **FIG. 22** depicts results of the 3rd degree burn wound healing study (Biological Example 6) comparing a pharmaceutical composition (Formulation 21 with **Compound 7**) to a saline control. Wound area (cm^2) was measured at 2-6 day intervals. Results from day 16 to day 30 are shown.

[0033] **FIG. 23** depicts results of the 3rd degree burn wound healing study (Biological Example 6) comparing a pharmaceutical composition (Formulation 26 with **Compound 8** + GO-HA) to a saline control. Wound area (cm^2) was measured at 2-6 day intervals. Results from day 16 to day 30 are shown.

[0034] **FIG. 24** depicts results of the 3rd degree burn wound healing study (Biological Example 6) comparing a pharmaceutical composition (Formulation 25 with **Compound 7** + GO-HA) to a saline control. Wound area (cm^2) was measured at 2-6 day intervals. Results from day 16 to day 30 are shown.

[0035] **FIG. 25** shows results of the closed excisional wound study (Biological Example 6): saline control, serum formulation (also called serum formulation control or serum control) as a vehicle, and GO-HA vehicle, from day 1 to day 21. The sutures were removed at day 13.

[0036] **FIG. 26** shows results of the closed excisional wound study (Biological Example 6): pharmaceutical compositions (Formulation 20 with **Compound 1**, Formulation 22 with **Compound 8**, and Formulation 23 with **Compound 10**), from day 1 to day 21. The sutures were removed at day 13.

[0037] **FIG. 27** shows results of the closed excisional wound study (Biological Example 6): pharmaceutical compositions (Formulation 24 with **Compound 1** + GO-HA, Formulation 26 with **Compound 8** + GO-HA, and Formulation 27 with **Compound 10** + GO-HA), from day 1 to day 21. The sutures were removed at day 13.

[0038] **FIG. 28** shows results of the closed excisional wound study (Biological Example 6): cross sectional tissue samples stained with trichrome blue. Wound sites treated with saline control, serum formulation control, GO-HA vehicle, Formulations 20, 22, and 23 (with **Compound 1**, **Compound 8**, **Compound 10**, respectively), and Formulations 24, 26, and 27 (with **Compound 1** + GO-HA, **Compound 8** + GO-HA, and **Compound 10** + GO-HA, respectively) are compared.

[0039] **FIG. 29** shows results of the closed excisional wound study (Biological Example 6): cross sectional tissue samples under polarized microscopy. Wound sites treated with saline control, serum formulation control, GO-HA vehicle, Formulations 20, 22, and 23 (with **Compound 1**, **Compound 8**, **Compound 10**, respectively), and Formulations 24, 26, and 27 (with **Compound 1** + GO-HA, **Compound 8** + GO-HA, and **Compound 10** + GO-HA, respectively) are compared.

[0040] **FIG. 30** shows results of closed excisional wound polarized image collagen infiltration (Biological Example 6). The number of wounds indicating collagen infiltration in

the scar are shown for A–saline (also called saline control); B–serum (also called serum formulation control); C–GO-HA vehicle (also called GO-HA control or GO-HA); D–(Formulation 20 with **Compound 1**); E–(Formulation 24 with **Compound 1** + GO-HA); F–(Formulation 22 with **Compound 8**); G-(Formulation 26 with **Compound 8** + GO-HA); H–(Formulation 23 with **Compound 10**); and J-(Formulation 27 with **Compound 10** + GO-HA).

[0041] **FIG. 31** shows results of the 3rd degree burn wound healing study (Biological Example 6) comparing a pharmaceutical composition (Formulation 21 with **Compound 7**, Formulation 25 with **Compound 7** + GO-HA, Formulation 22 with **Compound 8**, and Formulation 26 with **Compound 8** + GO-HA) to a saline control and GO-HA vehicle. Histology demonstrated that the pharmaceutical compositions promoted regeneration of tissue (improved organized reticular collagen and rete ridge formation) and reduced scar formation as compared to saline control and GO-HA vehicle.

[0042] **FIG. 32** shows inhibition of the Wnt transcription signaling pathway activity by a compound of Formula (I) (**Compound 1**) compared to a compound of Formula (I) and GO-HA (GO-HA + **Compound 1**) (Biological Example 3).

[0043] **FIG. 33** shows inhibition of the Wnt transcription signaling pathway activity by a compound of Formula (I) (**Compound 7**) compared to a compound of Formula (I) and GO-HA (GO-HA + **Compound 7**) (Biological Example 3).

[0044] **FIG. 34** shows inhibition of the Wnt transcription signaling pathway activity by a compound of Formula (I) (**Compound 8**) compared to a compound of Formula (I) and GO-HA (GO-HA + **Compound 8**) (Biological Example 3).

SUMMARY

[0045] Disclosed herein are pharmaceutical compositions comprising a WNT inhibitor, a glycol, one or more solubilizers, and saline or water. The pharmaceutical composition optionally comprises a graphene oxide-hyaluronic acid conjugate, one or more phospholipids, one or more carriers, stabilizer, buffer, and/or preservative. The pharmaceutical composition is suitable for topical administration.

[0046] In a first aspect, provided is a pharmaceutical composition comprising a WNT inhibitor which is 2-(4-(trifluoromethyl)phenyl)-1,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound A**) and a graphene oxide-hyaluronic acid conjugate (GO-HA), wherein the pharmaceutical composition is a liposomal composition (in some embodiments a water-based liposomal composition), and preferably wherein the pharmaceutical composition comprises one or more phospholipids. Alternatively, the WNT inhibitor in the first aspect is

according to the Compound of Formula (I) as defined in the fourth aspect or any embodiment thereof.

[0047] In a second aspect, provided is a pharmaceutical composition comprising a WNT inhibitor (in one or more embodiments, Compound A or a Compound of Formula (I) or any embodiments thereof), a graphene oxide-hyaluronic acid conjugate (GO-HA), a glycol, one or more solubilizers, one or more optional thickeners, one or more phospholipids, one or more carriers, an optional stabilizer, an optional buffer, an optional preservative, and saline or water. In one or more embodiments, provided is a pharmaceutical composition, comprising:

a Wnt inhibitor which is 2-(4-(trifluoromethyl)phenyl)-1,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound A**) preferably in an amount from about 0.01 wt% to about 2 wt%;

a graphene oxide-hyaluronic acid conjugate (GO-HA), preferably in an amount from about 0.01 wt% to about 2 wt%;

a glycol, preferably in an amount from about 2 wt% to about 10 wt%, or in an amount from about 3 wt% to about 6 wt%;

one or more solubilizers, preferably in an amount from about 0.5 wt% to about 10 wt%, preferably about 1 wt% to about 6 wt%;

one or more optional thickeners, and when present, preferably in an amount from about 0.5 wt% to about 2.5 wt%;

one or more phospholipids, preferably in an amount from about 0.1 wt% to about 4 wt%;

one or more carriers, preferably in an amount from about 2 wt% to about 10 wt%, preferably about 3 wt% to about 6 wt% when the one or more carrier is ethanol;

an optional stabilizer, and when present, preferably in an amount from about 0.1 wt% to about 0.5 wt%;

an optional buffer, and when present, preferably in an amount from about 0.2 wt% to about 2.0 wt%;

an optional preservative, and when present, preferably in an amount from about 0.025 wt% to about 1.5 wt%; and

saline or water; and

wherein the wt% of the WNT inhibitor, GO-HA, glycol, one or more solubilizers, one or more optional thickeners (when present), one or more phospholipids, one or more carriers, optional stabilizer, (when present) optional buffer (when present), optional preservative (when present), and saline or water is 100 wt%; or wherein the wt% of the WNT inhibitor, GO-HA, glycol, one or more solubilizers, one or more

phospholipids, one or more carriers, optional stabilizer, (when present) optional buffer (when present), optional preservative (when present), and saline or water is 100 wt%;

wherein the wt% ratio of the WNT inhibitor to GO-HA is about 0.1:1 to about 12:1.

[0048] In a third aspect, provided is a pharmaceutical composition, comprising

a Wnt inhibitor which is 2-(4-(trifluoromethyl)phenyl)-1,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound A**) preferably in an amount from about 0.01 wt% to about 2 wt%;

a graphene oxide-hyaluronic acid conjugate (GO-HA), preferably in an amount from about 0.01 wt% to about 2 wt%;

a glycol, preferably in an amount from about 2 wt% to about 10 wt%, or in an amount from about 3 wt% to about 6 wt%;

one or more solubilizers, preferably in an amount from about 0.5 wt% to about 10 wt%, preferably about 1 wt% to about 6 wt%;

one or more optional thickeners, and when present, preferably in an amount from about 0.5 wt% to about 2.5 wt%;

one or more phospholipids, preferably in an amount from about 0.1 wt% to about 4 wt%;

one or more carriers, preferably in an amount from about 2 wt% to about 10 wt%, preferably about 3 wt% to about 6 wt% when the one or more carrier is ethanol;

an optional stabilizer, and when present, preferably in an amount from about 0.1 wt% to about 0.5 wt%;

an optional buffer, and when present, preferably in an amount from about 0.2 wt% to about 2.0 wt%;

an optional preservative, and when present, preferably in an amount from about 0.025 wt% to about 1.5 wt%; and

saline or water; and

wherein the wt% of the WNT inhibitor, GO-HA, glycol, one or more solubilizers, one or more optional thickeners (when present), one or more phospholipids, one or more carriers, optional stabilizer (when present), optional buffer (when present), optional preservative (when present), and saline or water is 100 wt%; or wherein the wt% of the WNT inhibitor, GO-HA, glycol, one or more solubilizers, one or more phospholipids, one or more carriers, optional stabilizer (when present), optional buffer (when present), optional preservative (when present), and saline or water is 100 wt%;

wherein the wt% ratio of the WNT inhibitor to GO-HA is about 0.1:1 to about 12:1; and

wherein at least one of the stabilizer, buffer, and preservative is present; or wherein each of the stabilizer, buffer, and preservative is present.

Alternatively, the WNT inhibitor in the third aspect is according to the Compound of Formula (I) as defined in the fourth aspect or any embodiment thereof.

[0049] In a fourth aspect, provided is provided herein is a pharmaceutical composition comprising:

a WNT inhibitor of Formula (I), preferably in an amount from about 0.01 wt% to about 2 wt%;

an optional graphene oxide-hyaluronic acid conjugate (GO-HA), and when present, preferably in an amount from about 0.01 wt% to about 2 wt%;

a glycol, preferably in an amount from about 2 wt% to about 10 wt%, preferably about 3 wt% to about 6 wt%;

one or more solubilizers, preferably in an amount from about 0.5 wt% to about 10 wt%, preferably about 1 wt% to about 6 wt%;

one or more optional thickeners, and when present, preferably in an amount from about 0.5 wt% to about 2.5 wt%;

one or more optional phospholipids, and when present, preferably in an amount from about 0.1 wt% to about 4 wt%;

one or more optional carriers, and when present, preferably in an amount from about 2 wt% to about 10 wt% or in an amount from about 3 wt% to about 6 wt% when the one or more carrier is ethanol;

an optional stabilizer, and when present, preferably in an amount from about 0.1 wt% to about 0.5 wt%;

an optional buffer, and when present, preferably in an amount from about 0.2 wt% to about 2.0 wt%;

an optional preservative, and when present, preferably in an amount from about 0.025 wt% to about 1.5 wt%; and

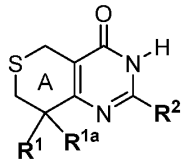
saline or water; and

wherein the wt% of the WNT inhibitor, optional GO-HA (when present), glycol, one or more solubilizers, one or more optional thickeners (when present), one or more optional phospholipids (when present), one or more optional carriers (when present), optional stabilizer (when present), optional buffer (when present), optional preservative (when present), and water or saline is 100 wt%; wherein the wt% of

the WNT inhibitor, optional GO-HA (when present), glycol, one or more solubilizers, one or more optional phospholipids (when present), one or more optional carriers (when present), optional stabilizer (when present), optional buffer (when present), optional preservative (when present), and water or saline is 100 wt%;

wherein the wt% ratio of the WNT inhibitor to GO-HA, when present, is about 0.1:1 to about 12:1; and

wherein the WNT inhibitor of Formula (I) is according to:



(I),

or a single stereoisomer or mixture of stereoisomers thereof; a single tautomer or mixture of tautomers thereof; and/or a pharmaceutically acceptable salt thereof;

wherein:

R^1 is hydrogen, deuterium, C_1-C_3alkyl, -OH, -O-C_1-C_3alkyl, -CH_2OH, or -B(OH)$_2$;

R^{1a} is hydrogen, deuterium, or C_1-C_3alkyl;

R^2 is:

(a) phenyl substituted with **R^3** and additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups;

(b) phenyl substituted with 5- or 6-membered heteroaryl where the 5- or 6-membered heteroaryl is substituted with **R^3** and additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups and where the phenyl is additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups;

(c) phenyl optionally substituted with 1, 2, or 3 **R^{3a}** groups and additionally substituted with -phenyl-**R^3** where the phenyl in -phenyl-**R^3** is optionally substituted with 1, 2, or 3 **R^{3a}** groups;

(d) 5- or 6-membered heteroaryl substituted with **R^3** and additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups;

(e) 5- or 6-membered heteroaryl optionally substituted with 1, 2, or 3 **R^{3a}** groups and additionally substituted with -phenyl-**R^3** where the phenyl is additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups;

(f) 5- or 6-membered heteroaryl optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups and additionally substituted with –(5- or 6-membered heteroaryl)-$\mathbf{R^3}$ where the 5- or 6-membered heteroaryl in –(5- or 6-membered heteroaryl)-$\mathbf{R^3}$ is optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups;

(g) C_3-C_6cycloalkyl substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1 or 2 $\mathbf{R^{3a}}$ groups;

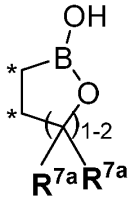
(h) C_3-C_6cycloalkyl substituted with 5- or 6-membered heteroaryl where the 5- or 6-membered heteroaryl is substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups, and where the C_3-C_6cycloalkyl is optionally substituted with 1 or 2 $\mathbf{R^{3a}}$ groups;

(j) C_3-C_6cycloalkyl substituted with phenyl where the phenyl is substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups, and where the cycloalkyl is optionally substituted with 1 or 2 $\mathbf{R^{3a}}$ groups;

(k) 3- to 8-membered heterocycloalkyl substituted with phenyl or substituted with 5- or 6-membered heteroaryl, where the phenyl and the 5- or 6-membered heteroaryl are substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups; or

(m) -CH=CH-$\mathbf{R^5}$ where $\mathbf{R^5}$ is phenyl or 5- or 6- membered heteroaryl, where the phenyl and the 5- or 6-membered heteroaryl are substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups;

$\mathbf{R^3}$ is independently selected from -$B(OH)_2$, cyano, halo, halo-C_1-C_6alkyl, -(C_0-C_6alkylene)-O-$\mathbf{R^4}$, or 5- to 10-membered heterocyclic wherein the 5- to 10-membered heterocyclic is optionally substituted with cyano; or when $\mathbf{R^2}$ is (a), then $\mathbf{R^3}$ and one $\mathbf{R^{3a}}$, when on adjacent carbons, together with the carbons to which they are attached form:



(a-1),

where the * indicate the carbons shared with the phenyl ring and where the remaining optional R^{3a} on the phenyl ring portion are as defined below, and each R^{7a} is independently hydrogen or C_1-C_6alkyl;

each R^{3a} is independently selected from cyano, halo, -OH, C_1-C_6alkyl, halo-C_1-C_6alkyl, and C_1-C_6alkoxy; and

R^4 is hydroxy-C_1-C_6alkyl, C_1-C_6alkoxy-C_1-C_6alkyl, or C_1-C_6alkoxycarbonyl-NH-C_1-C_6alkyl.

[0050] In a fifth aspect, provided is a method of treating a disease, disorder, or condition associated with Wnt signaling pathway activity, comprising administering topically a pharmaceutical composition according to any one of the first, second, third, and fourth aspects, or any of one or more embodiments herein to a mammal in need thereof.

DETAILED DESCRIPTION

[0051] The present disclosure provides for pharmaceutical compositions and methods of administration thereof to: induce healing of a wound (including a chronic wound, an acute wound, an alkali-burned corneal wound, and an incisional wound—open or closed), healing of a burn (including first, second, and third degree burns), or healing of a lesion (including lesions caused by HPV and/or a virus selected from the *Poxviridae* family of viruses); treat an inflammatory dermatitis disease (including acne, psoriasis, rosacea, and scleroderma), a cartilage disease, a bone disease, organ fibrosis, or cancer (including melanoma and breast cancer); induce tissue regeneration (including but not limited to regeneration of damaged elastic cartilage); induce bacteriostasis; induce bacterial growth inhibition; maintain bacteriostasis; induce antifungal activity; induce neovascularization (in tissues in need thereof); induce reinnervation (of a de-nerved body part); inhibit osteoclast differentiation; enhance osteoblast differentiation; and/or inhibit bone destruction associate with breast cancer.

[0052] The wound may include but is not limited to one or more selected from the group consisting of a chronic wound, an acute wound, and alkali-burned corneal wound. The inflammatory dermatitis disease may include but is not limited to one or more selected from the group consisting of acne, psoriasis, rosacea, and scleroderma. The cartilage disease may include but is not limited to one or more selected from the group consisting of osteoarthritis, rheumatoid arthritis, internal derangement of the joints, and degenerative cartilage disease. The bone disease may include but is not limited to a bone disease with impaired bone formation, e.g., osteoporosis.

[0053] The organ fibrosis may include but is not limited to one or more selected from the group consisting of lung fibrosis, heart fibrosis, liver fibrosis, and kidney fibrosis.

[0054] The cancer may include but is not limited to melanoma, breast cancer, and/or prostate cancer.

[0055] Further provided herein are Wnt inhibitors, pharmaceutical compositions comprising the Wnt inhibitors, methods of preparing the Wnt inhibitors, and methods of using the Wnt inhibitors and pharmaceutical compositions in treating wounds, in particular, the enhanced tissue regeneration following treatment of a wound. Also provided herein are methods of treating conditions associated with Wnt transcription products or Wnt signaling pathway activity in a mammal comprising administering a therapeutically effective amount of a Wnt inhibitor or pharmaceutical composition to a mammal. In one or more embodiments, the mammal is a human.

Definitions

[0056] When referring to the compounds provided herein, the following terms have the following meanings unless indicated otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. In the event that there is a plurality of definitions for a term herein, those in this section prevail unless stated otherwise. Unless specified otherwise, where a term is defined as being substituted, the groups in the list of substituents are themselves unsubstituted. For example, a substituted alkyl group can be substituted, for example, with a cycloalkyl group, and the cycloalkyl group is not further substituted unless specified otherwise.

[0057] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X". As used herein, and unless otherwise specified, the terms "about" and "approximately," when used in connection with temperatures, doses, amounts, or weight percent of ingredients of a composition or a dosage form, mean a dose, amount, or weight percent that is recognized by those of ordinary skill in the art to provide a pharmacological effect equivalent to that obtained from the specified dose, amount, or weight percent. Specifically, the terms "about" and "approximately," when used in this context, contemplate a dose, amount, or weight percent within 15%, within 10%, within 5%, within 4%, within 3%, within 2%, within 1%, or within 0.5% of the specified dose, amount, or weight percent.

[0058] The terms "a" or "an," as used in herein means one or more, unless context clearly dictates otherwise. For example, "pharmaceutically acceptable carrier" includes one or more ingredients as provided herein.

[0059] It is to be understood that the description of compounds, compositions, formulations, and methods of treatment described herein include "comprising," "consisting of," and "consisting essentially of" some or any embodiments. In one or more embodiments, for all compositions described herein, and all methods using a composition described herein, the compositions can either comprise the listed components or steps or can "consist essentially of" the listed components or steps. When a composition is described as "consisting essentially of" the listed components, the composition contains the components listed, and may contain other components which do not substantially affect the basic and novel properties (in one or more embodiments, the condition being treated), but do not contain any other components which substantially affect the basic and novel properties (in one or more embodiments, condition being treated) other than those components expressly listed; or, if the composition does contain extra components other than those listed which substantially affect the basic and novel properties (in one or more embodiments, the condition being treated), the composition does not contain a sufficient concentration or amount of the extra components to substantially affect the basic and novel properties (in one or more embodiments, the condition being treated). When a method is described as "consisting essentially of" the listed steps, the method contains the steps listed, and may contain other steps that do not substantially affect the basic and novel properties (in one or more embodiments, the condition being treated), but the method does not contain any other steps which substantially affect the basic and novel properties (in one or more embodiments, the condition being treated) other than those steps expressly listed. As a non-limiting specific example, when a composition is described as "consisting essentially of" a component, the composition may additionally contain any amount of pharmaceutically acceptable carriers, vehicles, or diluents and other such components which do not substantially affect the basic and novel properties (in one or more embodiments, the condition being treated).

[0060] As used herein for GO-HA linker "alkyl" refers to straight or branched hydrocarbon. An alkyl may be linear, branched, cyclic, or a combination thereof, and may contain, for example, from one to sixty carbon atoms, in one or more embodiments, 2-25 carbons. Examples of alkyl groups include but are not limited to ethyl, ethyl, propyl, isopropyl, cyclopropyl, butyl isomers (e.g. n-butyl, iso-butyl, tert-butyl, etc.) cyclobutyl isomers (e.g. cyclobutyl, methylcyclopropyl, etc.), pentyl isomers, cyclopentyl isomers, hexyl isomers, cyclohexyl isomers, and the like.

[0061] The term "alkyl," as used herein for a Wnt inhibitor of Formula (I), unless otherwise specified, refers to a saturated straight or branched, monovalent hydrocarbon. In one or more embodiments, the alkyl group is a primary, secondary, or tertiary hydrocarbon. An alkyl may be linear or branched, and may contain, for example, from one to eight carbon atoms. In one or more embodiments, the alkyl group has one to six carbon atoms, *i.e.,* C_1 to C_6 alkyl (C_1-C_6alkyl). In one or more embodiments, the alkyl is a C_1-C_3alkyl. In one or more embodiments, the alkyl group is selected from the group consisting of methyl, ethyl, propyl, isopropyl, butyl, isobutyl, secbutyl, *t*-butyl, pentyl, isopentyl, neopentyl, hexyl, isohexyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. Examples of alkyl groups include but are not limited to methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl isomers (e.g. n-butyl, iso-butyl, tert-butyl, etc.) pentyl isomers, hexyl isomers, and the like.

[0062] As used herein for the GO-HA linker, the term "linear alkyl" refers to a chain of carbon and hydrogen atoms (e.g., ethane, propane, butane, pentane, hexane, etc.).

[0063] As used herein for the GO-HA linker, the term "branched alkyl" refers to a chain of carbon and hydrogen atoms, without double or triple bonds that contains a fork, branch, and/or split in the chain. "Branching" refers to the divergence of a carbon chain, whereas "substitution" refers to the presence of non-carbon/non-hydrogen atoms in a moiety.

[0064] The term "alkylene," as used herein, unless otherwise specified, refers to a divalent alkyl group, as defined herein for either the GO-HA linker or as defined for a Wnt inhibitor of formula (I), as applicable.

[0065] As used herein for the GO-HA linker, the term "cycloalkyl" refers to a completely saturated mono- or multi-cyclic hydrocarbon ring system. When composed of two or more rings, the rings may be joined together in a fused, bridged or spiro-connected fashion. A cycloalkyl group may be unsubstituted, substituted, branched, and/or unbranched. Typical cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like. If substituted, the substituent(s) may be an alkyl (but not substituted alkyl) or selected from those indicated above with regard to substitution of an alkyl group unless otherwise indicated. Unless specified otherwise (e.g., substituted cycloalkyl group, heterocyclyl, cycloalkoxy group, halocycloalkyl, cycloalkylamine, thiocycloalkyl, etc.), an alkyl group contains carbon and hydrogen atoms exclusively. In some or any embodiments, the cycloalkyl group includes three to six carbon atoms, *i.e.,* C_3 to C_6 cycloalkyl. In some or any embodiments, the cycloalkyl has 3, 4, 5, or 6 (C_{3-6}) 3, 4, or 5 (C_{3-5}); 3 or 4 (C_{3-4}); 3 (C_3); 4 (C_4); or 5 (C_5) carbon atoms. In some or any embodiments, the cycloalkyl group is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl. In some or any embodiments, the cycloalkyl group is

cyclopropyl, cyclobutyl, or cyclopentyl. In one or more embodiments, the cycloalkyl group is cyclobutyl.

[0066] The term "C_3-C_{10}-cycloalkyl," as used herein for the Wnt inhibitor of Formula (I), refers to a monovalent, saturated, monocyclic hydrocarbon or bicyclic (fused, bridged, or spirocyclic) ring. In some or any embodiments, the terms "fused cycloalkyl" and "spirocycloalkyl" are embodiments of the cycloalkyl group. In some or any embodiments, the cycloalkyl group includes three to six carbon atoms, *i.e.*, C_3 to C_6 cycloalkyl ("C_3-C_6-cycloalkyl"). In some or any embodiments, the cycloalkyl has 3, 4, or 5 (C_{3-5}); 3 or 4 (C_{3-4}); 3 (C_3); 4 (C_4); or 5 (C_5) carbon atoms. In some or any embodiments, the cycloalkyl group is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl. In some or any embodiments, the cycloalkyl group is cyclopropyl, cyclobutyl, or cyclopentyl. In some or any embodiments, the cycloalkyl group is cyclopropyl. In some or any embodiments, the cycloalkyl group is cyclobutyl. In some or any embodiments, the cycloalkyl group is cyclopentyl. In some or any embodiments, the cycloalkyl group is bicyclo[3.2.1]octyl, bicyclo[2.2.2]octyl, bicyclo[3.3.1]nonyl, bicyclo[3.3.2.]decyl, bicyclo[2.2.2]octyl, or adamantyl. In one or more embodiments, the cycloalkyl group is cyclobutyl.

[0067] The term "alkoxy" and "alkyloxy," as used herein, and unless otherwise specified, refer to the group –OR′ where R′ is alkyl. In one or more embodiments, alkoxy is C_1-C_6alkoxy. In one or more embodiments, alkoxy is C_1-C_3alkoxy, such as -O-C_1-C_3alkyl. Alkoxy and alkyloxy groups include, in one or more embodiments, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexyloxy, 1,2-dimethylbutoxy, and the like.

[0068] The term "alkoxyalkyl," as used herein for a Wnt inhibitor of Formula (I), and unless otherwise specified, refers to an alkyl group, as defined herein, substituted with one or two -OR′ groups where each R′ is alkyl, as defined herein, and is independently selected. In some or any embodiments, alkoxyalkyl is C_1-C_6alkoxy-C_1-C_6alkyl.

[0069] The term "alkoxycarbonyl-NH-alkyl," as used herein for a Wnt inhibitor of Formula (I), and unless otherwise specified, refers to an alkyl group substituted with -NH-C(O)O(alkyl), wherein alkyl is as defined herein. In some or any embodiments, alkoxycarbonyl-NH-alkyl is C_1-C_6alkoxycarbonyl-NH-C_1-C_6alkyl.

[0070] As used herein "alkenyl," as used herein for the GO-HA linker, means a straight or branched chain hydrocarbon having at least 2 carbon atoms, which contains at least one carbon-carbon double bond.

[0071] As used herein "alkynyl," as used herein for the GO-HA linker, means a straight or branched chain hydrocarbon having at least 2 carbon atoms, which contains at least one carbon-carbon triple bond.

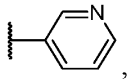
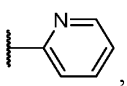
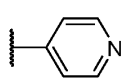
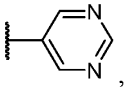
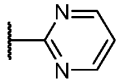
[0072] As used herein "amine" or "amino" as used herein for the GO-HA linker are represented by a formula $-NA_1A_2$, where A_1 and A_2 are, independently, hydrogen or alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group where each of these groups are as described herein for the GO-HA linker. In one or more embodiments amine (or amino) refers to any of NH_2, NH(alkyl), NH(aryl), $N(alkyl)_2$, N(alkyl)(aryl), and $N(aryl)_2$.

[0073] The term "aryl," as used herein, and unless otherwise specified, refers to a monovalent C_6- C_{15} carbocyclic ring system which comprises at least one aromatic ring wherein the aryl ring system is mono, di, or tricyclic. The aryl may be attached to the main structure through any of its rings, *i.e.* any aromatic or nonaromatic ring. In some or any embodiments, the aryl group may be a bridged (where chemically feasible) or non-bridged, spirocyclic (where chemically feasible) or not spirocyclic, and/or fused or not fused multicyclic group. In some or any embodiments, aryl is C_6-C_{10} aryl. In some or any embodiments, aryl is C_6 aryl, *i.e.* phenyl. In some or any embodiments, aryl is phenyl, naphthyl, indanyl, fluorenyl, 6,7,8,9-tetrahydro-5*H*-benzo[7]annulenyl, or tetrahydronaphthyl. When aryl is substituted, it can be substituted on any ring, *i.e.* on any aromatic or nonaromatic ring comprised by aryl.

[0074] The term "haloalkyl," as used herein, and unless otherwise specified, refers to an alkyl group substituted with 1, 2, 3, 4, or 5 halo groups. In some or any embodiments, the haloalkyl is a halo-C_1-C_6alkyl. In some or any embodiments, the haloalkyl is $-CF_3$, $-CH_2F$, $-CHF_2$, or $-CH_2CF_3$.

[0075] The terms "halogen" and "halo," as used herein, and unless otherwise specified, are synonymous and refer to chloro, bromo, fluoro, or iodo.

[0076] The term "heteroaryl," as used herein, and unless otherwise specified, refers to a monocyclic aromatic ring system or multicyclic aromatic ring system wherein one or more (in some or any embodiments, 1, 2, 3, or 4) of the ring atoms is a heteroatom independently selected from O, $S(O)_{0-2}$, NH, and N, and the remaining ring atoms are carbon atoms, and where the ring may be optionally substituted as described herein. The heteroaryl group is bonded to the rest of the molecule through any atom in the ring system, valency rules permitting. In some or any embodiments, each ring of a heteroaryl group can contain one or two O atoms, one or two S atoms, and/or one to four N atoms, or a combination thereof, provided that the total number of heteroatoms in each ring is four or less and each ring contains at least one carbon

atom. In some or any embodiments, the heteroaryl has from 5 to 20, from 5 to 15, from 5 to 6 ring atoms, or from 5 to 10 ring atoms. When heteroaryl is substituted, it can be substituted on any ring. In one or more embodiments, heteroaryl is a 5- to 10-membered heteroaryl. In one or more embodiments, heteroaryl is a 5- or 6-membered heteroaryl. In one or more embodiments, heteroaryl is a 6-membered heteroaryl. In one or more embodiments, heteroaryl is , , , , or , wherein indicates the point of attachment of the heteroaryl to the rest of the molecule.

[0077] In some or any embodiments, monocyclic heteroaryl groups include, but are not limited to, furanyl, imidazolyl, isothiazolyl, isoxazolyl, oxadiazolyl, oxadiazolyl, oxazolyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridyl, pyrimidinyl, pyrrolyl, thiadiazolyl, thiazolyl, thienyl, tetrazolyl, triazinyl and triazolyl. In some or any embodiments, bicyclic heteroaryl groups include, but are not limited to, benzofuranyl, benzimidazolyl, benzoxazolyl, benzoisoxazolyl, benzopyranyl, benzothiadiazolyl, benzothiazolyl, benzoisothiazolyl, benzothienyl, benzotriazolyl, furopyridyl, thienopyridyl, imidazopyridinyl, imidazothiazolyl, indolizinyl, indolyl, indazolyl, isobenzofuranyl, isobenzothienyl, isoindolyl, isoquinolinyl, isothiazolyl, naphthyridinyl, oxazolopyridinyl, phthalazinyl, pteridinyl, purinyl, pyridopyridyl, pyrrolopyridyl, quinolinyl, quinoxalinyl, or quinazolinyl. In some or any embodiments, tricyclic heteroaryl groups include, but are not limited to, acridinyl, benzindolyl, carbazolyl, dibenzofuranyl, perimidinyl, phenanthrolinyl, phenanthridinyl, and phenazinyl. In some or any embodiments, heteroaryl is indolyl, furanyl, pyridinyl, pyrimidinyl, imidazolyl, or pyrazolyl; each of which is optionally substituted with 1, 2, 3, or 4 groups as defined throughout the specification, including in one or more embodiments with group(s) independently selected from C_1-C_6alkyl, hydroxy, halo, halo-C_1-C_6alkyl, C_1-C_6alkoxy, cyano, or phenyl.

[0078] The term "heterocyclic," as used herein, and unless otherwise specified, refers to a monovalent monocyclic non-aromatic ring system or a monovalent multicyclic ring system that contains at least one non-aromatic ring; wherein one or more (in some or any embodiments, 1, 2, 3, or 4) of the monocyclic non-aromatic ring atoms is a heteroatom independently selected from O, $S(O)_{0-2}$, and N, and the remaining ring atoms are carbon atoms; and wherein one or more (in some or any embodiments, 1, 2, 3, or 4) of any of the ring atoms in the multicyclic ring system is a heteroatom(s) independently selected from O, $S(O)_{0-2}$, and N, and the remaining ring atoms are carbon. The term "heterocyclic" does not include fully aromatic

ring(s), *i.e.* does not include imidazole, pyrimidine, pyridine, and the like. In some or any embodiments, the heterocyclic ring comprises one or two heteroatom(s) which are independently selected from nitrogen and oxygen. In some or any embodiments, the heterocyclic ring comprises one or two heteroatom(s) which are oxygen. In some or any embodiments, the heterocyclic ring comprises one or two heteroatom(s) which are nitrogen (where the nitrogen is substituted as described in any aspect or embodiment described herein). In some or any embodiments, heterocyclic is multicyclic and comprises one heteroatom in a non-aromatic ring, or comprises one heteroatom in an aromatic ring, or comprises two heteroatoms in an aromatic ring, or comprises two heteroatoms where one is in an aromatic ring and the other is in a non-aromatic ring. In some or any embodiments, the heterocyclic group has from 3 to 20, 3 to 15, 3 to 10, 3 to 8, 4 to 7, or 5 to 6 ring atoms. In one or more embodiments, the heterocyclic is a 4- to 10-membered heterocyclic. In one or more embodiments, the heterocyclic is a 5- to 10-membered heterocyclic. In some or any embodiments, the heterocyclic is a monocyclic, bicyclic, tricyclic, or tetracyclic ring system. In some or any embodiments, the heterocyclic group may be a bridged or non-bridged, spirocyclic or not spirocyclic, and/or fused or not fused multicyclic group. One or more of the nitrogen and sulfur atoms may be optionally oxidized, one or more of the nitrogen atoms may be optionally quaternized, one or more of the carbon atoms may be optionally replaced with . Some rings may be partially or fully saturated, or aromatic provided that heterocyclic is not fully aromatic. The monocyclic and multicyclic heterocyclic rings may be attached to the main structure at any heteroatom or carbon atom which results in a stable compound. The multicyclic heterocyclic may be attached to the main structure through any of its rings, including any aromatic or nonaromatic ring, regardless of whether the ring contains a heteroatom. In some or any embodiments, heterocyclic is "heterocycloalkyl" which is 1) a saturated monovalent monocyclic group which contains at least one ring heteroatom, as described herein, or 2) a saturated monovalent bi- or tri-cyclic group in which at least one ring contains at least one heteroatom as described herein. In some or any embodiments, heterocyclic is 3- to 6-membered heterocycloalkyl. In some or any embodiments, heterocyclic is 3- to 8-membered heterocycloalkyl. In some or any embodiments, heterocyclic is 3- to 9- membered heterocycloalkyl. When heterocyclic and heterocycloalkyl are substituted, they can be substituted on any ring, i.e. on any aromatic or nonaromatic ring comprised by heterocyclic and heterocycloalkyl. In some or any embodiments, such heterocyclic includes, but are not limited to, azepinyl, benzodioxanyl, benzodioxolyl, 3,4-dihydro-2H-benzo[b][1,4]oxazinyl,

3,4-dihydro-2H-benzo[b][1,4]dioxepinyl, 1,3-dihydroisobenzofuranyl, benzofuranonyl, benzopyranonyl, benzopyranyl, dihydrobenzofuranyl, benzotetrahydrothienyl, 2,2-dioxo-1,3-dihydrobenzo[c]thienyl, benzothiopyranyl, benzoxazinyl, β-carbolinyl, chromanyl, chromonyl, cinnolinyl, coumarinyl, decahydroquinolinyl, decahydroisoquinolinyl, dihydro-benzimidazolonyl (including but not limited to 2-oxo-1,3-dihydro-2H-benzo[d]imidazol-1-yl), dihydrobenzisothiazinyl, dihydrobenzisoxazinyl, dihydrofuryl, dihydroisoindolyl, dihydropyranyl, dihydropyrazolyl, dihydropyrazinyl, dihydropyridinyl, dihydropyrimidinyl, dihydropyrrolyl, dioxolanyl, 1,4-dithianyl, furanonyl, imidazolidinyl, 2,4-dioxo-imidazolidinyl, imidazolinyl, indolinyl, 2-oxo-indolinyl, isobenzotetrahydrofuranyl, isobenzotetrahydrothienyl, isochromanyl, isocoumarinyl, isoindolinyl, 1-oxo-isoindolinyl, 1,3-dioxo-isoindolinyl, isothiazolidinyl, isoxazolidinyl, 3-oxo-isoxazolidinyl, morpholinyl, 3,5-dioxo-morpholinyl, octahydroindolyl, octahydroisoindolyl, 1-oxo-octahydroisoindolyl, 1,3-dioxo-hexahydroisoindolyl, oxo-oxadiazolyl (including but not limited to 5-oxo-1,2,4-oxadiazol-3-yl), oxazolidinonyl, oxazolidinyl, oxiranyl, piperazinyl, 2,6-dioxo-piperazinyl, piperidinyl, 2,6-dioxo-piperidinyl, 4-piperidonyl, pyrazolidinyl, pyrazolinyl, pyrrolidinyl, pyrrolinyl, 2-oxopyrrolidinyl, 2,5-dioxopyrrolidinyl, quinuclidinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydropyranyl, tetrahydrothienyl, thiamorpholinyl, thiomorpholinyl, 3,5-dioxo-thiomorpholinyl, thiazolidinyl, 2,4-dioxo-thiazolidinyl, tetrahydroquinolinyl, phenothiazinyl, phenoxazinyl, xanthenyl, 1,3,5-trithianyl, or 1,3-dihydro-imidazopyridin-2-onyl. In some or any embodiments, heterocyclic is benzo-1,4-dioxanyl, benzodioxolyl, indolinyl, 2-oxo-indolinyl, pyrrolidinyl, piperidinyl, 2,3-dihydrobenzofuranyl, decahydroquinolinyl, dihydrocyclopentapyridyl, dihydropyranopyridyl, tetrahydronaphthyridyl, 2,2-dioxo-3,4-dihydro-thiopyrano-pyridyl, dihydrofuropyridyl, dihydropyrrolopyridyl, 2,2-dioxo-1,3-dihydro-thieno-pyridyl, or tetrahydrocyclopropacyclopentapyridyl, ; each of which is optionally substituted with 1, 2, 3, or 4 groups as defined throughout the specification, including in some or any embodiments with group(s) independently selected from halo, alkyl, and phenyl. In some or any embodiments, heterocycloalkyl is pyrrolidinyl. In some or any embodiments, heterocycloalkyl is an N-linked heterocycloalkyl.

[0079] The term “hydroxyalkyl” as used herein, unless otherwise specified, refers to an alkyl, as defined herein, substituted by 1, 2, or 3 hydroxy groups. In one or more embodiments, the hydroxy group is a primary, secondary, or tertiary alcohol. In one or more embodiments, the hydroxyalkyl group includes one to ten carbons, *i.e.*, C_1 to C_{10} hydroxyalkyl. In one or more embodiments, the hydroxyalkyl group includes one or two alcohol (hydroxy) groups, provided

that they are not on the same carbon. In one or more embodiments, the hydroxyalkyl group is hydroxyC_1-C_6alkyl. In one or more embodiments, the hydroxyalkyl group is hydroxyC_1-C_3alkyl. In one or more embodiments, the hydroxyalkyl group is selected from the group consisting of hydroxymethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, hydroxypentyl, and hydroxyhexyl. In one or more embodiments, the hydroxyalkyl group is a C_1-C_6hydroxyalkyl. In one or more embodiments, the hydroxyalkyl group is selected from the group consisting of hydroxymethyl (-CH_2OH), 1-hydroxyethyl, 2-hydroxyethyl, 1-hydroxypropan-2-yl, and 2-hydroxypropan-2-yl.

[0080] The term "oxo" as used herein and unless otherwise specified, refers to a keto group (C=O). An oxo group that is a substituent of a nonaromatic carbon results in a conversion of a -CH_2- to -C=O. An oxo group that is a substituent of an aromatic carbon results in a conversion of -CH- to -C=O. When a substituent is oxo, then two hydrogens on the atom are replaced. When an oxo group substitutes aromatic moieties, the corresponding partially unsaturated ring replaces the aromatic ring. For example, a pyridyl group substituted by an oxo group is a pyridone. The person of ordinary skill in the art will appreciate that in one or more embodiments that such a group, e.g. pyridone and 2,4(1*H*,3*H*)-dioxo-pyrimidinyl, can exist in its tautomeric form, e.g. hydroxypyridine and 2,4-dihydroxypyrimidinyl, respectively.

[0081] The term "oxo" as used herein and unless otherwise specified, refers to a keto group (C=O). An oxo group that is a substituent of a nonaromatic carbon results in a conversion of a -CH_2- to -C=O. An oxo group that is a substituent of an aromatic carbon results in a conversion of -CH- to -C=O. When a substituent is oxo, then two hydrogens on the atom are replaced. When an oxo group substitutes aromatic moieties, the corresponding partially unsaturated ring replaces the aromatic ring. For example, a pyridyl group substituted by an oxo group is a pyridone. The person of ordinary skill in the art will appreciate that in some embodiments that such a group, e.g. pyridone and 2,4(1*H*,3*H*)-dioxo-pyrimidinyl, can exist in its tautomeric form, e.g. hydroxypyridine and 2,4-dihydroxypyrimidinyl, respectively.

[0082] As used herein "regeneration" means the renewal or growth of destroyed or devitalized tissue from the remnant tissue. It is a reparative attempt of the body, and in the context of wound represents the migration, differentiation, or replication of cells or transformation of progenitor cells into the appropriate cell types for the respective tissue which may include sebaceous cells, hair follicles, nerve cells, chondrocytes.

[0083] As used herein "wound" means an injury to tissue or skin caused by scrapes, cuts, abrasion, surgical procedures (e.g., caused by minimally invasive surgery, laparoscopic surgery, robotic surgery, incisional biopsies, general surgery, and cosmetic surgery), denuded

skin, burns, ulcers (e.g., diabetic ulcers, ulcers from vascular insufficiency, pressure sores, and burns), or other skin problems (e.g., allergies). Wound may range from superficial (e.g., affecting merely the epidermis) to more traumatic (e.g., lesions which affect layers of skin or tissue at depths which are beneath the epidermis). Wounds may be of any length or shape, e.g., in one or more embodiments, wounds are straight, jagged or curved.

[0084] In one or more embodiments, the term "pharmaceutically acceptable carrier" includes any and all and/or one or more solvents, co-solvents, complexing agents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, excipients, diluents, disintegrants, lubricants, adjuvants, and the like which are not biologically or otherwise undesirable. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. In addition, various adjuvants such as are commonly used in the art may be included. These and other such compounds are described in the literature, e.g., in the Merck Index (Merck & Company, Rahway, N.J.), considerations for the inclusion of various components in pharmaceutical compositions are described, (e.g., Gilman et al. (Eds.), 2010, Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 12th Ed., The McGraw-Hill Companies).

[0085] In one or more embodiments, the term "pharmaceutically acceptable salt" refers to salts that retain the biological effectiveness and properties of the compounds provided herein and which are not biologically or otherwise undesirable. In many cases, the compounds provided herein are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto. Pharmaceutically acceptable acid addition salts can be formed with inorganic acids and organic acids. Inorganic acids from which salts can be derived include, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Organic acids from which salts can be derived include, for example, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like. Pharmaceutically acceptable base addition salts can be formed with inorganic and organic bases. Inorganic bases from which salts can be derived include, for example, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Of these, the inorganic bases from which salts can be derived can include ammonium, potassium, sodium, calcium and magnesium salts. Organic bases from

which salts can be derived include, for example, primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, basic ion exchange resins, and the like, specifically such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. Many such salts are known in the art, as described in WO 87/05297.

[0086] In one or more embodiments, the term "pharmaceutically acceptable salt," as used herein, and unless otherwise specified, refers to any salt of a compound provided herein which retains its biological properties and which is not toxic or otherwise desirable for pharmaceutical use. Such salts may be derived from a variety of organic and inorganic counter-ions well known in the art. Such salts include, but are not limited to: (1) acid addition salts formed with organic or inorganic acids such as hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, sulfamic, acetic, trifluoroacetic, trichloroacetic, propionic, hexanoic, cyclopentylpropionic, glycolic, glutaric, pyruvic, lactic, malonic, succinic, sorbic, ascorbic, malic, maleic, fumaric, tartaric, citric, benzoic, 3-(4-hydroxybenzoyl)benzoic, picric, cinnamic, mandelic, phthalic, lauric, methanesulfonic, ethanesulfonic, 1,2-ethane-disulfonic, 2-hydroxyethanesulfonic, benzenesulfonic, 4-chlorobenzenesulfonic, 2-naphthalenesulfonic, 4-toluenesulfonic, camphoric, camphorsulfonic, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic, glucoheptonic, 3-phenylpropionic, trimethylacetic, *tert*-butylacetic, lauryl sulfuric, gluconic, benzoic, glutamic, hydroxynaphthoic, salicylic, stearic, cyclohexylsulfamic, quinic, muconic acid and the like acids; and (2) base addition salts formed when an acidic proton present in the parent compound either (a) is replaced by a metal ion, *e.g.*, an alkali metal ion, an alkaline earth ion or an aluminum ion, or alkali metal or alkaline earth metal hydroxides, such as sodium, potassium, calcium, magnesium, aluminum, lithium, zinc, and barium hydroxide, ammonia or (b) coordinates with an organic base, such as aliphatic, alicyclic, or aromatic organic amines, such as ammonia, methylamine, dimethylamine, diethylamine, picoline, ethanolamine, diethanolamine, triethanolamine, ethylenediamine, lysine, arginine, ornithine, choline, N,N′-dibenzylethylene-diamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, *N*-methylglucamine tris(hydroxymethyl)-aminomethane, piperazine, tetramethylammonium hydroxide, and the like.

[0087] In one or more embodiments, pharmaceutically acceptable salts further include, in some or any embodiments, and without limitation, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium salts and the like. When the compound contains a basic functionality, salts of non-toxic organic or inorganic acids, such as hydrohalides, *e.g.* hydrochloride and hydrobromide, sulfate, phosphate, sulfamate, nitrate, acetate,

trifluoroacetate, trichloroacetate, propionate, hexanoate, cyclopentylpropionate, glycolate, glutarate, pyruvate, lactate, malonate, succinate, sorbate, ascorbate, malate, maleate, fumarate, tartarate, citrate, benzoate, 3-(4-hydroxybenzoyl)benzoate, picrate, cinnamate, mandelate, phthalate, laurate, methanesulfonate (mesylate), ethanesulfonate, 1,2-ethane-disulfonate, 2-hydroxyethanesulfonate, benzenesulfonate (besylate), 4-chlorobenzenesulfonate, 2-naphthalenesulfonate, 4-toluenesulfonate, camphorate, camphorsulfonate, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylate, glucoheptonate, 3-phenylpropionate, trimethylacetate, *tert*-butylacetate, lauryl sulfate, gluconate, benzoate, glutamate, hydroxynaphthoate, salicylate, stearate, cyclohexylsulfamate, quinate, muconate and the like.

[0088] In one or more embodiments, a "therapeutically effective amount" or "pharmaceutically effective amount" of a compound as provided herein, is one which is sufficient to achieve the desired effect and may vary according to the nature and severity of the disease condition, and the potency of the compound. "Therapeutically effective amount" is also intended to include one or more of the compositions of the present disclosure so as to result in the increased regeneration of tissue subject to a wound. The combination of compounds is preferably a synergistic combination. Synergy, as described in the art (for example, Chou, 2010, Canc. Res. 70(2):440-446), occurs when the effect of the compounds when administered in combination is greater than the additive effect of the compounds when administered alone as a single agent. In general, a synergistic effect is most clearly demonstrated at sub-optimal concentrations of the compounds. This amount can further depend upon the patient's height, weight, sex, age and medical history.

[0089] The term "mammal" specifically includes humans, cattle, horses, dogs, and cats, but also includes many other mammalian species like pigs, rats, mice, primates (e.g., a monkey such as a cynomolgous monkey, a chimpanzee and a human). In one or more embodiments, the mammal is a human.

[0090] The term "subject" refers to a mammal, as provided herein, as well as to a cell or biological sample.

[0091] The term "substantially free of" stereoisomers with respect to a composition refers to a composition that includes at least 85% or 90% by weight, in some or any embodiments 95%, 98%, 99% or 100% by weight, of a designated stereoisomer of a compound in the composition. In some or any embodiments, in the methods and compounds provided herein, the compounds are substantially free of stereoisomers.

[0092] Similarly, the term "isolated" with respect to a composition refers to a composition that includes at least 85%, 90%, 95%, 98%, 99% to 100% by weight, of a specified compound, the remainder comprising other chemical species or stereoisomers.

[0093] The term "isotopic composition," as used herein, and unless otherwise specified, refers to the amount of each isotope present for a given atom, and "natural isotopic composition" refers to the naturally occurring isotopic composition or abundance for a given atom. Atoms containing their natural isotopic composition may also be referred to herein as "non-enriched" atoms. Unless otherwise designated, the atoms of the compounds recited herein are meant to represent any stable isotope of that atom. For example, unless otherwise stated, when a position is designated specifically as "H" or "hydrogen," the position is understood to have hydrogen at its natural isotopic composition. An isotopic composition may be included in a pharmaceutical composition disclosed herein.

[0094] The term "isotopic enrichment," as used herein, and unless otherwise specified, refers to the percentage of incorporation of an amount of a specific isotope at a given atom in a molecule in the place of that atom's natural isotopic abundance. In some or any embodiments, deuterium enrichment of 1% at a given position means that 1% of the molecules in a given sample contain deuterium at the specified position. Because the naturally occurring distribution of deuterium is about 0.0156%, deuterium enrichment at any position in a compound synthesized using non-enriched starting materials is about 0.0156%. The isotopic enrichment of the compounds provided herein can be determined using conventional analytical methods known to one of ordinary skill in the art, including mass spectrometry and nuclear magnetic resonance spectroscopy.

[0095] The term "isotopically enriched," as used herein, and unless otherwise specified, refers to an atom having an isotopic composition other than the natural isotopic composition of that atom. "Isotopically enriched" may also refer to a compound containing at least one atom having an isotopic composition other than the natural isotopic composition of that atom.

[0096] As used herein, and unless otherwise specified, the term "IC_{50}" refers to an amount, concentration or dosage of a particular test compound that achieves a 50% inhibition of a maximal response in an assay that measures such response.

[0097] In one or more embodiments, the terms "therapeutic agent" and "therapeutic agents" refer to any agent(s) which can be used in the treatment or prevention of a disorder or one or more symptoms thereof. In some or any embodiments, the term "therapeutic agent" includes a compound provided herein. In some or any embodiments, a therapeutic agent is an

agent which is known to be useful for, or has been or is currently being used for the treatment or prevention of a disorder or one or more symptoms thereof.

[0098] In one or more embodiments, "treating" or "treatment" of any condition or disorder refers, in some or any embodiments, to ameliorating a condition or disorder that exists in a subject. In another embodiment, "treating" or "treatment" includes ameliorating one or more physical parameter, which may be indiscernible by the subject. In yet another embodiment, "treating" or "treatment" includes modulating the condition or disorder, either physically (e.g., stabilization of a discernible symptom) or physiologically (e.g., stabilization of a physical parameter) or both. In yet another embodiment, "treating" or "treatment" includes delaying the onset of the condition or disorder. In yet another embodiment, "treating" or "treatment" includes the reduction or elimination of either the condition or one or more symptoms of the condition, or to retard the progression of the condition or of one or more symptoms of the condition, or to reduce the severity of the condition or of one or more symptoms of the condition.

Pharmaceutical Composition

[0099] Provided herein are compositions that can induce improved wound healing and tissue regeneration. Provided herein are compositions that can treat wounds and/or conditions associated with Wnt transcription products or Wnt signaling pathway activity, and in particular enhance tissue regeneration following treatment of a wound. In one or more embodiments, the composition is a pharmaceutical composition.

[00100] Further provided herein are compositions that can modulate the activity of the Wnt signaling pathway or Wnt transcription (products). The compositions can be formulated as described herein and used for the treatment of conditions associated with Wnt transcription products or Wnt signaling pathway activity.

[00101] In one or more embodiments, the condition associated with Wnt transcription products or Wnt signaling pathway activity is a chronic wound, an acute wound, an alkali-burned corneal wound, a burn, a lesion (including lesions caused by HPV and/or a virus selected from the *Poxviridae* family of viruses), an inflammatory dermatitis disease (including acne, psoriasis, rosacea, and scleroderma), a cartilage disease (including osteoarthritis, rheumatoid arthritis, internal derangement of the joints, and degenerative cartilage disease), a bone disease (including osteoporosis), organ fibrosis (including lung fibrosis, heart fibrosis, liver fibrosis, and kidney fibrosis), cancer (including melanoma, breast cancer, and prostate cancer), a de-nerved body part in need of reinnervation, tissue in need of regeneration

(including damaged elastic cartilage), bacterial growth in need of inhibition, fungal growth in need of inhibition, tissue in need of neovascularization, osteoclast differentiation in need of inhibition, osteoblast differentiation disorders (where inhibition of osteoblast differentiation is needed), and/or bone destruction associated with breast cancer.

[00102] In one or more embodiments, the condition associated with Wnt transcription products or Wnt signaling pathway activity is a chronic wound, an acute wound, an alkali-burned corneal wound, an incisional wound (open or closed), a burn, a lesion (including lesions caused by HPV and/or a virus selected from the *Poxviridae* family of viruses), an inflammatory dermatitis disease (including acne, psoriasis, rosacea, and scleroderma), a cartilage disease (including osteoarthritis, rheumatoid arthritis, internal derangement of the joints, and degenerative cartilage disease), a bone disease (including osteoporosis), organ fibrosis (including lung fibrosis, heart fibrosis, liver fibrosis, and kidney fibrosis), cancer (including melanoma, breast cancer, and prostate cancer), a de-nerved body part in need of reinnervation, tissue in need of regeneration (including damaged elastic cartilage), bacterial growth in need of inhibition, fungal growth in need of inhibition, tissue in need of neovascularization, osteoclast differentiation in need of inhibition, osteoblast differentiation disorders (where inhibition of osteoblast differentiation is needed), and/or bone destruction associated with breast cancer.

[00103] In one or more embodiments, the condition associated with Wnt transcription products or Wnt signaling pathway activity selected from a chronic wound, an acute wound, an alkali-burned corneal wound, an incisional wound (open or closed), a burn, a lesion (including lesions caused by HPV and/or a virus selected from the *Poxviridae* family of viruses), an inflammatory dermatitis disease (including acne, psoriasis, rosacea, and scleroderma), cancer (including melanoma and breast cancer), a de-nerved body part in need of reinnervation, tissue in need of regeneration (including damaged elastic cartilage), bacterial growth in need of inhibition, fungal growth in need of inhibition, and tissue in need of neovascularization.

[00104] In one or more embodiments, a pharmaceutical composition for inhibiting Wnt transcription or Wnt signaling pathway activity is provided, comprising: a WNT inhibitor; a graphene oxide (GO) and hyaluronic acid (HA) conjugate (GO-HA), wherein GO and HA are covalently linked via a linker, where the GO-HA is optional; a glycol; one or more solubilizers; one or more phospholipids, where the one or more phospholipids are optional; one or more carriers, where the one or more carriers are optional; a buffer, where the buffer is optional; a preservative, where the preservative is optional; and saline or water, wherein the wt% of the

WNT inhibitor, optional GO-HA (when present), glycol, one or more solubilizers, one or more optional phospholipids (when present), one or more optional carriers (when present), optional stabilizer (when present), optional buffer (when present), optional preservative (when present), and water or saline is 100 wt%; wherein the wt% ratio of the WNT inhibitor to the GO-HA, when present, is about 0.1:1 to about 12:1; wherein the one or more phospholipid and the GO-HA are not optional when the WNT inhibitor is (4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**Compound A**); and wherein the pharmaceutical composition is suitable for topical administration.

[00105] In one or more embodiments, the pharmaceutical composition comprises the WNT inhibitor in an amount from about 0.01 wt% to about 2 wt%; the optional GO-HA, when present, in an amount from about 0.01 wt% to about 2 wt%; the glycol in amount from about 2 wt% to about 10 wt%; the one or more solubilizers in an amount from about 0.5 wt% to about 10 wt%; the one or more optional phospholipids, when present, in an amount from about 0.1 wt% to about 4 wt%; the one or more optional carriers, when present, in an amount from about 2 wt% to about 10 wt%; the optional stabilizer, when present, in an amount from about 0.1 wt% to about 0.5 wt%; the optional buffer, when present, in an amount from about 0.2 wt% to about 2.0 wt%; the optional preservative, when present, in an amount from about 0.025 wt% to about 1.5 wt%; saline or water; wherein the wt% of the WNT inhibitor, optional GO-HA (when present), glycol, one or more solubilizers, one or more optional phospholipids (when present), one or more optional carriers (when present), optional stabilizer (when present), optional buffer (when present), optional preservative (when present), and water or saline is 100 wt%.

Embodiment A

[00106] **Embodiment A1.** Provided is a pharmaceutical composition comprising a WNT inhibitor where the pharmaceutical composition is a liposomal composition (in some embodiments a water-based liposomal composition).

[00107] **Embodiment A2.** In an embodiment of Embodiment A1, provided is a pharmaceutical composition comprising a WNT inhibitor which is 2-(4-(trifluoromethyl)phenyl)-1,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound A**) and a graphene oxide-hyaluronic acid conjugate (GO-HA), wherein the pharmaceutical composition is a liposomal composition (in some embodiments a water-based liposomal composition), and preferably wherein the pharmaceutical composition comprises one or more phospholipids.

[00108] **Embodiment A3.** In an embodiment of Embodiment A1 or A2, the pharmaceutical composition comprises a matrix component comprising a graphene oxide (GO) and hyaluronic acid (HA) conjugate (GO-HA), wherein the GO and HA are covalently linked via a linker; XAV939; water or saline; and a glycol. In an embodiment of Embodiment A1 or A2, the pharmaceutical composition comprises a matrix component comprising a graphene oxide (GO) and hyaluronic acid (HA) conjugate (GO-HA), wherein the GO and HA are covalently linked via a linker; XAV939; water or saline; and butylene glycol.

[00109] **Embodiment A4.** In an embodiment of any one of Embodiments A1 to A3, the pharmaceutical composition comprises a matrix component comprising a graphene oxide (GO) and hyaluronic acid (HA) conjugate (GO-HA), wherein the GO and HA are covalently linked via a linker; XAV939; water or saline; and one or more solubilizers. In an embodiment of any one of Embodiments A1 to A3, the pharmaceutical composition comprises a matrix component comprising a graphene oxide (GO) and hyaluronic acid (HA) conjugate (GO-HA), wherein the GO and HA are covalently linked via a linker; XAV939; water or saline; and a polysorbate 20.

[00110] **Embodiment A5.** In an embodiment of any one of Embodiments A1 to A4, the pharmaceutical composition comprises a matrix component comprising a graphene oxide (GO) and hyaluronic acid (HA) conjugate (GO-HA), wherein the GO and HA are covalently linked via a linker; XAV939; water or saline; and one or more phospholipids. In an embodiment of any one of Embodiments A1 to A4, the pharmaceutical composition comprises a matrix component comprising a graphene oxide (GO) and hyaluronic acid (HA) conjugate (GO-HA), wherein the GO and HA are covalently linked via a linker; XAV939; water or saline; and phosphatidylcholine.

[00111] **Embodiment A6.** In an embodiment of any one of Embodiments A1 to A5, the pharmaceutical composition comprises a matrix component comprising a graphene oxide (GO) and hyaluronic acid (HA) conjugate (GO-HA), wherein the GO and HA are covalently linked via a linker; XAV939; water or saline; and one or more carriers. In an embodiment of any one of Embodiments A1 to A5, the pharmaceutical composition comprises a matrix component comprising a graphene oxide (GO) and hyaluronic acid (HA) conjugate (GO-HA), wherein the GO and HA are covalently linked via a linker; XAV939; water or saline; and ethanol.

[00112] **Embodiment A7.** In an embodiment of any one of Embodiments A1 to A6, the pharmaceutical composition comprises a matrix component comprising a graphene oxide (GO) and hyaluronic acid (HA) conjugate (GO-HA), wherein the GO and HA are covalently linked via a linker; XAV939; water or saline; and one or more optional thickeners.

[00113] **Embodiment A7a.** In an embodiment of any one of Embodiments A1 to A7, one or more thickeners are present.

[00114] **Embodiment A7b.** In an embodiment of any one of Embodiments A1 to A7a, the pharmaceutical composition comprises a matrix component comprising a graphene oxide (GO) and hyaluronic acid (HA) conjugate (GO-HA), wherein the GO and HA are covalently linked via a linker; XAV939; water or saline; and hydroxypropylcellulose.

[00115] **Embodiment A7c.** In an embodiment of any one of Embodiments A1 to A7, the one or more thickeners are not present.

[00116] **Embodiment A8.** In an embodiment of any one of Embodiments A1 to A7, the pharmaceutical composition comprises XAV939, a glycol, one or more solubilizers, one or more phospholipids, one or more carriers, GO-HA, one or more optional thickeners, and water or saline.

[00117] **Embodiment A8a.** In an embodiment of any one of Embodiments A1 to A7b and A8, the pharmaceutical composition comprises XAV939, butylene glycol, a polysorbate 20, phosphatidylcholine, ethanol, GO-HA, and water or saline.

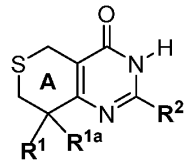
[00118] **Embodiment A8b.** In an embodiment of any one of Embodiments A1 to A7b and A8, the pharmaceutical composition comprises XAV939, butylene glycol, a polysorbate 20, phosphatidylcholine, ethanol, GO-HA, water or saline, and hydroxypropylcellulose.

Wnt Inhibitor

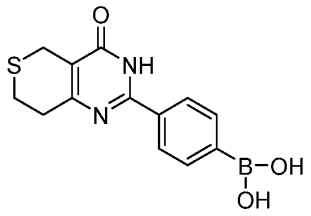
[00119] The aspects and embodiments described herein include pharmaceutical compositions comprising the recited Wnt inhibitor compounds as well as a pharmaceutically acceptable salt thereof and/or an isomer thereof. For instance, aspects and embodiments described herein include pharmaceutical compositions with a single stereoisomer of mixture of stereoisomers thereof, and/or a pharmaceutically acceptable salt thereof.

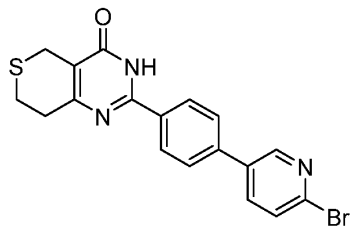
[00120] Included herein, if chemically possible, are all stereoisomers of the compounds, including diastereomers and enantiomers. Also included are mixtures of possible stereoisomers in any ratio, including, but not limited to, racemic mixtures. Unless stereochemistry is explicitly indicated in a structure at a particular atom, the structure is intended to embrace all possible stereoisomers of the compound depicted. If stereochemistry is explicitly indicated for one portion or portions of a molecule, but not for another portion or portions of a molecule, the structure is intended to embrace all possible stereoisomers for the portion or portions where stereochemistry is not explicitly indicated. It will be apparent that certain structures recite specific stereochemistry at particular atoms.

Wnt Inhibitor Embodiment B

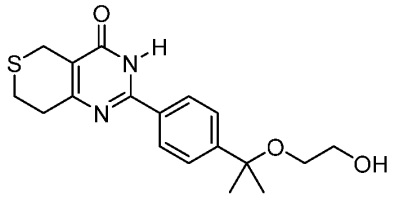
[00121] **Embodiment B1:** In one or more embodiments, the pharmaceutical composition comprises a WNT inhibitor according to a Wnt inhibitor of Formula (I):  (I), or a single stereoisomer or mixture of stereoisomers thereof; a single tautomer or mixture of tautomers thereof; and/or a pharmaceutically acceptable salt thereof; wherein Formula (I) is as defined in the fourth aspect or any embodiments thereof.

[00122] **Embodiment B2:** In one or more embodiments, a pharmaceutical composition herein includes a Wnt inhibitor that is an inhibitor of the Wnt pathway.

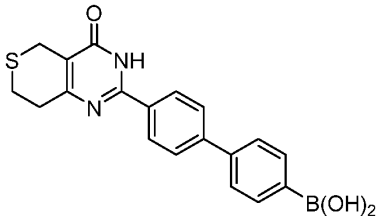
[00123] **Embodiment B3:** In one or more embodiments of the pharmaceutical composition described herein, including any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor of Formula (I) is an inhibitor of the Wnt pathway, with a chemical name (4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**Compound 1**). The structure of **Compound 1** is:  . In one or more embodiments, including any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor of Formula (I) is **Compound 1** or any prodrug, pharmaceutically acceptable salt, metabolite, polymorph, solvate, hydrate, stereoisomer, or a single tautomer or mixture of tautomers thereof.

[00124] **Embodiment B4:** In one or more embodiments of the pharmaceutical composition described herein, including any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor of Formula (I) is an inhibitor of the Wnt pathway, with a chemical name 2-(4-(6-bromopyridin-3-yl) phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano [4,3-d]pyrimidin-4-one (**Compound 7**). The structure of **Compound 7** is:  . In one or more embodiments, including any one of the first, second, third, fourth, and fifth aspects, the

Wnt inhibitor of Formula (I) is **Compound 7** or any prodrug, pharmaceutically acceptable salt, metabolite, polymorph, solvate, hydrate, stereoisomer, or a single tautomer or mixture of tautomers thereof.

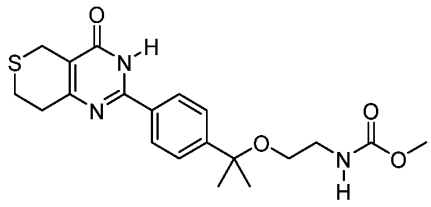
[00125] Embodiment B5: In one or more embodiments of the pharmaceutical composition herein, including any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor of Formula (I) is an inhibitor of the Wnt pathway, with a chemical name 2-(4-(2-(2-hydroxyethoxy)propan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 8**). The structure of **Compound 8** is: 

. In one or more embodiments, including any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor of Formula (I) is **Compound 8** or any prodrug, pharmaceutically acceptable salt, metabolite, polymorph, solvate, hydrate, stereoisomer, or a single tautomer or mixture of tautomers thereof.

[00126] Embodiment B6: In one or more embodiments of the pharmaceutical composition described herein, including any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor of Formula (I) is an inhibitor of the Wnt pathway, with a chemical name (4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**Compound 10**). The structure of **Compound 10** is: 

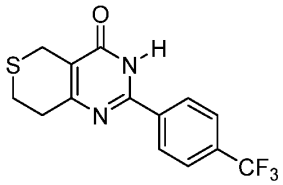
. In one or more embodiments, including any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor of Formula (I) is **Compound 10** or any prodrug, pharmaceutically acceptable salt, metabolite, polymorph, solvate, hydrate, stereoisomer, or a single tautomer or mixture of tautomers thereof.

[00127] Embodiment B7: In one or more embodiments of the pharmaceutical composition described herein, including any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor of Formula (I) is an inhibitor of the Wnt pathway, with a chemical name methyl (2-((2-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**Compound 18**). The structure of **Compound 18** is:



. In one or more embodiments, including any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor of Formula (I) is **Compound 18** or any prodrug, pharmaceutically acceptable salt, metabolite, polymorph, solvate, hydrate, stereoisomer, or a single tautomer or mixture of tautomers thereof.

[00128] Embodiment B8: In one or more embodiments of the pharmaceutical composition described herein, including any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor of Formula (I) is an inhibitor of the Wnt pathway, with a chemical name 2-(4-(trifluoromethyl)phenyl)-1,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound A**). The structure of **Compound A** is:

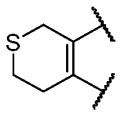


. In one or more embodiments, including any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor of Formula (I) is **Compound A** or any prodrug, pharmaceutically acceptable salt, metabolite, polymorph, solvate, hydrate, stereoisomer, or a single tautomer or mixture of tautomers thereof.

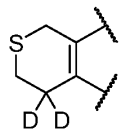
Wnt Inhibitor Embodiment C

[00129] Embodiment C1: In one or more embodiments, including any one of the first, second, third, fourth, and fifth aspects, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^1}$ and $\mathbf{R^{1a}}$ are independently selected from is hydrogen and deuterium.

[00130] Embodiment C2: In one or more embodiments, including any one of the first, second, third, fourth, and fifth aspects and **Embodiment C1**, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein ring **A** is



or



, and wherein ≈ designates attachment to the remainder of the Wnt inhibitor of Formula (I).

Wnt Inhibitor Embodiments where R^2 is (a)

[00131] Embodiment C3: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 and C2, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R^2** is phenyl substituted with **R^3** and additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups.

[00132] Embodiment C4: In one or more embodiments, including of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C3, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein

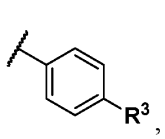
R^3 is attached to the para-position of the phenyl ring; or

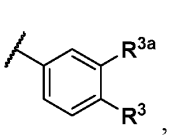
R^3 and one **R^{3a}**, when on adjacent carbons, together with the carbons to which they are attached form ring (a-1) and

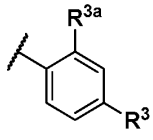
where the phenyl portion is optionally substituted with the remaining **R^{3a}** groups.

[00133] Embodiment C5: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C4, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein when **R^2** is (a), **R^3** is not halo or haloalkyl.

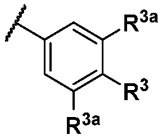
[00134] Embodiment C6: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C4, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein when **R^2** is (a), **R^3** is $B(O)_2$ or $-(C_0\text{-}C_6\text{alkylene})\text{-}O\text{-}\mathbf{R^4}$.

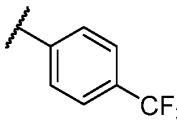
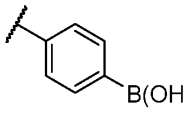
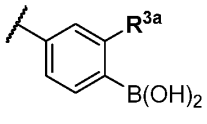
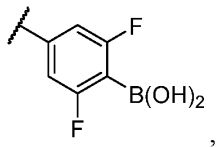
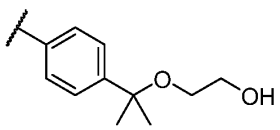
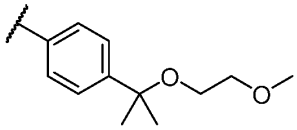
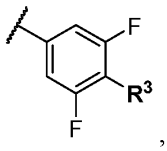
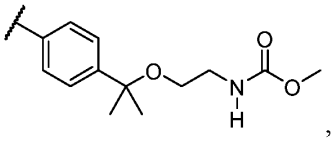
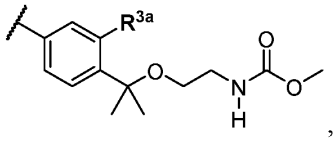
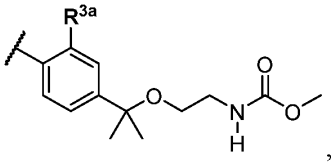
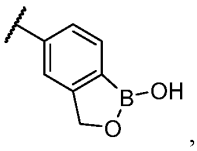
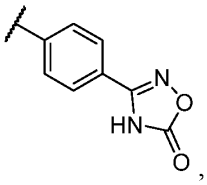
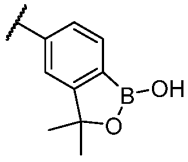
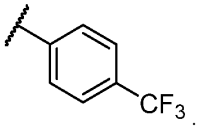
[00135] Embodiment C7: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C6, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R^2** is selected from the group consisting of 

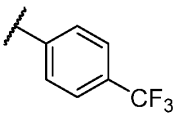
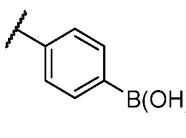
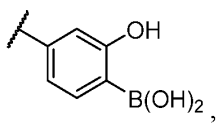
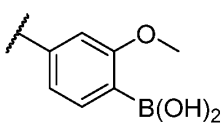
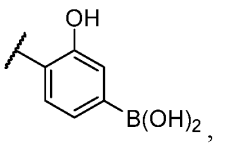
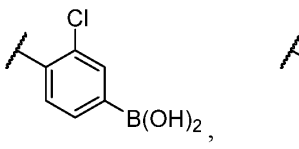
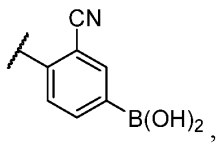
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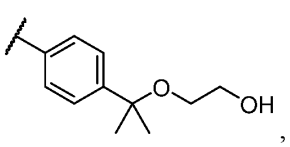
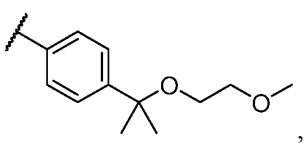
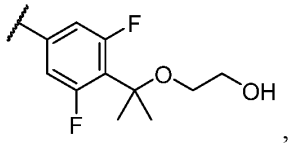
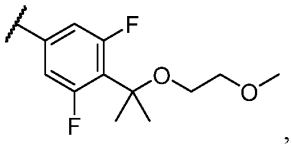
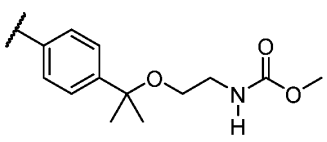
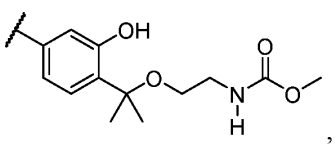
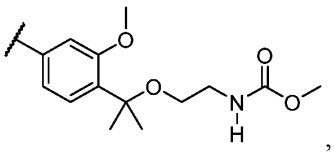
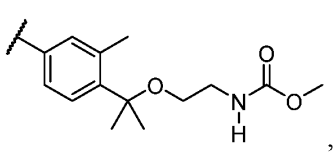
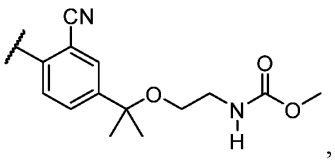
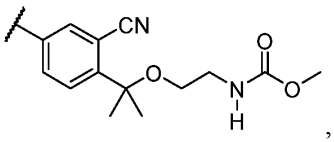
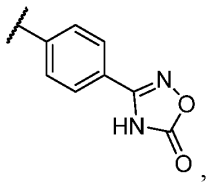
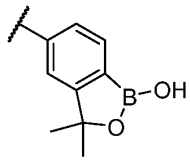
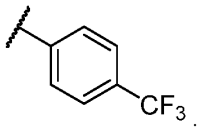
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. In one or more embodiments, $\mathbf{R^3}$ is not halo or haloalkyl. In one or more embodiments, $\mathbf{R^3}$ is $B(O)_2$ or $-(C_0\text{-}C_6\text{alkylene})\text{-}O\text{-}\mathbf{R^4}$.

[00136] Embodiment C8: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C4, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is selected from the group consisting of , , , , , , , , , , , , and . In one or more embodiments, $\mathbf{R^2}$ is not .

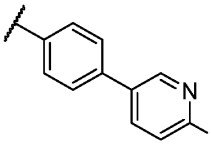
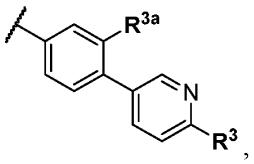
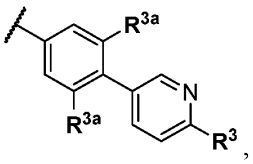
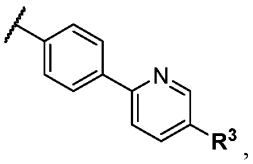
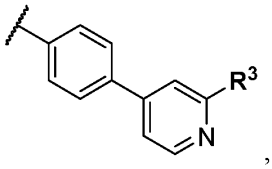
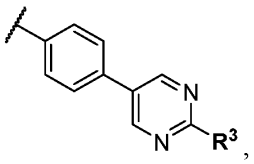
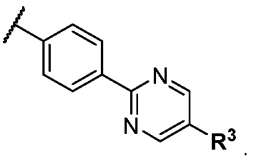
[00137] Embodiment C9: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C4, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is selected from the group consisting of , , , , , , ,

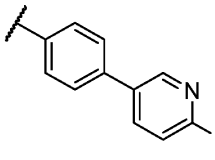
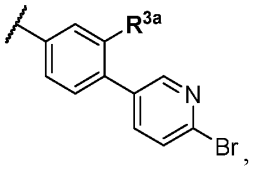
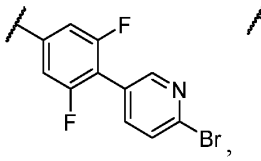
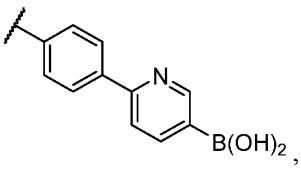
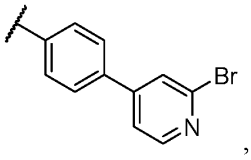
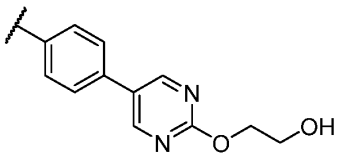
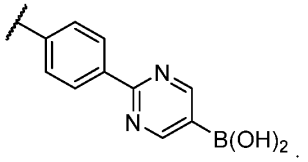











, and . In one or more embodiments, **R²** is not .

Wnt Inhibitor Embodiments where R^2 is (b)

[00138] Embodiment C10: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 and C2, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R²** is phenyl substituted with 5- or 6-membered heteroaryl where the 5- or 6-membered heteroaryl is substituted with **R³** and additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups and where the phenyl is additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups.

[00139] Embodiment C11: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2 and C10, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R²** is phenyl substituted at its para-position with 5- or 6-membered heteroaryl where the 5- or 6-membered heteroaryl is substituted with **R³** and additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups and where the phenyl is additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups; and when the 5- or 6-membered heteroaryl is a 6-membered heteroaryl then **R³** is substituted on the para-position of the 6-membered heteroaryl.

[00140] **Embodiment C12:** In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, C10, and C11, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is selected from the group consisting of , , , , , , and .

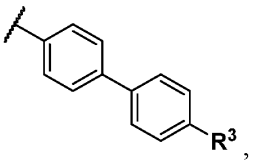
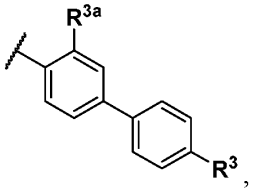
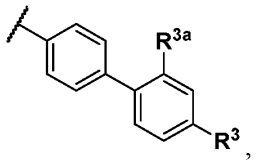
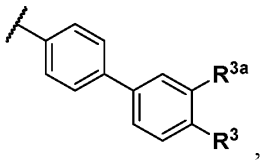
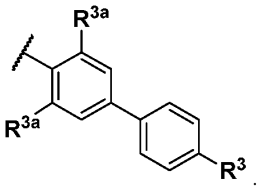
[00141] **Embodiment C13:** In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, and C10 to C12, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is selected from the group consisting of , , , , , , and .

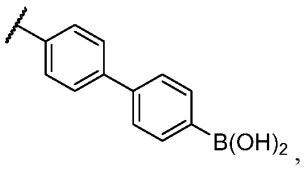
Wnt Inhibitor Embodiments where R^2 is (c)

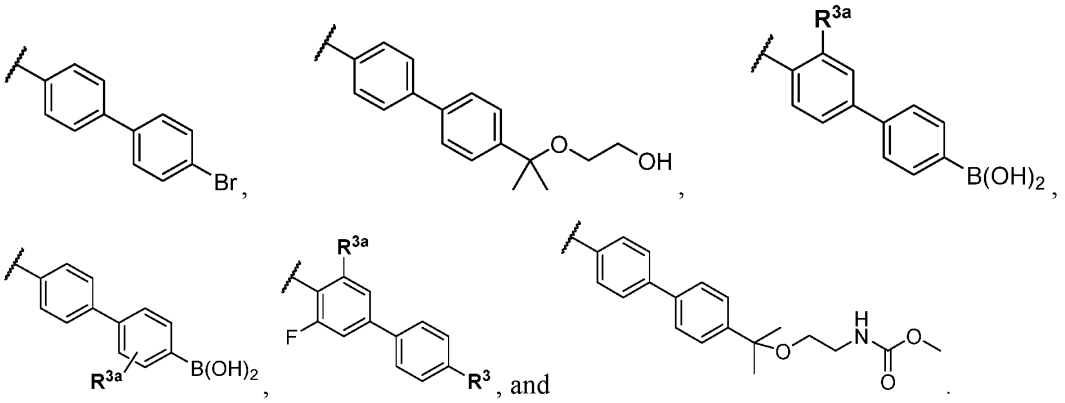
[00142] **Embodiment C14:** In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 and C2, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is phenyl optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups and additionally substituted

with -phenyl-$\mathbf{R^3}$ where the phenyl in -phenyl-$\mathbf{R^3}$ is optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups.

[00143] Embodiment C15: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, and C14, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is phenyl optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups and additionally substituted at its para-position with -phenyl-$\mathbf{R^3}$ where the phenyl in -phenyl-$\mathbf{R^3}$ is optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups and where the $\mathbf{R^3}$ is in the para-position of the phenyl in -phenyl-$\mathbf{R^3}$.

[00144] Embodiment C16: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, C14, and C15, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is selected from the group consisting of , , , , and .

[00145] Embodiment C17: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, C14, and C15, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is selected from the group consisting of ,



Wnt Inhibitor Embodiments where R^2 is (d)

[00146] Embodiment C18: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 and C2, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is 5- to 6- membered heteroaryl substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups; optionally wherein the $\mathbf{R^3}$ is at the para-position of the 6-membered heteroaryl.

[00147] Embodiment C19: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, and C18, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein when $\mathbf{R^2}$ is (d), $\mathbf{R^3}$ is not halo or haloalkyl.

[00148] Embodiment C20: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, and C18, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein when $\mathbf{R^2}$ is (d), $\mathbf{R^3}$ is $B(O)_2$ or $-(C_0\text{-}C_6\text{alkylene})\text{-}O\text{-}\mathbf{R^4}$.

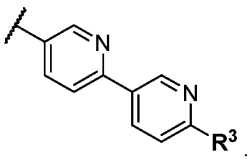
Wnt Inhibitor Embodiments where R^2 is (e)

[00149] Embodiment C22: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 and C2, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is 5- or 6- membered heteroaryl optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups and additionally substituted with -phenyl-$\mathbf{R^3}$ where the phenyl is additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups; optionally wherein the -phenyl-$\mathbf{R^3}$ is at the para-position of the 6-membered heteroaryl; and optionally wherein the $\mathbf{R^3}$ is at the para-position of the phenyl.

Wnt Inhibitor Embodiments where R^2 is (f)

[00150] **Embodiment C23:** In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 and C2, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is 5- or 6-membered heteroaryl optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups and additionally substituted with –(5- or 6-membered heteroaryl)-$\mathbf{R^3}$ where the 5- or 6-membered heteroaryl in –(5- or 6-membered heteroaryl)-$\mathbf{R^3}$ is optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups; optionally wherein the -(6-membered heteroaryl)-$\mathbf{R^3}$ is at the para-position of the first 6-membered heteroaryl; and optionally wherein the $\mathbf{R^3}$ is at the para-position of the 6-membered heteroaryl to which it is attached.

[00151] **Embodiment C24:** In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, and C23, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein

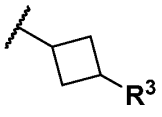
$\mathbf{R^2}$ is 

.

Wnt Inhibitor Embodiments where R^2 is (g)

[00152] **Embodiment C25:** In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 and C2, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is C_3-C_6cycloalkyl substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1 or 2 $\mathbf{R^{3a}}$ groups.

[00153] **Embodiment C26:** In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, and C25, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein

$\mathbf{R^2}$ is 

, optionally wherein $\mathbf{R^3}$ is 5- to 10-membered heterocyclic optionally substituted with cyano.

Wnt Inhibitor Embodiments where R^2 is (h)

[00154] Embodiment C27: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 and C2, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is C_3-C_6cycloalkyl substituted with 5- or 6-membered heteroaryl where the 5- or 6-membered heteroaryl is substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups, and where the cycloalkyl is optionally substituted with 1 or 2 $\mathbf{R^{3a}}$ groups.

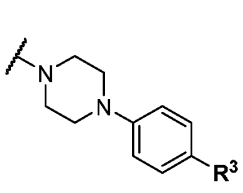
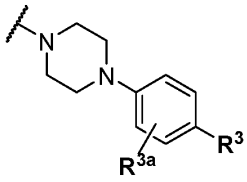
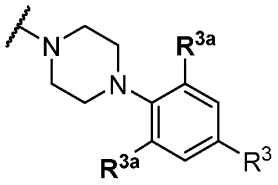
Wnt Inhibitor Embodiments where R^2 is (i)

[00155] Embodiment C28: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 and C2, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is C_3-C_6cycloalkyl substituted with phenyl where the phenyl is substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups, and where the cycloalkyl is optionally substituted with 1 or 2 $\mathbf{R^{3a}}$ groups.

Wnt Inhibitor Embodiments where R^2 is (j)

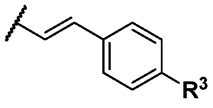
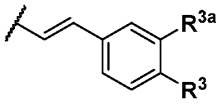
[00156] Embodiment C29: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 and C2, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is 3- to 8-membered heterocycloalkyl substituted with phenyl or 5- or 6- membered heteroaryl, where the phenyl and the 5- to 6- membered heteroaryl are substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups.

[00157] Embodiment C30: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, and C29, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein

$\mathbf{R^2}$ is selected from the group consisting of , , and .

Wnt Inhibitor Embodiments where R^2 is (k)

[00158] Embodiment C31: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 and C2, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is -CH=CH-$\mathbf{R^5}$ where $\mathbf{R^5}$ is phenyl or 5- or 6- membered heteroaryl, where the phenyl and the 5- or 6- membered heteroaryl are substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups.

[00159] Embodiment C32: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, and C31, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^2}$ is selected from the group consisting of  and .

Wnt Inhibitor Additional Embodiments

[00160] Embodiment C33: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C5, C7, C10 to C12, C14 to C16, C18 to C19, and C22 to C32 and any embodiments provided above when $\mathbf{R^2}$ is (a)-(k), provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^3}$ is cyano, -$B(OH)_2$, or -(C_0-C_6alkylene)-O-$\mathbf{R^4}$. In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^4}$ is hydroxy-C_1-C_6alkyl (in one or more embodiments, hydroxyethyl). In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^4}$ is C_1-C_6alkoxy-C_1-C_6alkyl (in one or more embodiments, C_1-C_6alkoxy-C_2alkyl). In one or more embodiments, provided is a pharmaceutical composition

comprising a Wnt inhibitor of Formula (I), wherein **R⁴** is C_1-C_6alkoxycarbonyl-NH-C_1-C_6alkyl (in one or more embodiments, C_1-C_6alkoxycarbonyl-NH-C_2alkyl).

[00161] Embodiment C34: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C5, C7, C10 to C12, C14 to C16, C18 to C19, and C22 to C32 and any embodiments provided above when R^2 is (a)-(k), provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R³** is cyano.

[00162] Embodiment C35: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C5, C7, C10 to C12, C14 to C16, C18 to C19, and C22 to C32 and any embodiments provided above when R^2 is (a)-(k), provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R³** is -$B(OH)_2$.

[00163] Embodiment C36: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C5, C7, C10 to C12, C14 to C16, C18 to C19, and C22 to C32 and any embodiments provided above when R^2 is (a)-(k), provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R³** is -(C_0-C_6alkylene)-O-**R⁴**. In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R⁴** is hydroxy-C_1-C_6alkyl (in one or more embodiments, hydroxyethyl). In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R⁴** is C_1-C_6alkoxy-C_1-C_6alkyl (in one or more embodiments, C_1-C_6alkoxy-C_2alkyl). In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R⁴** is C_1-C_6alkoxycarbonyl-NH-C_1-C_6alkyl (in one or more embodiments, C_1-C_6alkoxycarbonyl-NH-C_2alkyl).

[00164] Embodiment C37: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C5, C7, C10 to C12, C14 to C16, C18 to C19, and C22 to C32 and any embodiments provided above when R^2 is (a)-(k), provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R³** is -(C_1-C_6alkylene)-O-**R⁴**. In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R⁴** is hydroxy-C_1-C_6alkyl (in one or more embodiments, hydroxyethyl). In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein **R⁴** is C_1-C_6alkoxy-C_1-C_6alkyl (in one or more embodiments, C_1-C_6alkoxy-C_2alkyl). In one or more embodiments, provided is a pharmaceutical composition

comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^4}$ is C_1-C_6alkoxycarbonyl-NH-C_1-C_6alkyl (in one or more embodiments, C_1-C_6alkoxycarbonyl-NH-C_2alkyl).

[00165] Embodiment C38: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, C10 to C12, C14 to C16, and C22 to C24 and any embodiments provided above when $\mathbf{R^2}$ is (b), (c), (e), or (f) provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^3}$ is halo, cyano, -$B(OH)_2$, or -(C_0-C_6alkylene)-O-$\mathbf{R^4}$. In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^4}$ is hydroxy-C_1-C_6alkyl (in one or more embodiments, hydroxyethyl). In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^4}$ is C_1-C_6alkoxy-C_1-C_6alkyl (in one or more embodiments, C_1-C_6alkoxy-C_2alkyl). In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^4}$ is C_1-C_6alkoxycarbonyl-NH-C_1-C_6alkyl (in one or more embodiments, C_1-C_6alkoxycarbonyl-NH-C_2alkyl).

[00166] Embodiment C39: In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1, C2, C10 to C12, C14 to C16, and C22 to C24 and any embodiments provided above,

when $\mathbf{R^2}$ is (b), (c), (e), or (f), provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^3}$ is halo, cyano, -$B(OH)_2$, or -(C_0-C_6alkylene)-O-$\mathbf{R^4}$; or

when $\mathbf{R^2}$ is (b), (c), (e), or (f), provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^3}$ is cyano, -$B(OH)_2$, or -(C_0-C_6alkylene)-O-$\mathbf{R^4}$.

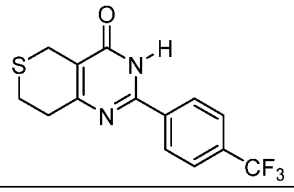
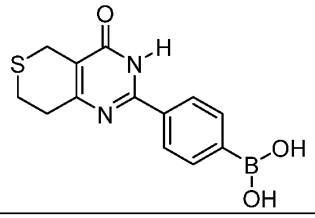
[00167] Embodiment C40: In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects and Embodiments C1 to C5, C7, C10 to C12, C14 to C16, C18 to C19, and C22 to C32 wherein $\mathbf{R^4}$ is hydroxy-C_1-C_6alkyl (in one or more embodiments, hydroxyethyl). In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^4}$ is C_1-C_6alkoxy-C_1-C_6alkyl (in one or more embodiments, C_1-C_6alkoxy-C_2alkyl). In one or more embodiments, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), wherein $\mathbf{R^4}$ is C_1-C_6alkoxycarbonyl-NH-C_1-C_6alkyl (in one or more embodiments, C_1-C_6alkoxycarbonyl-NH-C_2alkyl).

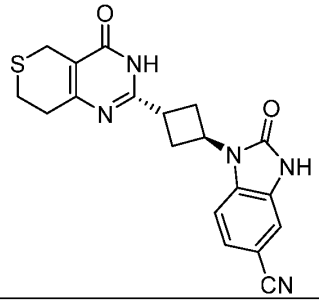
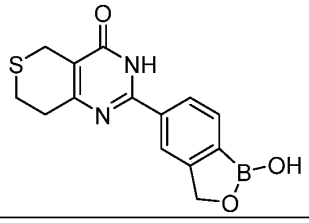
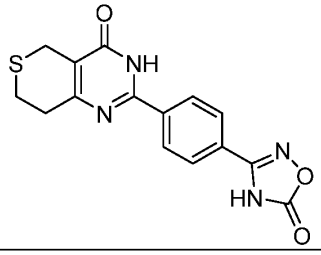
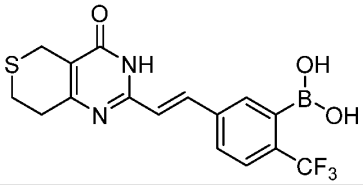
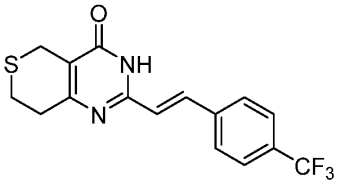
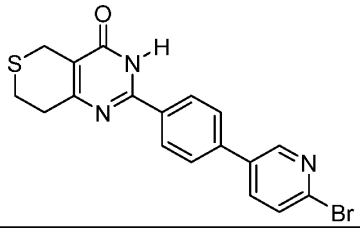
[00168] Provided herein are any one of claims 1-3 and 5-49 wherein the WNT inhibitor is according to any one of Embodiments C1 to C40.

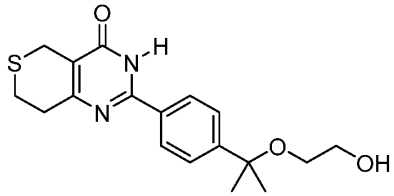
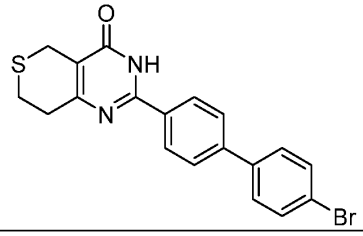
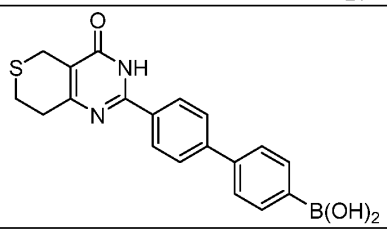
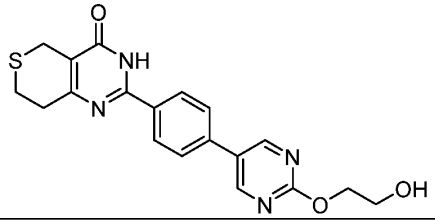
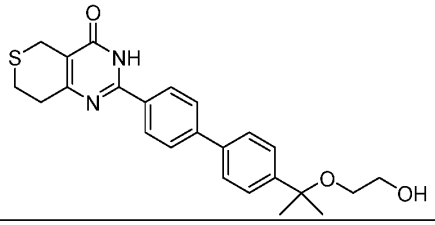
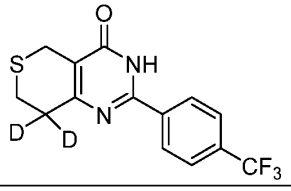
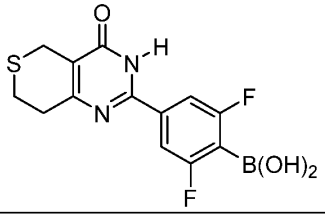
[00169] **Embodiment C41:** In one or more embodiments, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects, provided is a pharmaceutical composition comprising a Wnt inhibitor of Formula (I), selected from the group consisting of the compounds provided in Table 1.

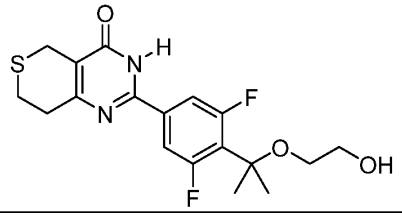
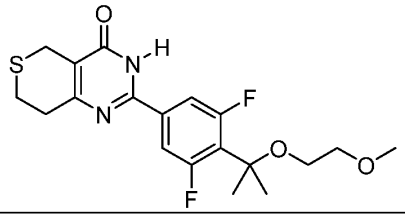
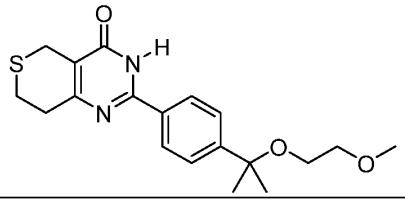
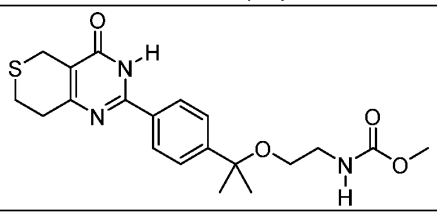
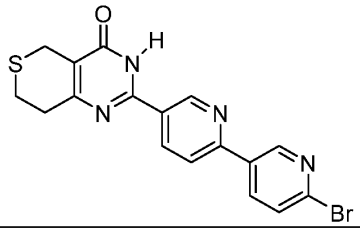
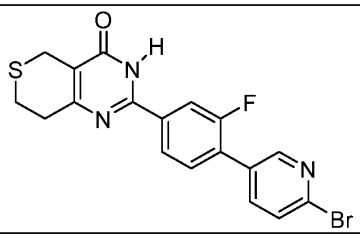
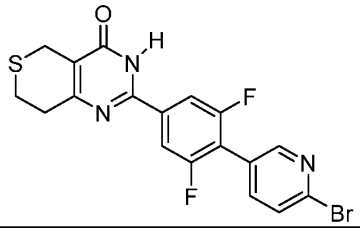
[00170] **Embodiment C42:** In one or more embodiments of the pharmaceutical composition, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor is according to a Wnt inhibitor of Formula (I), the compound is selected from any of **Compounds 1-44** or a pharmaceutically acceptable salt thereof, from Table 1. In one or more embodiments of the pharmaceutical composition, the Wnt inhibitor is according to a Wnt inhibitor of Formula (I), the compound is selected from any of **Compounds 1-44**, or an isomer thereof, from Table 1. In one or more embodiments of the pharmaceutical composition, the Wnt inhibitor is according to a Wnt inhibitor of Formula (I), the compound is selected from any of **Compounds 1-44**, or a single stereoisomer of mixture of stereoisomers thereof, and/or a pharmaceutically acceptable salt thereof, from Table 1. In one or more embodiments of the pharmaceutical composition, the Wnt inhibitor is according to a Wnt inhibitor of Formula (I), the compound is selected from any of **Compounds 1-44**, or a prodrug, pharmaceutically acceptable salt, metabolite, polymorph, solvate, hydrate, stereoisomer, or tautomer thereof, from Table 1.

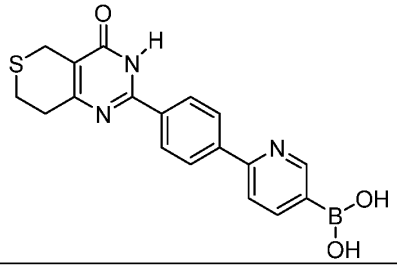
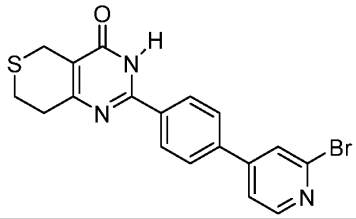
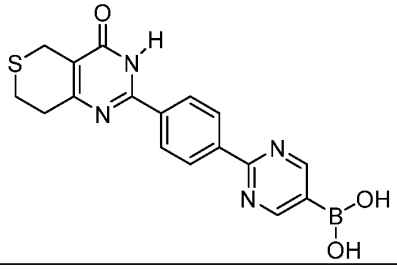
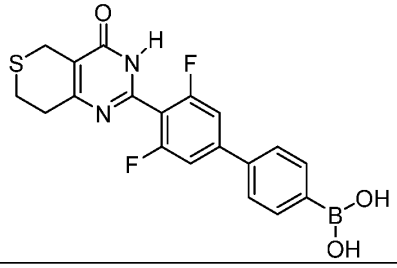
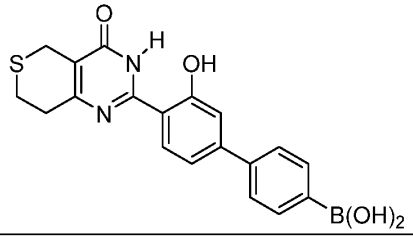
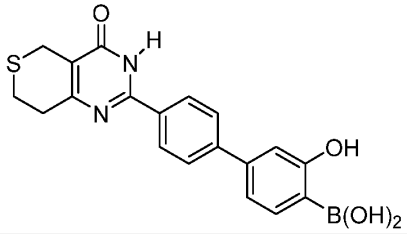
Table 1. Wnt inhibitor compounds

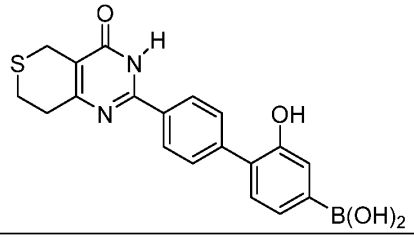
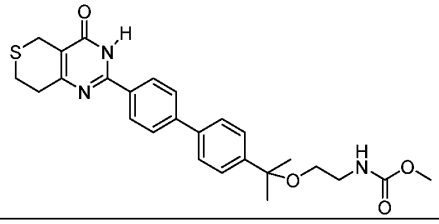
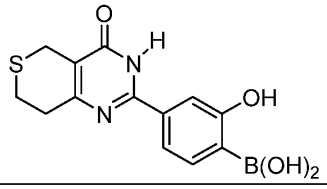
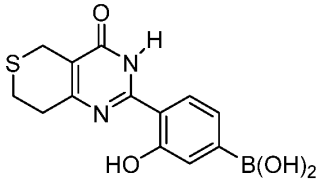
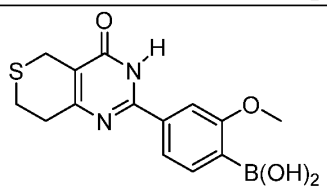
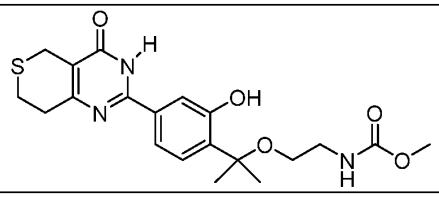
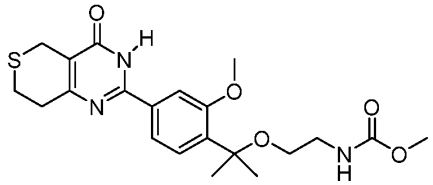
Compound Number	Structure
A (XAV939)	
1	

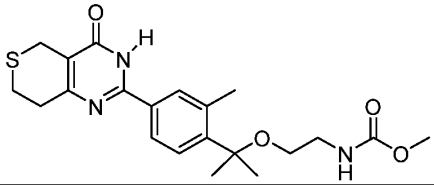
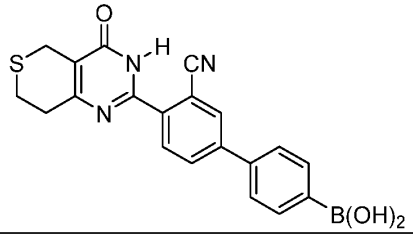
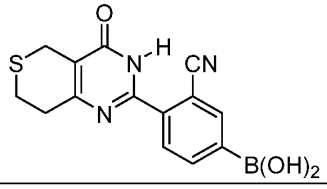
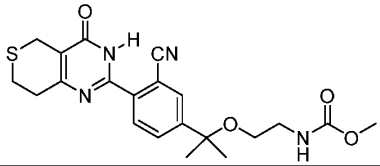
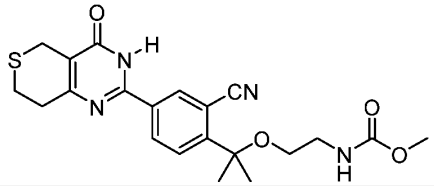
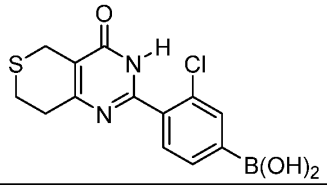
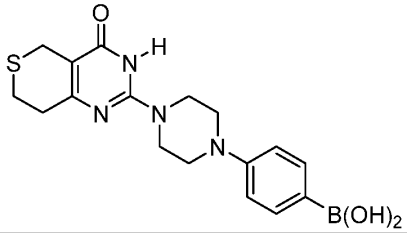
Compound Number	Structure
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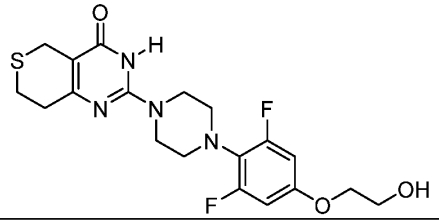
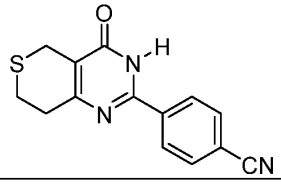
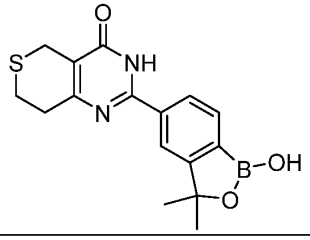
Compound Number	Structure
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Compound Number	Structure
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Compound Number	Structure
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Compound Number	Structure
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Compound Number	Structure
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Compound Number	Structure
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43	
44	

Wnt Inhibitor – Amounts

[00171] In one or more embodiments of the pharmaceutical composition, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor, such as a Wnt inhibitor of Formula (I) (for example, **Compound 1**, **Compound 7**, **Compound 8**, **Compound 10**, **Compound 18**) can constitute from about 0.001 wt% to about 5 wt% of the total composition (including water). For example, the WNT inhibitor can be about 0.005 wt% to about 5 wt%, from about 0.005 wt% to about 4.5 wt%, from about 0.005 wt% to about 4 wt%, from about 0.005 wt% to about 3.5 wt%, from about 0.005 wt% to about 3 wt%, from about 0.005 wt% to about 2.5 wt%, from about 0.005 wt% to about 2 wt%, from about 0.005 wt% to about 1.5 wt%, from about 0.005 wt% to about 1 wt%, from about 0.005 wt% to about 0.1 wt% of the total composition wt%.

[00172] In one or more embodiments of the pharmaceutical composition, including the Wnt Inhibitor of any one of the first, second, third, fourth, and fifth aspects, the Wnt inhibitor, such as a Wnt inhibitor of Formula (I) (for example, **Compound 1**, **Compound 7**, **Compound 8**, **Compound 10**, **Compound 18**) can constitute from about 0.01 wt% to about 5 wt% of the total composition (including water). For example, the WNT inhibitor can be from about 0.01 wt% to about 5 wt%, 0.01 wt% to about 4.5 wt%, from about 0.01 wt% to about 4 wt%, from

about 0.01 wt% to about 3.5 wt%, from about 0.01 wt% to about 3 wt%, from about 0.01 wt% to about 2.5 wt%, from about 0.01 wt% to about 2 wt%, from about 0.01 wt% to about 1.5 wt%, from about 0.01 wt% to about 1 wt%, or from about 0.01wt% to about 0.1 wt%, from about 0.02 wt% to about 1 wt%, or from about 0.05 wt% to about 0.5 wt% of the total composition wt%.

[00173] The concentration of WNT inhibitor in the pharmaceutical composition can be from about 0.05 to about 20 mg/mL, from 0.05 to 20 mg/mL, from about 0.05 to about 15 mg/mL, from 0.05 to 15 mg/mL, from about 0.05 to about 10 mg/mL, from 0.05 to 10 mg/mL, from about 0.1 to about 20 mg/mL, from 0.1 to 20 mg/mL, from about 0.1 to about 15 mg/mL, from 0.1 to 15 mg/mL, from about 0.1 to about 10 mg/mL, from 0.1 to 10 mg/mL, from about 0.1 to about 5 mg/mL, or from 0.1 to 5 mg/mL of the total composition wt%.

[00174] In one or more embodiments, the concentration of WNT inhibitor in the pharmaceutical composition is from about 0.1 to about 10 mg/mL, or from 0.1 to 10 mg/mL.

[00175] In one or more embodiments, the concentration of XAV939 when present in the pharmaceutical composition is from about 0.1 mg/mL to about 10 mg/mL, or from 0.1 mg/mL to 10 mg/mL of the total composition wt%.

Wnt Inhibitor – Optically Active Compounds

[00176] It is appreciated that compounds provided herein have several chiral centers and may exist in and be isolated in optically active and racemic forms. It is to be understood that any racemic, optically-active, diastereomeric, tautomeric, or stereoisomeric form, mixture, or combination thereof, of a compound provided herein, which possess the useful properties described herein is within the scope of the present disclosure. It being well known in the art how to prepare optically active forms (in some or any embodiments, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase).

[00177] In some or any embodiments, methods to obtain optically active materials are known in the art and include at least the following.

i) physical separation of crystals - a technique whereby macroscopic crystals of the individual stereoisomers are manually separated. This technique can be used if crystals of the separate stereoisomers exist, *i.e.*, the material is a conglomerate, and the crystals are visually distinct;

ii) simultaneous crystallization - a technique whereby the individual stereoisomers are separately crystallized from a solution of the racemate, possible if the latter is a conglomerate in the solid state;

iii) enzymatic resolutions - a technique whereby partial or complete separation of a racemate by virtue of differing rates of reaction for the stereoisomers with an enzyme;

iv) enzymatic asymmetric synthesis - a synthetic technique whereby one or more step(s) of the synthesis uses an enzymatic reaction to obtain a stereoisomerically pure or enriched synthetic precursor of the desired stereoisomer;

v) chemical asymmetric synthesis - a synthetic technique whereby the desired stereoisomer is synthesized from an achiral precursor under conditions that produce asymmetry (*i.e.*, chirality) in the product, which may be achieved using chiral catalysts or chiral auxiliaries;

vi) diastereomer separations - a technique whereby a racemic compound is reacted with an enantiomerically pure reagent (the chiral auxiliary) that converts the individual enantiomers to diastereomers. The resulting diastereomers are then separated by chromatography or crystallization by virtue of their now more distinct structural differences and the chiral auxiliary later removed to obtain the desired enantiomer;

vii) first- and second-order asymmetric transformations - a technique whereby diastereomers from the racemate equilibrate to yield a preponderance in solution of the diastereomer from the desired enantiomer or where preferential crystallization of the diastereomer from the desired enantiomer perturbs the equilibrium such that eventually in principle all the material is converted to the crystalline diastereomer from the desired enantiomer. The desired enantiomer is then released from the diastereomer;

viii) kinetic resolutions - this technique refers to the achievement of partial or complete resolution of a racemate (or of a further resolution of a partially resolved compound) by virtue of unequal reaction rates of the stereoisomers with a chiral, non-racemic reagent or catalyst under kinetic conditions;

ix) stereospecific synthesis from non-racemic precursors - a synthetic technique whereby the desired stereoisomer is obtained from non-chiral starting materials and where the stereochemical integrity is not or is minimally compromised over the course of the synthesis;

x) chiral liquid chromatography - a technique whereby the stereoisomers of a racemate are separated in a liquid mobile phase by virtue of their differing interactions with a

stationary phase. The stationary phase can be made of chiral material or the mobile phase can contain an additional chiral material to provoke the differing interactions;

xi) chiral gas chromatography - a technique whereby the racemate is volatilized and stereoisomers are separated by virtue of their differing interactions in the gaseous mobile phase with a column containing a fixed non-racemic chiral adsorbent phase;

xii) extraction with chiral solvents - a technique whereby the stereoisomers are separated by virtue of preferential dissolution of one stereoisomer into a particular chiral solvent;

xiii) transport across chiral membranes - a technique whereby a racemate is placed in contact with a thin membrane barrier. The barrier typically separates two miscible fluids, one containing the racemate, and a driving force such as concentration or pressure differential causes preferential transport across the membrane barrier. Separation occurs as a result of the non-racemic chiral nature of the membrane which allows one stereoisomer of the racemate to pass through.

[00178] In some or any embodiments, provided is a pharmaceutical composition with a compound that comprises a substantially pure designated stereoisomer of the compound. In some or any embodiments, in the methods and compounds, the compounds are substantially free of other stereoisomer. In some or any embodiments, a pharmaceutical composition includes a compound that is at least 85%, 90%, 95%, 98%, 99% or 100% by weight, of the designated stereoisomer, the remainder comprising other chemical species or stereoisomers.

Wnt Inhibitor – Isotopically Enriched Compounds

[00179] Also provided herein are isotopically enriched compounds.

[00180] Isotopic enrichment (in some or any embodiments, deuteration) of pharmaceuticals to improve pharmacokinetics ("PK"), pharmacodynamics ("PD"), and toxicity profiles, has been demonstrated previously with some classes of drugs. See, for example, Lijinsky *et. al.*, Food Cosmet. Toxicol., 20: 393 (1982); Lijinsky *et. al.*, J. Nat. Cancer Inst., 69: 1127 (1982); Mangold *et. al.*, Mutation Res. 308: 33 (1994); Gordon *et. al.*, Drug Metab. Dispos., 15: 589 (1987); Zello *et. al.*, Metabolism, 43: 487 (1994); Gately *et. al.*, J. Nucl. Med., 27: 388 (1986); Wade D, Chem. Biol. Interact. 117: 191 (1999).

[00181] Isotopic enrichment of a drug can be used, in some or any embodiments, to (1) reduce or eliminate unwanted metabolites, (2) increase the half-life of the parent drug, (3) decrease the number of doses to achieve a desired effect, (4) decrease the amount of a dose that achieves a desired effect, (5) increase the formation of active metabolites, if any are formed, and/or (6) decrees the production of deleterious metabolites in specific tissues and/or

create a more effective drug and/or a safer drug for combination therapy, whether the combination therapy is intentional or not.

[00182] Replacement of an atom for one of its isotopes often will result in a change in the reaction rate of a chemical reaction. This phenomenon is known as the Kinetic Isotope Effect ("KIE"). For example, if a C–H bond is broken during a rate-determining step in a chemical reaction (i.e. the step with the highest transition state energy), substitution of a deuterium for that hydrogen will cause a decrease in the reaction rate and the process will slow down. This phenomenon is known as the Deuterium Kinetic Isotope Effect ("DKIE"). See, *e.g.,* Foster *et al.*, Adv. Drug Res., vol. 14, pp. 1-36 (1985); Kushner *et al.*, Can. J. Physiol. Pharmacol., vol. 77, pp. 79-88 (1999). The highest transition state energy may refer to the largest Gibbs free energy or largest Gibbs free energy difference relative to starting material or intermediate of interest in a reaction pathway depicted by a single energy axis.

[00183] The magnitude of the DKIE can be expressed as the ratio between the rates of a given reaction in which a C–H bond is broken, and the same reaction where deuterium is substituted for hydrogen. The DKIE can range from about 1 (no isotope effect) to large integers, such as 50 or more, meaning that the reaction can be fifty, or more, times slower when deuterium is substituted for hydrogen. High DKIE values may be due in part to a phenomenon known as tunneling, which is a consequence of the uncertainty principle. Tunneling is ascribed to the small mass of a hydrogen atom and occurs because transition states involving a proton can sometimes form in the absence of the requisite activation energy. Because deuterium has more mass than hydrogen, it statistically has a much lower probability of undergoing this phenomenon.

[00184] Tritium ("T") is a radioactive isotope of hydrogen, used in research, fusion reactors, neutron generators and radiopharmaceuticals. Tritium is a hydrogen atom that has 2 neutrons in the nucleus and has an atomic weight close to 3. It occurs naturally in the environment in low concentrations, commonly found as T_2O. Tritium decays slowly (half-life = 12.3 years) and emits a low energy beta particle that cannot penetrate the outer layer of human skin. Internal exposure is the main hazard associated with this isotope, yet ingesting in large amounts can pose a significant health risk. As compared with deuterium, a lesser amount of tritium must be consumed before it reaches a hazardous level. Substitution of tritium ("T") for hydrogen results in yet a stronger bond than deuterium and gives numerically larger isotope effects. Similarly, substitution of isotopes for other elements, including, but not limited to, ^{13}C or ^{14}C for carbon, ^{33}S, ^{34}S, or ^{36}S for sulfur, ^{15}N for nitrogen, and ^{17}O or ^{18}O for oxygen, may lead to a similar kinetic isotope effect.

[00185] For example, the DKIE was used to decrease the hepatotoxicity of halothane by presumably limiting the production of reactive species such as trifluoroacetyl chloride. However, this method may not be applicable to all drug classes. For example, deuterium incorporation can lead to metabolic switching. The concept of metabolic switching asserts that xenogens, when sequestered by Phase I enzymes, may bind transiently and re-bind in a variety of conformations prior to the chemical reaction (e.g., oxidation). This hypothesis is supported by the relatively vast size of binding pockets in many Phase I enzymes and the promiscuous nature of many metabolic reactions. Metabolic switching can potentially lead to different proportions of known metabolites as well as altogether new metabolites. This new metabolic profile may impart more or less toxicity.

[00186] In one or more embodiments, the compounds described herein may be used as radiopharmaceuticals such as, for example, imaging agents. In one instance, radiopharmaceuticals are positron emission tomography (PET) imaging agents. In such embodiments, substitution of radionuclides (e.g., positron emitting isotopes) for atoms in the compounds allows for the syntheses of radiopharmaceuticals that can function as imaging agents. In one or more embodiments, radionuclides which can be substituted in the compounds described herein include, and are not limited to, ^{18}F, ^{11}C, ^{13}N, ^{15}O, ^{76}Br, and ^{124}I. In one or more embodiments, the compound is isotopically enriched at one or more atoms, one atom, two atoms, or three atoms. In one or more embodiments, the compound is administered as an isotopic composition.

[00187] The animal body expresses a variety of enzymes for the purpose of eliminating foreign substances, such as therapeutic agents, from its circulation system. In some or any embodiments, such enzymes include the cytochrome P450 enzymes (“CYPs”), esterases, proteases, reductases, dehydrogenases, and monoamine oxidases, to react with and convert these foreign substances to more polar intermediates or metabolites for renal excretion. Some common metabolic reactions of pharmaceutical compounds involve the oxidation of a carbon-hydrogen (C–H) bond to either a carbon-oxygen (C–O) or carbon-carbon (C–C) pi-bond. The resultant metabolites may be stable or unstable under physiological conditions, and can have substantially different pharmacokinetic, pharmacodynamic, and acute and long-term toxicity profiles relative to the parent compounds. For many drugs, such oxidations are rapid. These drugs therefore often require the administration of multiple or high daily doses.

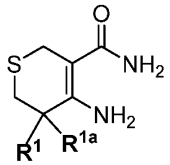
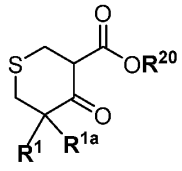
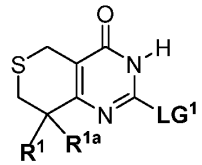
[00188] Therefore, isotopic enrichment at certain positions of a compound provided herein will produce a detectable KIE that will affect the pharmacokinetic, pharmacologic, and/or

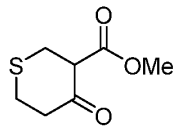
toxicological profiles of a compound provided herein in comparison with a similar compound having a natural isotopic composition.

Preparation of Wnt Inhibitor Compounds

[00189] The Wnt inhibitors provided herein can be prepared, isolated, or obtained by any method apparent to those of skill in the art. Wnt inhibitors provided herein can be prepared according to the Preparation Schemes provided below. Reaction conditions, steps and reactants not provided in the Preparation Schemes described herein would be apparent to, and known by, those skilled in the art.

[00190] Additional steps and reagents not provided in the Preparation Schemes described herein would be known to those of skill in the art. For example, intermediates and compounds could be prepared using the procedures known by one of ordinary skill in the art or as disclosed in U.S. Provisional Application Numbers: 63/417,257; 63/418,947; and 63/418,956 (wherein the synthetic methods disclosed therein are herein incorporated by reference in their entirety). Methods of preparation are described in detail in the Examples herein.

[00191] In one or more embodiments, provided is a method of preparing a Wnt inhibitor of Formula (I), comprising: a) contacting a compound of Formula (A):  with $\mathbf{R^{2'}}$-C(O)H; or b) contacting a compound of Formula (B):  with $\mathbf{R^{2'}}$-C(NH)NH_2, wherein R^{20} is Me or CD_3; or c) contacting a compound of Formula (C):  with $R^{2'}$-H, wherein $\mathbf{LG^1}$ is fluoro, chloro, bromo, iodo, triflate, mesylate, a triazole, a pyrazole, boronic acid, boronic ester, or aryl trifluoroborate; and optionally isolating the Wnt inhibitor of Formula (I); wherein $\mathbf{R^{2'}}$ is (b1) phenyl substituted with 5- or 6-membered heteroaryl where the 5- or 6-membered heteroaryl is substituted with $\mathbf{LG^1}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups and where the phenyl is additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups, (c1) phenyl optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups and additionally

substituted with -phenyl- **LG^1** where the phenyl in -phenyl- **LG^1** is optionally substituted with 1, 2, or 3 **R^{3a}** groups; (e1) 5- or 6-membered heteroaryl optionally substituted with 1, 2, or 3 **R^{3a}** groups and additionally substituted with -phenyl- **LG^1** where the phenyl is additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups; (f1) 5- or 6-membered heteroaryl optionally substituted with 1, 2, or 3 **R^{3a}** groups and additionally substituted with -(5- or 6-membered heteroaryl)- **LG^1** where the 5- or 6-membered heteroaryl in -(5- or 6-membered heteroaryl)- **LG^1** is optionally substituted with 1, 2, or 3 **R^{3a}** groups; (h1) C_3-C_6cycloalkyl substituted with NH_2 or OH and additionally optionally substituted with 1 or 2 **R^{3a}** groups; or (i1) C_3-C_6cycloalkyl substituted with phenyl where the phenyl is substituted with **LG^1** and the phenyl is additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups, and where the cycloalkyl is optionally substituted with 1 or 2 **R^{3a}** groups; and provided that the compound is not: methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate 

; or a salt thereof and/or a stereoisomer or mixture of stereoisomers thereof. In one or more embodiments, methods of preparing a Wnt inhibitor of Formula (I) include contacting under basic conditions; and all other groups are as defined herein in any embodiments; and optionally wherein **R^1** and **R^{1a}** are independently hydrogen or alkyl.

[00192] One of skill will understand that the order of steps for any process described herein may be changed. Other variations will be apparent to one of skill in the art and all such variations are contemplated within the scope of embodiments presented herein.

Active pharmaceutical agent

[00193] While the WNT inhibitors disclosed herein can be administered as the sole active pharmaceutical agent, the sole active pharmaceutical agent used to treat a particular disorder, or the sole active pharmaceutical agent in a therapeutically effective amount in a composition, they can also be used in combination with one or more other agents used in the treatment or suppression of certain disorders.

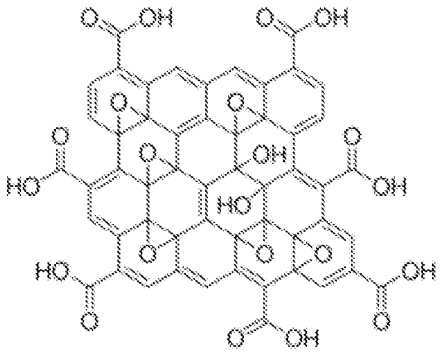
[00194] In one or more embodiments, the pharmaceutical compositions provided herein do not comprise an FGF agonist, a BMP inhibitor, a TGF-β agonist, and/or a DMP4 agonist.

[00195] In some or any embodiments, the WNT inhibitor is not a protein or peptide.

GO-HA

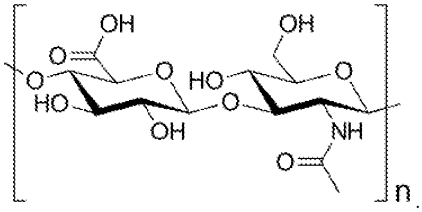
[00196] **Embodiment D1:** The covalently-linked GO and HA is also referred to herein as GO-HA conjugate or simply GO-HA. The GO-HA conjugate can be made according to procedures known to a person of ordinary skill in the art, including those disclosed in US-2019-0105398-A1.

[00197] Graphene oxide (GO) as used herein refers to an oxidized form of graphene, which is a single layer form of graphite. GO can be obtained by treating graphite with strong oxidizers. GO contains carbon, oxygen, and hydrogen in various amounts, depending on how it is made. It can be of length of several hundreds of nanometers up to several micrometers, its planar direction, and about 0.7-1.2 nm in thickness. GO can include various oxygen containing moieties, such as oxygen epoxide groups, carboxylic acid (-COOH), phenol, etc., when prepared using sulfuric acid (e.g. Hummer's method). An example GO structure is shown below.



[00198] In one or more embodiments, graphene oxide is modified by reacting with chloroacetic acid, herein referred to as "Modified GO" in some embodiments. In one or more embodiments, hydroxyl groups of the graphene oxide react with the chloroacetic acid, *i.e.* replacing -OH groups with $-OCH_2C(O)OH$ groups.

[00199] Hyaluronic acid (HA) is an anionic, highly hydrophilic, non-sulfated glycosaminoglycan, occurring naturally throughout the human body. It can be several thousands of carbohydrate units long and can bind to water giving it a gel of stiff viscous quality. An example structure of HA is provided below:



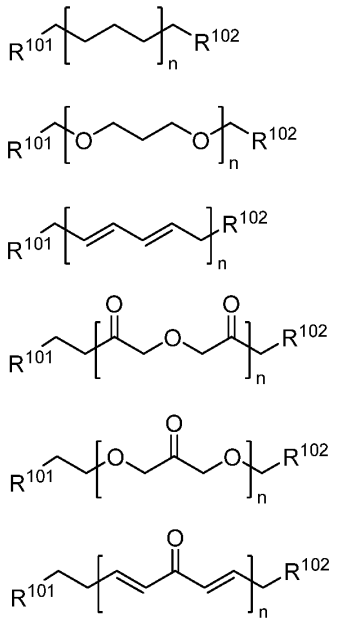
[00200] In a composition of present disclosure, the GO or Modified GO and HA are covalently linked to form a matrix component (or a carrier), which can serve to form a stable suspension of the Wnt inhibitor, including of Formula (I) (such as XAV939, **Compound 1**, **Compound 7**, **Compound 8**, **Compound 10**, and **Compound 18**) as well as providing other simultaneous benefits to wound healing.

[00201] **Embodiment D2:** The covalent linking can be accomplished by using a linker or linker moiety ("GO-HA linker"). In one or more embodiments, the GO-HA linker can include 2-25 carbons. In one or more embodiments, the GO-HA linker is linear. In one or more embodiments, the GO-HA linker is branched. The GO-HA linker can be saturated or unsaturated.

[00202] **Embodiment D3:** In one or more embodiments, the GO-HA linker can comprise a C_2-C_{25} alkylene group, where the carbons and hydrogens in the alkylene group can be substituted by oxygen or other atoms or groups such as hydroxy, carboxy, amino, alkyl, alkoxy, alkenyl, alkynyl, nitro, etc. In one or more embodiments, the GO-HA linker can comprise one or more $-CH_2CH_2O-$ units. In one or more embodiments, the GO-HA linker does not comprise dopamine. In one or more embodiments, the GO-HA linker does not comprise an aryl or heteroaryl group.

[00203] **Embodiment D4:** In one or more embodiments, the GO-HA linker comprises $-R_x-R_s-R_y-$, wherein R_x and R_y are each independently selected from the group consisting of -CO-, -COO-, - H-, - H- H-, - H- H- CO-, -CS-, -S-, -O-, and wherein R_s (which is also referred to as the spacer group in this application) can be an unsubstituted or substituted, saturated or unsaturated linear alkylene group having 2-20 backbone carbons. In one or more embodiments, both R_x and R_y are *- H- H-CO- (* denoting the ends of the linker distal to Rs).

[00204] **Embodiment D5:** In one or more embodiments, the spacer group in the GO-HA linker can be an unsubstituted or substituted, saturated or unsaturated linear alkylene group having 2-20 backbone carbons. For illustration and not limitation, the HA can be derivatized with one of the following spacer groups:



where R^{101} and R^{102} can be independently -CONHNH-, -S-, -NH-, -O-, or other nucleophiles, and n is an integer and can be for example, 1-20, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, etc. In one or more embodiments, the HA is derivatized with a spacer group comprising a dihydrazide (e.g. -NHNHC(O)-alkylene-CONHNH-), such as adipic acid dihydrazide (-NHNHC(O)$(CH_2)_4$CONHNH-). The derivatized HA can then be linked to GO or Modified GO.

[00205] **Embodiment D6a:** In some or any embodiments, the GO-HA conjugate is a conjugate of GO linked to HA via a linker comprising -CH_2-C(O)-NH-NH-C(O)-$(CH_2)_4$-C(O)NH-NH-.

[00206] **Embodiment D6:** In some or any embodiments, the GO-HA conjugate is a conjugate of GO linked to HA via the linker -CH_2-C(O)-NH-NH-C(O)-$(CH_2)_4$-C(O)NH-NH- where the HA is linked via an amide bond comprising the terminal NH of the linker and the GO is linked to the CH_2 end of the linker (in some embodiments through an ether bond with GO phenol).

Wnt Inhibitor to GO-HA weight ratio (wt% ratio)

[00207] In one or more embodiments of the pharmaceutical composition comprising GO-HA (for example, GO-HA of Embodiments D6 and D6a), the wt% ratio of the Wnt inhibitor, such as a Wnt inhibitor of Formula (I) (for example, XAV939, **Compound 1**, **Compound 7**, **Compound 8**, **Compound 10**, and **Compound 18**) to GO-HA can be from about 1:100 to 100:1, e.g., from about 1:2 to about 2:1 in the total composition. For example, the wt% ratio of

the Wnt inhibitor to GO-HA can be 1:100, about 1:90, about 1:80, about 1:70, about 1:60, about 1:50, about 1:40, about 1:30, about 1:20, about 1:10, about 1:5, about 1:1, about 5:1, about 10:1, about 20:1, about 30:1, about 40:1, about 50:1, about 60:1, about 70:1, about 80:1, about 90:1, or 100:1 in the total composition. In one or more embodiments, the wt% ratio of the Wnt inhibitor to GO-HA can be from about 0.1:1 to about 12:1, such as from 0.1:1, about 0.2:1, about 0.3:1, about 0.4:1, about 0.5:1, about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 11:1, or 12:1 in the total composition.

[00208] In one or more embodiments, the wt% ratio of WNT inhibitor:GO-HA in the pharmaceutical composition is from about 1:0.1 to about 1:1. For example, the wt% ratio of WNT inhibitor:GO-HA in the pharmaceutical composition is from about 1:0.2 to about 1:1, from about 1:0.3 to about 1:1, from about 1:0.4 to about 1:1, from about 1:0.5 to about 1:1, from about 0.6 to about 1:1, from about 0.7 to about 1:1, from about 0.8 to about 1:1, or from about 0.9 to about 1:1. In some or any embodiments, the WNT inhibitor is XAV939, **Compound 1**, **Compound 7**, **Compound 8**, **Compound 10**, or **Compound 18**. In some or any embodiments, the WNT inhibitor is XAV939.

GO-HA Amounts

[00209] In one or more embodiments, in the GO-HA conjugate (for example, GO-HA of Embodiments D6 and D6a), the weight ratio of GO:HA can be from about 1:1 to about 1:20, from about 1:6 to about 1:10. For example, the weight ratio of GO:HA in the GO-HA conjugate can be about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, about 1:10, about 1:11, about 1:12, about 1:13, about 1:14, about 1:15, about 1:16, about 1:17, about 1:18, about 1:19, or about 1:20.

[00210] In one or more embodiments, GO-HA (for example, GO-HA of Embodiments D6 and D6a) constitutes from about 0.001 wt % to about 5 wt% of the total composition. For example, the GO-HA can constitute from about 0.005 wt% to about 5 wt%, from about 0.005 wt% to about 4 wt%, from about 0.005 wt% to about 3 wt%, from about 0.005 wt% to about 2 wt%, from about 0.005 wt% to about 1 wt%, from about 0.005 wt% to about 0.5 wt%, from about 0.01 wt% to about 5 wt%, from about 0.01 wt% to about 4 wt%, from about 0.01 wt% to about 3 wt%, from about 0.01 wt% to about 2 wt%, from about 0.01 wt% to about 1 wt%, from about 0.01 wt% to about 0.5 wt%, from about 0.02 wt% to about 5 wt%, from about 0.02 wt% to about 4 wt%, from about 0.02 wt% to about 3 wt%, from about 0.02 wt% to about 2 wt%, from about 0.02 wt% to about 1 wt%, from about 0.02 wt% to about 0.5 wt%, from about 0.05 wt% to about 5 wt%, from about 0.05 wt% to about 4 wt%, from about 0.05 wt%

to about 3 wt%, from about 0.05 wt% to about 2 wt%, from about 0.05 wt% to about 1 wt%, or from about 0.05 wt% to about 0.5 wt% of the total composition wt%.

[00211] The concentration of GO-HA in the pharmaceutical composition can be from 0.005 to 25 mg/mL, about 0.005 to about 25 mg/mL, from about 0.005 to about 20 mg/mL, from 0.005 to 20 mg/mL, from about 0.005 to about 15 mg/mL, from 0.005 to 15 mg/mL, from about 0.005 to about 10 mg/mL, from 0.005 to 10 mg/mL, from about 0.005 to about 5 mg/mL, from 0.005 to 5 mg/mL, from about 0.01 to about 25 mg/mL, from 0.01 to 25 mg/mL, from about 0.01 to about 20 mg/mL, from 0.01 to 20 mg/mL, from about 0.01 to about 15 mg/mL, from 0.01 to 15 mg/mL, from about 0.01 to about 10 mg/mL, or from 0.01 to 10 mg/mL of the total composition wt%.

[00212] In one or more embodiments, the concentration of GO-HA in the pharmaceutical composition is from about 0.01 to about 10 mg/mL, or from 0.01 to 10 mg/mL of the total composition wt%.

Additional Components of the Composition

[00213] In the pharmaceutical compositions as described herein, other pharmaceutical or therapeutic compounds may be included in addition to a Wnt inhibitor, such as a Wnt inhibitor of Formula (I) (for example, XAV939, **Compound 1**, **Compound 7**, **Compound 8**, **Compound 10**, **Compound 18**). In other words, the pharmaceutical compositions, with Wnt inhibitors such as compound(s) of Formula (I) (for example, XAV939, **Compound 1**, **Compound 7**, **Compound 8**, **Compound 10**, **Compound 18**) present can also serve as a base dispersion medium in which other pharmaceutical or therapeutic agents, especially those which are hydrophobic, may be dispersed, e.g., for topical administration to a wound. These agents may include antifibrotic compounds such as pirfenidone, halofuginone, nintedanib, tocilizumab, rilonacept, etc., anti-cancer agents, anti-inflammatory agents, analgesics, antibiotics, Wnt inhibitors, Hedgehog pathway inhibitors, TGF-ß inhibitors, LOX inhibitors, etc.

[00214] In one or more embodiments, the pharmaceutical composition further comprises pharmaceutical carriers, excipients, compounds, or materials which enables the composition to be presented in topically administrable semi-solid aqueous gel forms. For example, carboxymethylcellulose can be used as a gel-forming agent. However, other cellulose derivatives such as microcrystalline cellulose as well as polysaccharides such as alginate, agarose, tragacanth, guar gum, and xanthum gum; are also suitable as gel-forming agents. The gel may be made thicker and/or stiffer by addition of a relatively resilient gel-forming material

such as a cross-linked fibrous protein, e.g. gelatin or collagen cross-linked with formaldehyde. In one or more embodiments, the pharmaceutical composition can be in a form of a cream, which can include those excipients suitable for a cream formulation, such as paraffin oil, vaseline, wax, organic esters such as cetyl palmitate, etc.

[00215] In one or more embodiments, the compositions can include a second medication or therapeutic agent to the wound, comprising one or more of: corticosteroid, a cytotoxic drug, an antibiotic, an antiseptic, nicotine, an anti-platelet drug, an NSAID, colchicine, an anti-coagulant, a vasoconstricting drug or an immunosuppressive, a growth factor, an antibody, a protease, a protease inhibitor, an antibacterial peptide, an adhesive peptide, a hemostatic agent, living cells, honey, or nitric oxide. These therapeutic agents can be delivered as separate dosage forms from the compositions described herein or may be included as additional components of the pharmaceutical compositions described herein, hence delivered together with the Wnt inhibitor of Formula (I) (or any embodiments thereof).

[00216] In some or any embodiments, the content of the pharmaceutical composition is described as being wherein: the wt% of the WNT inhibitor, GO-HA, glycol, one or more solubilizers, one or more phospholipids, one or more carriers, optional stabilizer, (when present), optional buffer (when present), optional preservative (when present), and saline or water is 100 wt%. Even though the total wt% of the recited components is described as being 100, it will be understood by the skilled person that the pharmaceutical composition can comprise additional ingredients, components, and/or agents in the composition, including any of those described in this section "*Additional Components of the Composition*."

[00217] In some or any embodiments, the second agent can be formulated or packaged with the compound provided herein. Of course, the second agent will be formulated with the compound provided herein when, according to the judgment of those of skill in the art, such co-formulation should not interfere with the activity of either agent or the method of administration. In some or any embodiments, the compound provided herein and the second agent are formulated separately. They can be packaged together, or packaged separately, for the convenience of the practitioner of skill in the art.

[00218] In clinical practice the active agents provided herein may be administered by any conventional route, in particular parenterally, rectally, orally, by inhalation (e.g. in the form of aerosols), or topically.

[00219] The composition(s) of the present disclosure described herein can be administered by applying the composition(s) topically on the wound. If the composition is included in a

medical device described herein which includes a substrate such as a patch or a pad, the medical device can be secured to the wound such that the composition contacts the wound.

[00220] Use may be made, as solid compositions for oral administration, of tablets, pills, hard gelatin capsules, powders, or granules. In these compositions, the active product is mixed with one or more inert diluents or adjuvants, such as sucrose, lactose, or starch.

[00221] These compositions can comprise substances other than diluents, for example a lubricant, such as magnesium stearate, or a coating intended for controlled release.

[00222] Use may be made, as liquid compositions for oral administration, of solutions which are pharmaceutically acceptable, suspensions, emulsions, syrups, and elixirs containing inert diluents, such as water or liquid paraffin. These compositions can also comprise substances other than diluents, in some or any embodiments, wetting, sweetening or flavoring products.

[00223] The compositions for parenteral administration can be emulsions or sterile solutions. Use may be made, as solvent or vehicle, of propylene glycol, a polyethylene glycol, vegetable oils, in particular olive oil, or injectable organic esters, in some or any embodiments, ethyl oleate. These compositions can also contain adjuvants, in particular wetting, isotonizing, emulsifying, dispersing and stabilizing agents. Sterilization can be carried out in several ways, in some or any embodiments, using a bacteriological filter, by radiation or by heating. They can also be prepared in the form of sterile solid compositions which can be dissolved at the time of use in sterile water or any other injectable sterile medium.

[00224] The compositions for rectal administration are suppositories or rectal capsules which contain, in addition to the active principle, excipients such as cocoa butter, semi-synthetic glycerides or polyethylene glycols.

[00225] The compositions can also be aerosols. For use in the form of liquid aerosols or sprays, the compositions can be stable sterile solutions or solid compositions dissolved at the time of use in apyrogenic sterile water, in saline or any other pharmaceutically acceptable vehicle. For use in the form of dry aerosols intended to be directly inhaled, the active principle is finely divided and combined with a water-soluble solid diluent or vehicle, in some or any embodiments, dextran, mannitol or lactose. In one or more embodiments, a pharmaceutical composition provided herein is a spray.

[00226] In some or any embodiments, a composition provided herein is a pharmaceutical composition or a single unit dosage form. Pharmaceutical compositions and single unit dosage forms provided herein comprise a therapeutically effective amount of one or more therapeutic agents (*e.g.*, a compound provided herein, or other therapeutic agent), and a typically one or more pharmaceutically acceptable carriers (e.g. excipients). In a specific embodiment and in

this context, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. In one or more embodiments, the term "carrier" includes a diluent, disintegrant, lubricant, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete)), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water can be used as a carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Examples of suitable pharmaceutical carriers are described in Remington: The Science and Practice of Pharmacy; Pharmaceutical Press; 22 edition (September 15, 2012).

[00227] Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well-known to those skilled in the art of pharmacy, and in some or any embodiments, suitable excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a mammal and the specific active ingredients in the dosage form. The composition or single unit dosage form, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

[00228] Lactose free compositions provided herein can comprise excipients that are well known in the art and are listed, in some or any embodiments, in the U.S. Pharmacopeia (USP 36–NF 31 S2). In general, lactose free compositions comprise an active ingredient, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Examples of lactose free dosage forms comprise an active ingredient, microcrystalline cellulose, pre gelatinized starch, and magnesium stearate.

[00229] Further provided are pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as stabilizers, include, but are not limited to, antioxidants.

[00230] Further provided are pharmaceutical compositions and dosage forms that comprise one or more chemical permeation enhancer. In some or any embodiments, chemical permeation

enhancers include but are not limited to one or more of ethanol, amides (such as Ozone, Laurocapram), alkyl and benzoate esters, fatty acid esters (such as isopropyl myristate, propylene glycol monocaprylate and propyleneglycomonolaurate), Transcutol (Registered name), fatty acids (oleic acid), glycols, pyrrolidone (N-methyl-2-pyrrolidone and 2-pyrrolidone, dimethylsulfoxide (DMSO), terpenes (such as essential oils comprising terpenes), phospholipids, and/or cyclodeextrines.

[00231] The pharmaceutical compositions and single unit dosage forms can take the form of solutions, suspensions, emulsion, sustained-release formulations, and the like. In one or more embodiments, the formulation suits the mode of administration. In a some or any embodiment, the pharmaceutical compositions or single unit dosage forms are sterile and in suitable form for administration to a mammal, in some or any embodiments, a human.

[00232] A pharmaceutical composition is formulated to be compatible with its intended route of administration. In some or any embodiments, routes of administration include, but are not limited to, parenteral, *e.g.*, intrathecal, epidural, local or regional for peripheral nerve block, intravenous, intradermal, subcutaneous, intramuscular, subcutaneous, oral, buccal, sublingual, inhalation, intranasal, transdermal, topical (including administration to the eye, and in one or more embodiments to the cornea), transmucosal, intra-tumoral, intra-synovial, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical (including administration to the eye, and in one or more embodiments to the cornea) administration to human beings. In a specific embodiment, a pharmaceutical composition is formulated in accordance with routine procedures for subcutaneous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. The composition may also include one or more solubilizers and a local anesthetic such as lignocamne to ease pain at the site of the injection.

[00233] In some or any embodiments, dosage forms include, but are not limited to: sprays, tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a mammal, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil in water emulsions, or a water in oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to

a mammal; and sterile solids (*e.g.*, crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a mammal.

[00234] The composition, shape, and type of dosage forms provided herein will typically vary depending on their use. In some or any embodiments, a dosage form used in the initial treatment of the disease, disorder, or condition may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the maintenance treatment of the same disease, disorder, or condition. These and other ways in which specific dosage forms encompassed herein will vary from one another will be readily apparent to those skilled in the art. See, *e.g.*, Remington: The Science and Practice of Pharmacy; Pharmaceutical Press; 22 edition (September 15, 2012).

Glycol

[00235] In one or more embodiments, the pharmaceutical composition comprises a glycol. In one or more embodiments, the glycol is a non-geminal diol or a geminal diol. In one or more embodiments, the glycol is a 3- or 4-carbon glycol. In one or more embodiments, the glycol is a propanediol, such as propylene glycol (for example, 1,2-propanediol). In one or more embodiments, the glycol is a butanediol, such as butylene glycol (for example, 1,3-butanediol). In one or more embodiments, the glycol is a polyethylene glycol. The glycol can be PEG-400 (polyethylene glycol 400), but is not limited thereto.

[00236] In one or more embodiments, the glycol is unconjugated to another molecule. For example, the glycol may be a 3- or 4-carbon glycol or a PEG, without further conjugation to another molecule.

[00237] Examples of conjugated glycols include, but are not limited to, glycol stearate and glycol laurate. As described in the following section, a conjugated glycol such as PEG-400 monostearate may be a solubilizer.

[00238] In one or more embodiments, the glycol is present in the pharmaceutical composition in an amount from about 0.1 wt% to about 20 wt% of that of the total composition. For example, the glycol can be from about 0.2 wt% to about 10 wt%, from about 0.5 wt% to about 10 wt%, from about 1 wt% to about 10 wt%, from about 2 wt% to about 10 wt%, from about 3 wt% to about 10 wt%, from about 3 wt% to about 9 wt%, from about 3 wt% to about 8 wt%, from about 3 wt% to about 7 wt%, from about 3 wt% to about 6 wt%, or from about 4 wt% to about 6 wt% of the total composition. In one or more embodiments, the glycol is present in the pharmaceutical composition in an amount from about 2 wt% to about 10 wt%,

preferably from about 3 wt% to about 5 wt% or from about 3 wt% to about 6 wt% or from about 4 wt% to about 5 wt% or about 4.6 wt%.

[00239] In some or any embodiments, the glycol is present in the pharmaceutical composition in an amount from about 2 wt% to less than 8 wt%; in some embodiments when the wound is a surface wound.

[00240] In some or any embodiments, the glycol is present in the pharmaceutical composition in an amount from about 2 wt% to about 10 wt%, in some embodiments, 8 wt% to about 10 wt%; in some embodiments when the wound is a burn wound where higher hydration may be desired.

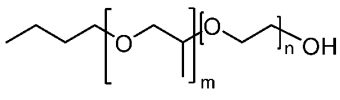
[00241] The glycol can be present in the pharmaceutical composition in a concentration from about 0.05 mg/mL to about 100 mg/mL, from about 1 mg/mL to about 100 mg/mL, from about 10 mg/mL to about 100 mg/mL, from about 20 mg/mL to about 100 mg/mL, from about 30 mg/mL to about 100 mg/mL, from about 40 mg/mL to about 100 mg/mL, from about 1 mg/mL to about 90 mg/mL, from about 10 mg/mL to about 90 mg/mL, from about 20 mg/mL to about 90 mg/mL, from about 30 mg/mL to about 90 mg/mL, from about 40 mg/mL to about 90 mg/mL, from about 1 mg/mL to about 80 mg/mL, from about 10 mg/mL to about 80 mg/mL, from about 20 mg/mL to about 80 mg/mL, from about 30 mg/mL to about 80 mg/mL, from about 40 mg/mL to about 80 mg/mL, from about 1 mg/mL to about 70 mg/mL, from about 10 mg/mL to about 70 mg/mL, from about 20 mg/mL to about 70 mg/mL, from about 30 mg/mL to about 70 mg/mL, from about 40 mg/mL to about 70 mg/mL, or from about 41 mg/mL to about 66 mg/mL.

Solubilizer

[00242] In one or more embodiments, the one or more solubilizers comprise: PPG-26 Buteth-26, PEG-40 hydrogenated castor oil (INCI name), and water; or the one or more solubilizers are one or more selected from the group consisting of hydroxypropyl cellulose, polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80. In one or more embodiments, the one or more solubilizers comprise Solubilisant LRI® (Sensient Cosmetic Technologies, Saint Ouen L'Aumone, France) or the one or more solubilizers are one or more selected from the group consisting of hydroxypropyl cellulose, polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80.

[00243] When the one or more solubilizer comprises PPG-26 Buteth-26, PEG-40 hydrogenated castor oil, and water, the one or more solubilizer allows water to be a substitute

for alcohol. A suitable example includes, but is not limited to, Solubilisant LRI® (Sensient Cosmetic Technologies, Saint Ouen L'Aumone, France).

[00244] PPG-26-Buteth-26 is a polyoxypropylene, polyoxyethylene ether of butyl alcohol, where PPG stands for polypropylene glycol. Suitable examples include, but are not limited to, Chemical Abstracts Service ("CAS") Registry Number 9065-63-8. In the CAS registry, PPG-26 Buteth-26 may also be called polyoxyethylene (26) polyoxypropylene (26) monobutyl ether, and polyoxypropylene (26) polyoxyethylene (26) monobutyl ether, and has the following structure: , where m is an integer representing a number of polyoxypropylene subunit, n is an integer representing a number of polyoxyethylene subunit, where n and m are included in the molecular weight. The number average molecular weight (M_n) may be about 3,900 g/mol (3,900 Daltons).

[00245] PEG-40 hydrogenated castor oil is a polyethylene derivative of castor oil. A suitable example of PEG-40 hydrogenated castor oil includes, but is not limited to, CAS Reg. No. 61788-85-0. PEG-40 hydrogenated castor oil may include polyethyleneglycol 400 (PEG-400) monostearate (a conjugated PEG).

[00246] In one or more embodiments, the one or more solubilizers can be one or two solubilizers, or two solubilizers.

[00247] In one or more embodiments, the one or more solubilizers is present in the pharmaceutical composition in an amount from about 0.1 wt% to about 20 wt % of that of the total composition. For example, the one or more solubilizers can be from about 0.2 wt% to about 10 wt%, or from about 0.5 wt% to about 10 wt%, or from about 1 wt% to about 10 wt%, or from about 1 wt% to about 9 wt%, or from about 1 wt% to about 8 wt%, or from about 1 wt% to about 7 wt%, or from about 1 wt% to about 6 wt%, or from about 2 wt% to about 10 wt%, or from about 2 wt% to about 8 wt%, or from about 2 wt% to about 6 wt% of the total composition. In one or more embodiments, the pharmaceutical composition comprise 3 wt% to 5 wt% or 2 wt% to 6 wt% of the one or more solubilizer (ex: 4.6% polysorbate 20, polysorbate 60, polysorbate 80, or solubilisant™).

[00248] The one or more solubilizer can be present in the pharmaceutical composition in a concentration from about 0.5 mg/mL to about 100 mg/mL, from about 1 mg/mL to about 100 mg/mL, from about 1 mg/mL to about 90 mg/mL, from about 1 mg/mL to about 80 mg/mL, from about 1 mg/mL to about 70 mg/mL, from about 5 mg/mL to about 100 mg/mL, from about 5 mg/mL to about 90 mg/mL, from about 5 mg/mL to about

80 mg/mL, from about 5 mg/mL to about 70 mg/mL, from about 6 mg/mL to about 70 mg/mL, or from about 6 mg/mL to about 66 mg/mL.

Phospholipid

[00249] In one or more embodiments, the one or more phospholipid can be phosphatidylcholine or lecithin. In one or more embodiments, the one or more phospholipid is phosphatidylcholine. In one or more embodiments, the one or more phospholipid is lecithin.

[00250] In one or more embodiments, the phosphatidylcholine is derived from soy (CAS Reg. No. 97281-47-5). In one or more embodiments, the phosphatidylcholine is a phosphatidylcholine mixture containing phosphatidylcholine in an amount from 90 wt% or greater, 91 wt% or greater, 92 wt% or greater, 93 wt% or greater, 94 wt% or greater, or greater than or equal to 94 wt%. A suitable example of the phosphatidylcholine mixture includes, but is not limited to, Phospholipon® 90G (Lipoid, Ludwigshafen/Rhine, Germany). Phospholipon® 90G is a mixture of ≥94 wt% phosphatidylcholine, with the remaining amount of the phosphatidylcholine mixture containing ascorbyl palmitate (CAS Reg. No. 137-66-6) and tocopherol (CAS Reg. No. 10191-41-0, (±)-α-Tocopherol, DL-all-rac-α-Tocopherol, Vitamin E).

[00251] In one or more embodiments, the one or more phospholipid is present in the pharmaceutical composition in an amount from about 0.1 wt% to about 20 wt% of that of the total composition. For example, the one or more phospholipid can be from about 0.1 wt% to about 15 wt%, or from about 0.1 wt% to about 10 wt%, or from about 0.1 wt% to about 9 wt%, or from about 0.1 wt% to about 8 wt%, or from about 0.1 wt% to about 7 wt%, or from about 0.1 wt% to about 6 wt%, or from about 0.1 wt% to about 5 wt%, or from about 0.1 wt% to about 4 wt%, or from about 0.1 wt% to about 3 wt%, or from about 0.1 wt% to about 2 wt%, or from about 0.1 wt% to about 1 wt% of the total composition. In one or more embodiments, the one or more phospholipid is present in the pharmaceutical composition in an amount from about 0.1 wt% to about 1.2 wt%. In one or more embodiments, the pharmaceutical composition comprises 0.1% to 2wt% of the one or more phospholipid. In one or more embodiments, the pharmaceutical composition comprises 1.2% of the one or more phospholipid.

Carrier

[00252] In one or more embodiments, the one or more carrier is a C_2-C_4 alcohol. In one or more embodiments, the carrier is a C_2-C_3 alcohol. In one or more embodiments, the one or more carrier is ethanol.

[00253] In one or more embodiments, the one or more carrier is present in the pharmaceutical composition in an amount from about 0.1 wt% to about 10 wt% of that of the total composition. For example, the one or more carrier can be from 1 wt% to 10 wt%, or from about 1 wt% to about 9 wt%, or from about 1 wt% to about 8 wt%, or from about 1 wt% to about 7 wt%, or from about 1 wt% to about 6 wt%, or from about 2 wt% to about 10 wt%, or from about 2 wt% to about 9 wt%, or from about 2 wt% to about 8 wt%, or from about 2 wt% to about 7 wt%, or from about 2 wt% to about 6 wt%, or from about 3 wt% to about 10 wt%, or from about 3 wt% to about 9 wt%, or from about 3 wt% to about 8 wt%, or from about 3 wt% to about 7 wt%, or from about 3 wt% to about 6 wt%, or from about 4 wt% to about 5 wt% of the total composition.

Stabilizer

[00254] In a pharmaceutical composition that includes GO-HA and a Wnt inhibitor, the inventors surprisingly discovered that GO-HA can oxidize a WNT inhibitor in some instances. For example, when GO-HA and XAV939 present in a pharmaceutical composition, such as a liposomal composition (in some embodiments a water-based liposomal composition), the XAV939 was oxidized by the GO-HA. The skilled person could not have predicted this based on the state of the art. Advantageously, the addition of a stabilizer in a pharmaceutical composition according to one or more embodiments prevents oxidation of a Wnt inhibitor, such as XAV939.

[00255] In one or more embodiments, the stabilizer can be an antioxidant. In one or more embodiments, the stabilizer is an amino acid. In one or more embodiments, the stabilizer is or comprises a sulfur-containing amino acid. Suitable stabilizers include, but are not limited to, methionine, cysteine, cystine, taurine, and glutathione. In one or more embodiments, the stabilizer is methionine.

[00256] In one or more embodiments, the stabilizer is present in the pharmaceutical composition in an amount from about 0.05 wt% to about 3 wt% of that of the total composition. For example, the stabilizer can be from about 0.05 wt% to about 2 wt%, or from about 0.05 wt% to about 1 wt%, or from about 0.05 wt% to about 0.5 wt%, or from about 0.1 wt% to about 3 wt%, or from about 0.1 wt% to about 2 wt%, or from about 0.1 wt% to about 1 wt%, or from about 0.1 wt% to about 0.5 wt%, or from about 0.1 wt% to about 0.4 wt%, or from about 0.1 wt% to about 3 wt%, or from about 0.1 wt% to about 0.2 wt%, or from about 0.2 wt% to about 0.5 wt%, or from about 0.3 wt% to about 0.5 wt%, or from about 0.4 wt% to about 0.5 wt%, or from about 0.3 wt% to about 0.4 wt%, or from about 0.3 wt% to about 0.5 wt% of

the total composition. In one or more embodiments, the stabilizer is present in the pharmaceutical composition in an amount from about 0.1 wt% to about 0.5 wt%.

Buffer

[00257] In one or more embodiments, the buffer is or comprises a C_4-C_8 organic acid. In one or more embodiments, the buffer is or comprises a C_6 organic acid. The buffer can be a weak acid. A suitable example of the buffer is or comprises citric acid. In one or more embodiments, the buffer is a C_4-C_8 organic acid. In one or more embodiments, the buffer is a C_6 organic acid. The buffer is a weak acid. A suitable example of the buffer is citric acid. In one or more embodiments, the buffer comprises a C_4-C_8 organic acid. In one or more embodiments, the buffer comprises a C_6 organic acid. The buffer comprises a weak acid. A suitable example of the buffer comprises citric acid.

[00258] A buffer can be added to maintain the pH of the pharmaceutical composition. The buffer can be sufficient to maintain an acidic or neutral pH. The buffer can be sufficient to maintain a pH of the pharmaceutical composition in a range from about 3 to about 7, such as from about 3.5 to about 7, from about 4 to about 7, from about 4.5 to about 7, from about 5 to about 7, from about 5.5 to about 7, from about 6 to about 7, from about 6.5 to about 7, from about 3 to about 6.5, from about 3.5 to about 6.5, from about 4 to about 6.5, from about 4.5 to about 6.5, from about 5 to about 6.5, from about 5.5 to about 6.5, from about 6 to about 6.5, from about 3 to about 6, from about 3.5 to about 6, from about 4 to about 6, from about 4.5 to about 6, from about 5 to about 6, from about 5.5 to about 6, from about 3 to about 5.5, from about 3.5 to about 5.5, from about 4 to about 5.5, from about 4.5 to about 5.5, from about 5 to about 5.5, from about 3 to about 5, from about 3.5 to about 5, from about 4 to about 5, from about 4.5 to about 5, from about 3 to about 4.5, from about 3.5 to about 4.5, from about 4 to about 4.5, from about 3 to about 4, from about 3.5 to about 4, or from about 3 to about 3.5. In one or more embodiments, the pharmaceutical composition pH is about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, or about 7.

[00259] In one or more embodiments, the buffer is present in the pharmaceutical composition in an amount from about 0.1 wt% to about 20 wt% of that of the total composition. For example, the buffer can be from about 0.1 wt% to about 10 wt%, or from about 0.1 wt% to about 8 wt%, or from about 0.1 wt% to about 6 wt%, or from about 0.1 wt% to about 4 wt%, or from about 0.1 wt% to about 3 wt%, or from about 0.1 wt% to about 2 wt%, or from about 0.1 wt% to about 1 wt%, or from about 0.2 wt% to about 10 wt%, or from about 0.2 wt% to about 8 wt%, or from about 0.2 wt% to about 6 wt%, or from about 0.2 wt% to about 4 wt%,

or from about 0.2 wt% to about 3 wt%, or from about 0.2 wt% to about 2 wt%, or from about 0.2 wt% to about 1 wt% of the total composition. In one or more embodiments, the stabilizer is present in the pharmaceutical composition in an amount from about 0.2 wt% to about 2.0 wt%.

Preservative

[00260] A preservative can be included in the pharmaceutical composition to prevent growth of bacteria, mold, yeast, and/or fungi. In one or more embodiments, the preservative comprises phenoxyethanol and ethylhexylglycerin, or is potassium sorbate.

[00261] When the preservative comprises phenoxyethanol and ethylhexylglycerin, the phenoxyethanol may be in an amount from about 85 wt% to 90 wt% and the phenoxyethanol may be in an amount from about 7 wt% to 13 wt% of the total preservative wt %. A suitable example of the preservative comprising phenoxyethanol and ethylhexylglycerin is Euxyl® PE 9010 (Ashland Global, Wilmington, Deleware, USA). The phenoxyethanol may be 2-phenoxyethanol. The ethylhexylglycerin may be 3-[(2-ethylhexyl)oxy]-1,2-propanediol.

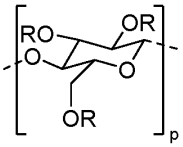
[00262] In one or more embodiments, the preservative is potassium sorbate. In one or more embodiments, the preservative is potassium sorbate in an amount from 0.5 wt% to 1.5 wt% of the total composition wt%.

[00263] In one or more embodiments, the preservative is Euxyl® PE 9010. In one or more embodiments, the preservative is Euxyl® PE 9010 in an amount from 0.5 wt% to 1.0 wt% of the total composition wt%.

[00264] In one or more embodiments, the preservative is present in the pharmaceutical composition in an amount from about 0.01 wt% to about 5 wt% of that of the total composition. For example, the preservative can be from about 0.01 wt% to about 4 wt%, or from about 0.01 wt% to about 3 wt%, or from about 0.01 wt% to about 2 wt%, or from about 0.01 wt% to about 1.5 wt%, or from about 0.01 wt% to about 1 wt%, or from about 0.01 wt% to about 0.5 wt%, or from about 0.025 wt% to about 4 wt%, or from about 0.025 wt% to about 3 wt%, or from about 0.025 wt% to about 2 wt%, or from about 0.025 wt% to about 1.5 wt%, or from about 0.025 wt% to about 1 wt%, or from about 0.025 wt% to about 0.5 wt%, or from about 0.5 wt% to about 5 wt%, or from about 0.5 wt% to about 4 wt%, or from about 0.5 wt% to about 3 wt%, or from about 0.5 wt% to about 2.5 wt%, or from about 0.5 wt% to about 2 wt%, or from about 0.5 wt% to about 1.5 wt%, or from about 0.5 wt% to about 1 wt%, of the total composition wt%. In one or more embodiments, the preservative can be from 0.5 wt% to 1.5 wt%, or from 0.5 wt% to 1.0 wt% of the total composition wt%.

Thickener

[00265] In one or more embodiments, the pharmaceutical composition further comprises one or more thickener. The one or more thickener contributes a congealing effect on the composition based on its concentration in the total composition and its critical temperature, among other things. The one or more thickener provides desired viscosity of the composition for certain skin delivery. For example, the one or more thickener can make the composition into a smooth film for easy application. It also reduces evaporation and allows the wound to stay moist longer, a factor that has been shown to improve healing and result in decreased scarring. The one or more thickener can be an aqueous based thickener. A suitable example of an aqueous based thickener is hydroxypropyl methyl cellulose (HPMC or hypromellose):



, where R is -H, $-CH_3$, or $-CH_2CH(OH)CH_3$, and p ranges from about 50,000 to 200,000 Mw. Hydroxypropyl methyl cellulose can also be called hydroxypropyl cellulose (or HPC). Another example of a thickener is xanthum gum.

[00266] For example, the one or more thickener can have a molecular weight range from 50,000 to 200,000 MW, or from about 60,000 MW to about 200,000 MW, or from about 80,000 to about 200,000 MW, or from about 90,000 to about 200,000 MW, or from about 50,000 to about 150,000 MW, or from about 60,000 to about 150,000 MW, or from about 70,000 to about 150,000 MW, or from about 80,000 to about 150,000 MW, or from about 90,000 to about 150,000 MW, or from about 50,000 to about 150,000 MW, or from about 60,000 to about 150,000 MW, or from about 70,000 to about 150,000 MW, or from about 80,000 to about 150,000 MW, or from about 90,000 to about 150,000 MW, or from about 50,000 to about 100,000 MW, or from about 60,000 to about 100,000 MW, or from about 70,000 to about 100,000 MW, or from about 80,000 to about 100,000 MW, or from about 90,000 to about 100,000 MW, or is about 90,000 MW, or is 90,000 MW. There are different grades of thickener available according to molecular weights of said thickener, or viscosity of thickener water solution(s) depending on thickener concentration.

[00267] In one or more embodiments, the one or more thickeners can be one or two thickeners, or two thickeners.

[00268] In one or more embodiments, the pharmaceutical composition does not comprise the one or more thickener. In some or more embodiments, the pharmaceutical composition

does not comprise the one or more thickener and the pharmaceutical composition is a spray formulation.

Saline or water

[00269] In one or more embodiments, the pharmaceutical composition comprises saline or water. The saline or water may be USP sterile grade, containing no antimicrobial agents. In one or more embodiments, the saline is from about 0.1 wt% to 1.0 wt% sodium chloride (NaCl) in water. For example, the saline can be from about 0.15 wt% to about 0.95 wt%, from about 0.20 wt% to about 0.90 wt%, from about 0.22 wt% to about 0.9 wt%, from about 0.3 wt% to about 0.9 wt%, from about 0.4 wt% to about 0.9 wt%, from about 0.5 wt% to about 0.9 wt%, from about 0.6 wt% to about 0.9 wt%, from about 0.7 wt% to about 0.9 wt%, or from about 0.8 wt% to about 0.9 wt% NaCl in water (Normal saline, USP sterile grade). In one or more embodiments, the saline is from about 0.22 wt% to about 0.9 wt% NaCl in water. In one or more embodiments, the saline is about 0.9 wt% NaCl in water, or 0.9 wt% NaCl in water. When all components, including additional active agents and unspecified additional components, of the pharmaceutical composition are accounted for in wt% of the composition except saline or water, the remaining wt% amount of the composition is saline or water.

Additional weight ratios (wt%)

[00270] In one or more embodiments, the wt% ratio of WNT inhibitor:one or more phospholipid in the pharmaceutical composition is from about 1:1 to about 1:50. For example, the wt% ratio of WNT inhibitor:one or more phospholipid in the pharmaceutical composition can be from about 1:5 to about 1:50, from about 1:10 to about 1:50, from about 1:15 to about 1:50, from about 1:20 to about 1:50, from about 1:25 to about 1:50, from about 1:30 to about 1:50, from about 1:35 to about 1:50, from about 1:40 to about 1:50, or from about 1:45 to about 1:50. In some embodiments, the WNT inhibitor is XAV939, **Compound 1**, **Compound 7**, **Compound 8**, **Compound 10**, or **Compound 18**. In some embodiments, the WNT inhibitor is XAV939.

[00271] In one or more embodiments, the wt% ratio of WNT inhibitor:one or more phospholipid in the pharmaceutical composition is from about 1:10 to about 1:30. For example, the wt% ratio of WNT inhibitor:one or more phospholipid in the pharmaceutical composition is from about 1:1 to about 1:50, from about 1:1 to about 1:25, from about 1:10 to about 1:25, from about 1:10 to about 1:24, from about 1:10 to about 1:23, from about 1:10 to about 1:22, from about 1:10 to about 1:21, from about 1:10 to about 1:20, from about 1:10 to about 1:19,

from about 1:10 to about 1:18, from about 1:10 to about 1:17, from about 1:10 to about 1:16, from about 1:10 to about 1:15, from about 1:10 to about 1:14, from about 1:10 to about 1:13, from about 1:10 to about 1:12, or from about 1:10 to about 1:11. In some or any embodiments, the WNT inhibitor is XAV939, **Compound 1**, **Compound 7**, **Compound 8**, **Compound 10**, or **Compound 18**. In some or any embodiments, the WNT inhibitor is XAV939.

[00272] In one or more embodiments of the pharmaceutical composition comprising one or more phospholipid, the wt% ratio of the Wnt inhibitor, such as a Wnt inhibitor of Formula (I) (for example, XAV939, **Compound 1**, **Compound 7**, **Compound 8**, **Compound 10**, and **Compound 18**) to the one or more phospholipid or the one or more phospholipid to the WNT inhibitor can be about 1:1, about 1:1.1, about 1:1.2, about 1:1.3, about 1:1.4, about 1:1.5, about 1:1.6, about 1:1.7, about 1:1.8, about 1:1.9, or about 1:2.

[00273] In one or more embodiments, the wt% of the one or more carrier(s):one or more phospholipid in the pharmaceutical composition is from about 1:1 to about 10:1. For example, the wt% ratio of the one or more carrier(s):one or more phospholipid in the pharmaceutical composition is from about 2:1 to about 10:1, from about 2:1 to about 9:1, from about 2:1 to about 9:1, from about 2:1 to about 8:1, from about 2:1 to about 7:1, from about 2:1 to about 6:1, from about 2:1 to about 5:1, from about 2:1 to about 4:1, from about 2:1 to about 3:1, from about 3:1 to about 10:1, from about 3:1 to about 9:1, from about 3:1 to about 8:1, from about 3:1 to about 7:1, from about 3:1 to about 6:1, from about 3:1 to about 5:1, or from about 3:1 to about 4:1.

[00274] In one or more embodiments, the wt% of the glycol:one or more solubilizer (solubilizers) are present in a wt% ratio in the pharmaceutical composition of about 1:0.1 to about 1:5. For example, the wt% of the glycol:one or more solubilizer in the pharmaceutical composition is from about 1:0.1 to about 1:4, from about 1:0.1 to about 1:3, from about 1:0.1 to about 1:2, from about 1:0.1 to about 1:1, from about 1:0.25 to about 1:5, from about 1:0.25 to about 1:4, from about 1:0.25 to about 1:3, from about 1:0.25 to about 1:2, from about 1:0.25 to about 1:1, from about 1:0.5 to about 1:5, from about 1:0.5 to about 1:4, from about 1:0.5 to about 1:3, from about 1:0.5 to about 1:2, from about 1:0.5 to about 1:1.5, or from about 1:0.5 to about 1:1.

[00275] In one or more embodiments, the wt% of the glycol:carrier (or carriers) are present in a wt% ratio in the pharmaceutical composition of about 1:0.1 to about 1:5. For example, the wt% of the glycol:carrier (or carriers) is from about 1:0.1 to about 1:4, from about 1:0.1 to about 1:3.5, from about 1:0.1 to about 1:3, from about 1:0.1 to about 1:2.5, from about 1:0.1 to about 1:2, from about 1:0.1 to about 1:1.5, from about 1:0.1 to about 1:1, from about 1:0.25 to

about 1:4, from about 1:0.25 to about 1:3.5, from about 1:0.25 to about 1:3, from about 1:0.25 to about 1:2.5, from about 1:0.25 to about 1:2, from about 1:0.25 to about 1:1.5, from about 1:0.25 to about 1:1, from about 1:0.5 to about 1:4, from about 1:0.5 to about 1:3.5, from about 1:0.5 to about 1:3, from about 1:0.5 to about 1:2.5, from about 1:0.5 to about 1:2, from about 1:0.5 to about 1:1.5, or from about 1:0.5 to about 1:1.

[00276] In one or more embodiments, the phrase "wherein the weight% of the WNT inhibitor, GO-HA, glycol, one or more solubilizers, one or more optional phospholipids, one or more optional carriers, an optional stabilizer, optional buffer, optional preservative, and water or saline is 100 wt%" does not exclude additional components being present in the composition.

Topical Administration

[00277] The pharmaceutical composition can be formulated for application to surfaces of the body, such as to the skin or mucous membranes. Thus, the pharmaceutical composition can be a topical, formulated for topical administration. Pharmaceutical compositions according to one or more embodiments herein can be applied directly to the skin, epicutaneously.

[00278] In one or more embodiments, the pharmaceutical composition is a topical solution. Suitable topical solutions include, but are not limited to, a drop, a rinse, and a spray. Examples include a serum (such as a skin serum), an eye drop, an ear drop, and an inhalant. The topical solution may be of lower viscosity compared with other topicals, including but not limited to creams and gels.

[00279] When the pharmaceutical composition is a spray, it may be applied (sprayed) directly to the target site. The spray may be configured to pass through a spray nozzle, atomizer, or orifice, which fragments the liquid into smaller liquid structures such a sheets, ligaments, streams, droplets, or combinations thereof. The spray may be configured for atomization, where the spray includes atomizing air passing through the stream of the spray resulting in fine liquid droplets, which is applied to the target site. The spray may be configured for vaporization, where the spray is a vapor, which is applied to the target site. The spray may be configured as an aerosol, where liquid droplets are suspended in air or another suitable gas, which is applied to the target site.

[00280] When topically administered, the pharmaceutical composition can provide a faster onset and more concentrated local effect on the target site than with other forms of administration. As a topical, the pharmaceutical composition bypasses the first-pass effect (first-pass metabolism), thereby preventing loss of individual components, such as an active

ingredient or drug, through sites of elimination during absorption. In other words, the pharmaceutical composition can bypass first-pass metabolism of the liver, intestinal lumen, or gut wall enzymes. Advantageously, the pharmaceutical composition as a topical retains the components of the composition during and after application to the extent that the effects of the pharmaceutical composition are realized, without being metabolized or separated.

[00281] Although permeability of skin varies with condition, anatomic site, age, and gender, benefits of the pharmaceutical composition as a topical (or as topically administered) include slow absorption through the skin, so the pharmaceutical composition remains at the target site. In the event of misapplication, the pharmaceutical composition can be washed off, thereby preventing unwanted side effects. By application to the target site, proprioception of the subject can be improved compared to other forms of administration.

[00282] Advantageously, the pharmaceutical composition according to one or more embodiments herein can prevent infection, prevent sunburn, reduce inflammation, and induce insensitivity to pain (anesthetic), each without causing rashes or itchiness, compared to without the pharmaceutical composition according to one or more embodiments.

[00283] Additional benefits of the topical composition and topical administration of embodiments of the present disclosure is low toxicity and high bioavailability of the beneficial components to the site of injury/wound.

Methods

[00284] The methods provided herein encompass administering pharmaceutical compositions containing one or more compound as described herein, including a Wnt Inhibitor of Formula (**I**) if appropriate in a salt form, either used alone or in the form of a combination with one or more compatible and pharmaceutically acceptable carriers, such as diluents or adjuvants, or with another agent for the treatment of wounds and/or conditions modulated by Wnt transcription products or Wnt signaling pathway activity.

[00285] In one or more embodiments, provided is a method for inhibiting Wnt transcription products or Wnt signaling pathway activity in a mammal comprising contacting the subject with an effective amount of a pharmaceutical composition according to one or more embodiments herein.

[00286] In one or more embodiments, provided is a method for treating a disease, disorder, or condition associated with Wnt transcription products or Wnt signaling pathway activity in a mammal in need thereof, comprising administering a pharmaceutical composition according to one or more embodiments herein.

[00287] In one or more embodiments, provided is a method for stimulating regeneration of tissue at a wound in a mammal in need thereof and wherein the wound is contacted with a pharmaceutical composition according to one or more embodiments herein.

[00288] In one or more embodiments, provided is a method for administering topically a pharmaceutical composition according to one or more embodiments herein, to a mammal in need thereof.

[00289] In one or more embodiments, a disease, disorder, or condition (to be treated in one or more embodiments of a method herein) is a chronic wound, an acute wound, an alkali-burned corneal wound, a burn, a lesion (including lesions caused by HPV and/or a virus selected from the *Poxviridae* family of viruses), an inflammatory dermatitis disease (including acne, psoriasis, rosacea, and scleroderma), a cartilage disease (including osteoarthritis, rheumatoid arthritis, internal derangement of the joints, and degenerative cartilage disease), a bone disease (including osteoporosis), organ fibrosis (including lung fibrosis, heart fibrosis, liver fibrosis, and kidney fibrosis), cancer (including melanoma, breast cancer, and prostate cancer), a de-nerved body part in need of reinnervation, tissue in need of regeneration (including damaged elastic cartilage), bacterial growth in need of inhibition, fungal growth in need of inhibition, tissue in need of neovascularization, osteoclast differentiation in need of inhibition, osteoblast differentiation disorders (where inhibition of osteoblast differentiation is needed), and/or bone destruction associated with breast cancer.

[00290] In one or more embodiments, a disease, disorder, or condition (to be treated in one or more embodiments of a method herein) is a chronic wound, an acute wound, an alkali-burned corneal wound, an incisional wound (open or closed), a burn, a lesion (including lesions caused by HPV and/or a virus selected from the *Poxviridae* family of viruses), an inflammatory dermatitis disease (including acne, psoriasis, rosacea, and scleroderma), a cartilage disease (including osteoarthritis, rheumatoid arthritis, internal derangement of the joints, and degenerative cartilage disease), a bone disease (including osteoporosis), organ fibrosis (including lung fibrosis, heart fibrosis, liver fibrosis, and kidney fibrosis), cancer (including melanoma, breast cancer, and prostate cancer), a de-nerved body part in need of reinnervation, tissue in need of regeneration (including damaged elastic cartilage), bacterial growth in need of inhibition, fungal growth in need of inhibition, tissue in need of neovascularization, osteoclast differentiation in need of inhibition, osteoblast differentiation disorders (where inhibition of osteoblast differentiation is needed), and/or bone destruction associated with breast cancer.

[00291] In one or more embodiments, a disease, disorder, or condition (to be treated in one or more embodiments of a method herein) is selected from a chronic wound, an acute wound, an alkali-burned corneal wound, an incisional wound (open or closed), a burn, a lesion (including lesions caused by HPV and/or a virus selected from the *Poxviridae* family of viruses), an inflammatory dermatitis disease (including acne, psoriasis, rosacea, and scleroderma), cancer (including melanoma and breast cancer), a de-nerved body part in need of reinnervation, tissue in need of regeneration (including damaged elastic cartilage), bacterial growth in need of inhibition, fungal growth in need of inhibition, and tissue in need of neovascularization.

[00292] In one or more embodiments, provided is a method of inducing bacteriostasis associated with Wnt transcription products or Wnt signaling pathway activity, comprising administering a pharmaceutical composition according to one or more embodiments herein to a mammal in need thereof.

[00293] In one or more embodiments, provided is a method for treating an open excisional wound in a mammal in need thereof, comprising administering a pharmaceutical composition according to one or more embodiments herein.

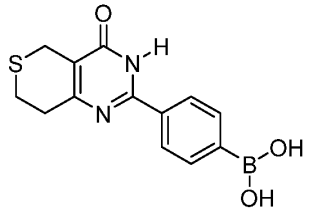
[00294] In one or more embodiments, provided is a method for treating a closed excisional wound in a mammal in need thereof, comprising administering a pharmaceutical composition according to one or more embodiments herein.

[00295] In one or more embodiments, provided is a method for treating a burn in a mammal in need thereof, comprising administering a pharmaceutical composition according to one or more embodiments herein.

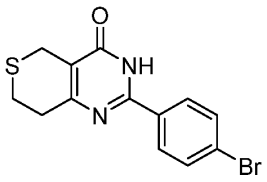
Synthetic Examples

Compound 1

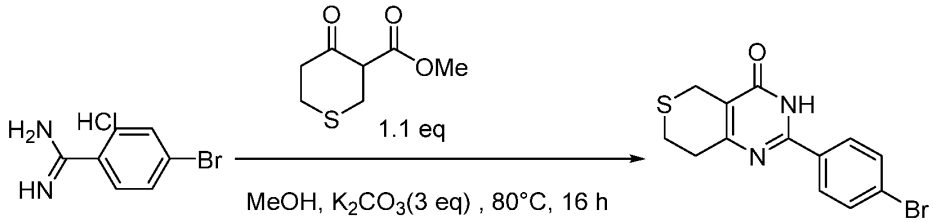
Synthesis of (4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (Compound 1)



Synthesis of 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one

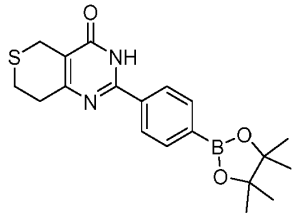


[00296] *Scheme 1A*

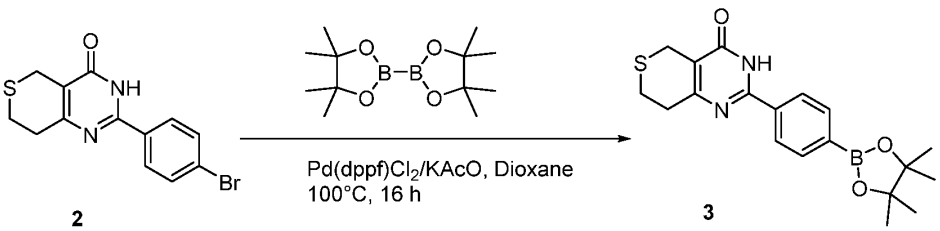


[00297] A mixture of 4-bromobenzimidamide hydrochloride (**1**) (22.0 g, 93.41 mmol, 1.0 eq), methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (1.1 g, 102.76 mmol, 1.1 eq), and K_2CO_3 (38.73 g, 280.23 mmol, 3.0 eq) in MeOH (660 mL) was stirred at 80°C for 16 h. The mixture was cooled and concentrated *in vacuo*. The solid was triturated with water (500 mL), and then filtered and dried to afford 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**2**) (26.4 g, 81.7 mmol). LC-MS: calculated for $C_{13}H_{11}BrN_2OS$: 323.2; found: 325.0.

Synthesis of 2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one

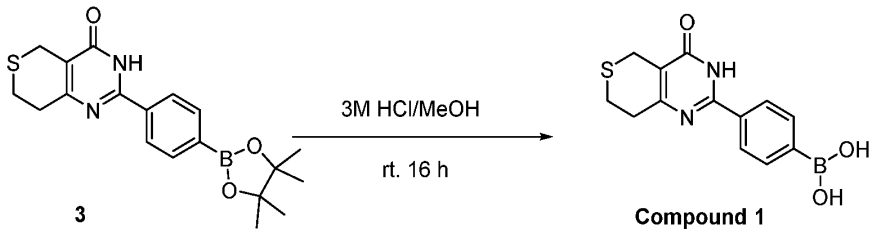


[00298] *Scheme 1B*



[00299] The degassed mixture of 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**2**) (20 g, 61.88 mmol, 1.0 eq), $(BPin)_2$ (31.43 g, 123.76 mmol, 2.0 eq), $Pd(dppf)Cl_2$ (2.26 g, 3.09 mmol, 0.05 eq), and KOAc (30.36 g, 309.40 mmol, 5.0 eq) in dioxane (200 mL) was stirred at 100°C for 16 h under Argon. The mixture was concentrated *in vacuo*. The crude product was purified on a silica gel column (PE:EtOAc = 5:1 to 1:1) to afford 2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (21.73 g, 58.72 mmol). LC-MS: calculated for $C_{19}H_{23}BN_2O_3S$: 370.1; found: 371.1.

[00300] *Scheme 1C*

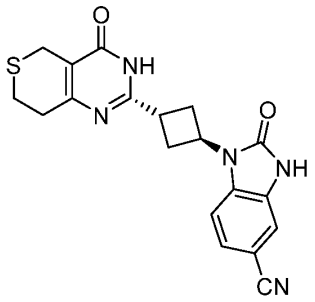


3 Compound 1

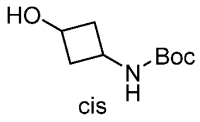
[00301] A solution of 2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (10.0 g, 370.27 mmol, 1.0 eq) in 3M HCl/MeOH (100 mL) was stirred at 25°C for 16 h. The mixture was concentrated under vacuum to afford a crude product which was triturated with THF (150 mL) under reflux and filtered to afford (4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**Compound 1**). LC-MS: calculated for $C_{13}H_{13}BN_2O_3S$: 288.1; found: 289.1. ^1H NMR (400 MHz, MeOD): δ 7.95 (s, 4H), 3.67 (s, 2H), 3.10 (m, 2H), 3.02 (m, 2H).

Compound 2

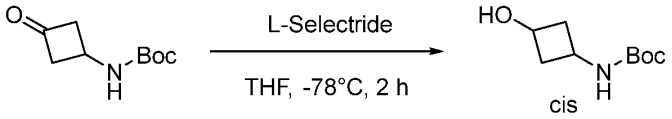
Synthesis of 2-oxo-1-((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)-2,3-dihydro-1H-benzo[d]imidazole-5-carbonitrile (Compound 2)



Synthesis of (Z)-tert-butyl (3-hydroxycyclobutyl)carbamate



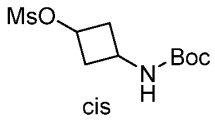
[00302] *Scheme 2A*



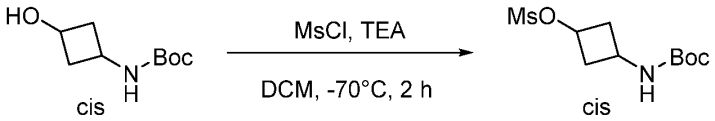
1 **2**

[00303] A solution of tert-butyl (3-oxocyclobutyl)carbamate (**1**) (2.5 g, 13.5 mmol, 1.0 eq) in 80 mL THF was cooled to -78°C and treated with a 1 N solution of L-Selectride (16.2 mL, 16.2 mmol, 1.2 eq) in THF. After stirring for 1 h the reaction was quenched with 5 mL water and warmed to rt. The reaction mixture was concentrated and purified by silica gel chromatography (EA:PE = 0~50%) provided the (Z)-tert-butyl (3-hydroxycyclobutyl)carbamate (**2**) (1.8 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 4.68 (brs, 1H), 4.05-3.98 (m, 1H), 3.67-3.65 (m, 1H), 2.78-2.75 (m, 2H), 2.08 (brs, 1H), 1.81-1.78 (m, 2H), 1.44 (s, 9H).

Synthesis of (Z)-3-((tert-butoxycarbonyl)amino)cyclobutyl methanesulfonate



[00304] *Scheme 2B*

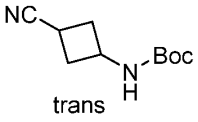


2 **3**

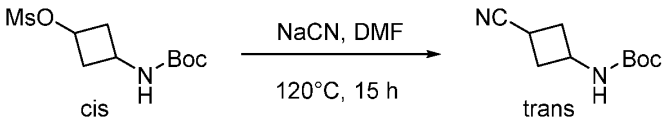
[00305] Methanesulfonyl chloride (1.3 g, 11.6 mmol, 1.2 eq) was added dropwise to a -70°C solution of (Z)-tert-butyl (3-hydroxycyclobutyl)carbamate (**2**) (1.8 g, 9.6 mmol, 1.0 eq) and TEA (1.5 g, 14.8 mmol, 1.5 eq) in dichloromethane (60 mL). The resulting solution was stirred for 2 hours at -70°C, the mixture was diluted with 100 mL of water. The resulting solution was extracted with dichloromethane (3x60 mL) and the organic layers combined. The resulting mixture was washed with brine (100 mL), dried over anhydrous sodium sulfate and concentrated to give (Z)-3-((tert-butoxycarbonyl)amino)cyclobutyl methanesulfonate (**3**)

(2.5 g). ^{1}H NMR (400 MHz, $CDC1_3$): δ (ppm): 4.75-4.67 (m, 2H), 3.85-3.82 (m, 1H), 2.98 (s, 3H), 2.93-2.88 (m, 2H), 2.22-2.14 (m, 2H), 1.44 (s, 9H).

Synthesis of (E)-tert-butyl (3-cyanocyclobutyl)carbamate



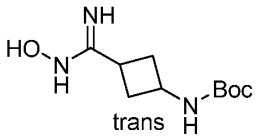
[00306] *Scheme 2C*



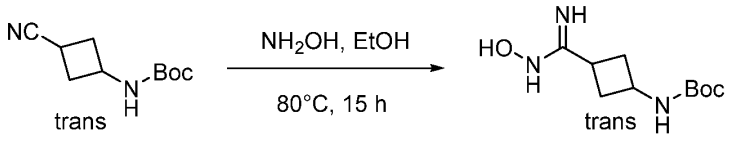
3 **4**

[00307] (Z)-3-((tert-butoxycarbonyl)amino)cyclobutyl methanesulfonate (**3**) (1.2 g, 4.5 mmol, 1.0 eq) in DMF (30 mL) was treated with NaCN (665.7 mg, 13.6 mmol, 3.0 eq) and the reaction was heated to 120°C for 15 h, the mixture was diluted with 50 mL of water. The resulting solution was extracted with EA (2x60 mL) and the organic layers combined. The resulting mixture was washed with brine (60 mL), dried over anhydrous sodium sulfate and concentrated to give (E)-tert-butyl (3-cyanocyclobutyl)carbamate (**4**) (730.0 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 4.77 (br s, 1H), 4.41-4.39 (m, 1H), 3.06-3.01 (m, 1H), 2.74-2.41 (m, 2H), 2.27-2.24 (m, 2H), 1.45 (s, 9H).

Synthesis of (E)-tert-butyl (3-(N-hydroxycarbamimidoyl)cyclobutyl)carbamate



[00308] *Scheme 2D*

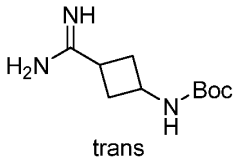


4 **5**

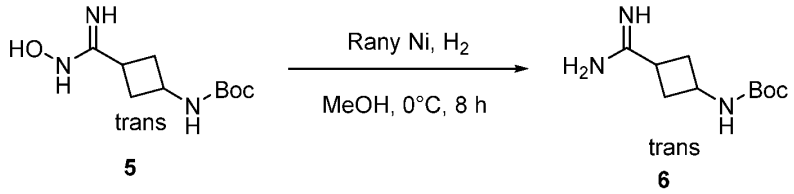
[00309] To a solution of (E)-tert-butyl (3-cyanocyclobutyl)carbamate (**4**) (730.0 mg, 3.7 mmol, 1.0 eq) in EtOH (25 mL) was added aqueous hydroxylamine (2.2 g/2.0 mL, 33.3 mmol, 9.0 eq) under N_2. The mixture was heated to 80°C and stirred for 15 h. The reaction mixture was concentrated and purified by Prep-HPLC to give (E)-tert-butyl

(3-(N-hydroxycarbamimidoyl)cyclobutyl)carbamate (**5**) (520.0 mg). LCMS (ESI): m/z 230.2 $[M+H]^+$.

Synthesis of (E)-tert-butyl (3-carbamimidoylcyclobutyl)carbamate

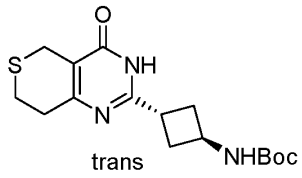


[00310] *Scheme 2E*

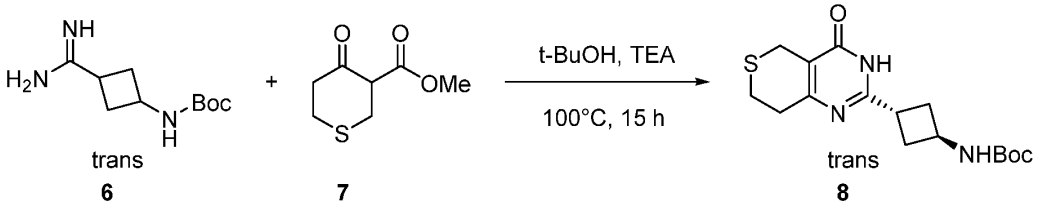


[00311] To a solution of (E)-tert-butyl (3-(N-hydroxycarbamimidoyl)cyclobutyl)carbamate (**5**) (520.0 mg, 2.3 mmol, 1.0 eq) in MeOH (100 mL) was added Raney-Ni (200 mg, 2.3 mmol, 1.0 eq) under N_2. The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 at 0°C for 8 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give (E)-tert-butyl (3-carbamimidoylcyclobutyl)carbamate (**6**) (480.0 mg). LCMS (ESI): m/z 214.2 $[M+H]^+$.

Synthesis of (E)-tert-butyl ((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)carbamate



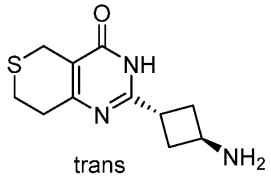
[00312] *Scheme 2F*



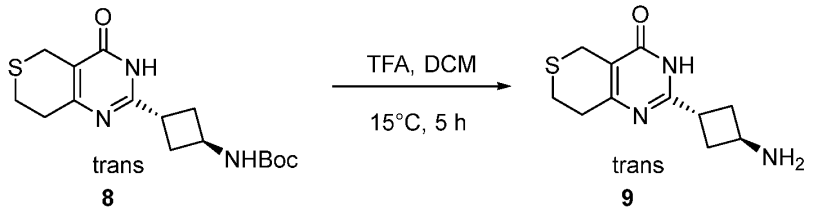
[00313] To a solution of (E)-tert-butyl (3-carbamimidoylcyclobutyl)carbamate (**6**) (480.0 mg, 2.3 mmol, 1.0 eq) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**7**) (431.3 mg, 2.5 mmol, 1.1 eq) in *t*-BuOH (30 mL) was added TEA (1.3 g, 12.8 mmol, 5.6 eq) in one portion at 15°C under N_2. The mixture was heated to 100°C and stirred for 15 h. The reaction was

concentrated in vacuo. The residue was purified by silica gel column chromatography (DCM/MeOH = 100%~95%) to give (E)-tert-butyl ((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)carbamate (**8**) (630.1 mg). LCMS (ESI): m/z 338.1 $[M+H]^+$.

Synthesis of (E)- 2-((1r,3r)-3-aminocyclobutyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one

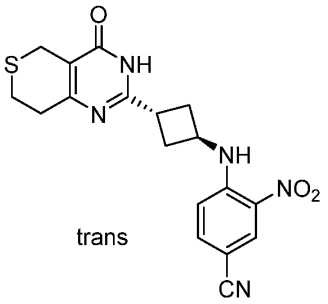


[00314] *Scheme 2G*

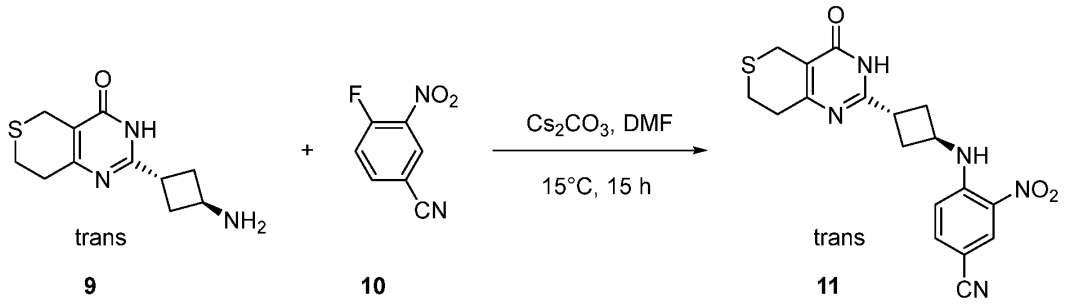


[00315] To a stirred solution of (**8**) (630 mg, 1.9 mmol, 1.0 eq) in DCM (30.0 mL) was added TFA (3.0 mL, 39.5 mmol, 20.8 eq) at 25°C. The reaction mixture was stirred at 25°C under N_2 atmosphere for 5 h. The reaction mixture was concentrated in vacuum to give (E)-2-((1r,3r)-3-aminocyclobutyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**9**) (656.5 mg). LCMS (ESI): m/z 238.0 $[M+H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6): δ (ppm): 8.41 (brs, 3H), 3.75-3.71 (m, 1H), 3.47 (s, 2H), 3.43-3.80 (m, 1H), 2.90-2.87 (m, 4H), 2.66-2.53 (m, 4H).

Synthesis of (E)-3-nitro-4-(((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)amino)benzonitrile

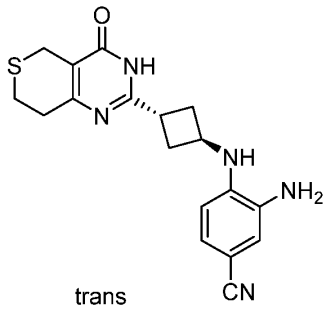


[00316] *Scheme 2H*

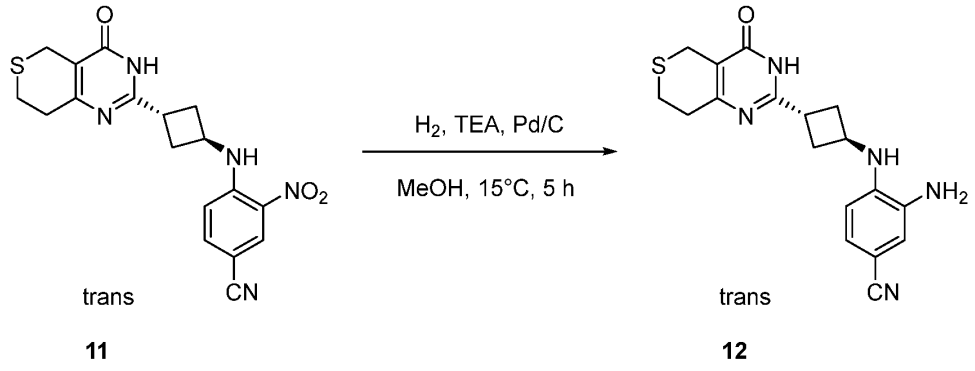


[00317] To a solution of (**9**) (443.1 mg, 1.9 mmol, 1.0 eq) and 4-fluoro-3-nitrobenzonitrile (**10**) (248.3 mg, 1.5 mmol, 0.8 eq) in DMF (30 mL) was added Cs_2CO_3 (1.5 g, 4.6 mmol, 2.4 eq) in one portion at 15°C under N_2. The reaction mixture was stirred at 15°C under N_2 atmosphere for 15 h, the mixture was diluted with 50 mL of water. The resulting solution was extracted with EA (2x60 mL) and the organic layers combined. The resulting mixture was washed with brine (60 mL), dried over anhydrous sodium sulfate and concentrated to give (E)-3-nitro-4-(((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)amino)benzonitrile (**11**) (500.9 mg). LCMS (ESI): m/z 384.0 $[M+H]^+$.

Synthesis of (E)-3-amino-4-(((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)amino)benzonitrile

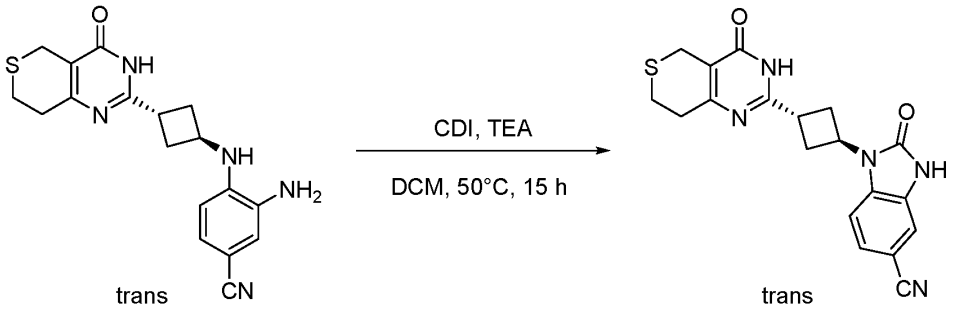


[00318] *Scheme 2J*



[00319] To a solution of (**11**) (200.0 mg, 0.52 mmol, 1.0 eq) and TEA (1.0 mL, 7.2 mmol, 13.8 eq) in MeOH (200 mL) was added Pd/C (200 mg) under N_2.The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 at 15°C for 5 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give (E)-3-amino-4-(((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)amino)benzonitrile (**12**) (180.0 mg). LCMS (ESI): m/z 354.2 $[M+H]^+$.

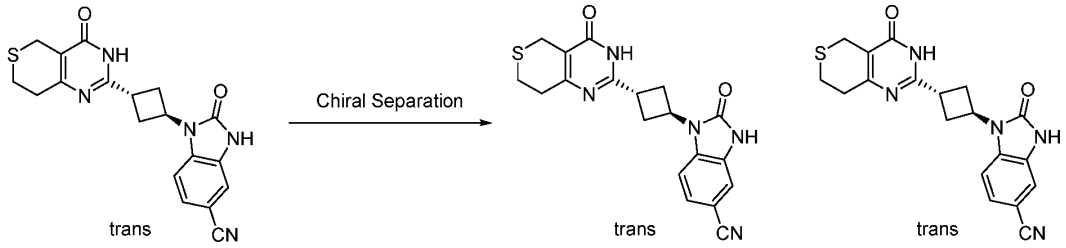
[00320] *Scheme 2K*



12 **Compound 2**

[00321] (E)-3-amino-4-(((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)amino)benzonitrile (**12**) (180.0 mg, 0.51 mmol, 1.0 eq) and TEA (3 mL, 21.7 mmol, 42.6 eq) in dichloromethane (DCM) (30 mL) was treated with 1,1'-carbonyldiimidazole (CDI) (918.3 mg, 5.7 mmol, 11.2 eq) and the reaction was heated to 50°C for 15 h, the mixture was diluted with 50 mL of water. The resulting solution was extracted with EA (2x60 mL) and the organic layers combined. The resulting mixture was washed with brine (60 mL), dried over anhydrous sodium sulfate, the organic layers were concentrated and purified by prep-HPLC to give (E)- 2-oxo-1-((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)-2,3-dihydro-1H-benzo[d]imidazole-5-carbonitrile (**Compound 2**) (102.0 mg). LCMS (ESI): m/z 380.0 $[M+H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6): δ (ppm): 11.40 (s, 1H), 8.05 (d, J=8.4 Hz, 1H), 7.58 (dd, J=8.0 Hz, J=1.2 Hz, 1H), 7.40 (d, J=1.2 Hz, 1H), 5.02-4.92 (m, 1H), 3.47 (s, 2H), 3.31-3.24 (m, 1H), 3.10-3.02 (m, 2H), 2.93-2.85 (m, 4H), 2.71-2.63 (m, 2H).

[00322] *Scheme 2L*

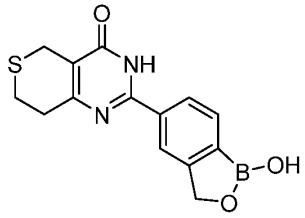


Compound 2 **Compound 2-P1** **Compound 2-P2**

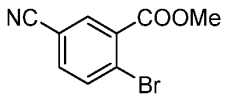
[00323] (**Compound 2**) (150 mg) was purified by chiral-HPLC to give (**Compound 2**-P1) and (**Compound 2**-P2). LCMS (ESI): m/z 380.0 $[M+H]^+$. ^{1}H-NMR (**Compound 2**-P1, 400MHz, DMSO-d_6): δ (ppm): 7.49 (s, 2H), 7.38 (s, 1H), 5.10-5.04 (m, 1H), 3.58-3.50 (m, 1H), 3.47 (s, 2H), 3.17-3.09 (m, 2H), 2.93-2.85 (m, 4H), 2.71-2.63 (m, 2H). ^{1}H-NMR (**Compound 2**-P2, 400MHz, DMSO-d_6): δ (ppm): 12.49 (s, 1H), 11.39 (s, 1H), 8.09 (d, J=8.4 Hz, 1H), 7.58 (dd, J=8.4 Hz, J=1.6 Hz, 1H), 7.40 (d, J=1.6 Hz, 1H), 5.02-4.92 (m, 1H), 3.47 (s, 2H), 3.31-3.24 (m, 1H), 3.10-3.01 (m, 2H), 2.93-2.90 (m, 4H), 2.71-2.63 (m, 2H).

Compound 3

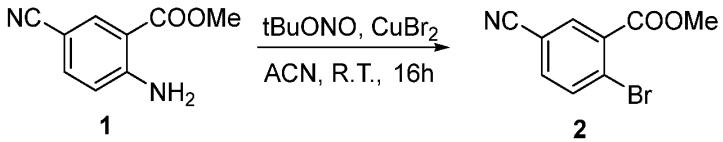
Synthesis of 2-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yl)-7,8-dihydro-3H-thiopyrano [4,3-d]pyrimidin-4(5H)-one (Compound 3)



Synthesis of methyl 2-bromo-5-cyanobenzoate



[00324] *Scheme 3A*

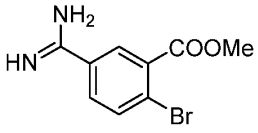


1 **2**

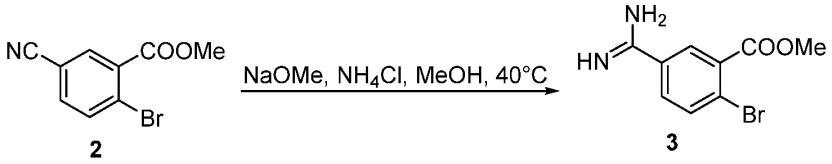
[00325] A mixture of $CuBr_2$ (3.04 g, 13.6 mmol, 1.2 eq) in CAN (50 mL) was added to tBuONO (1.64 g, 15.9 mmol, 1.4 eq) at 0°C. The mixture was stirred for 5 min. Methyl 2-amino-5-cyanobenzoate (**1**) (2.0 g, 11.4 mmol, 1 eq) was added in portions. The mixture was

stirred for 16 h at room temperature and made acidic (pH = 2) by the addition of 1 M HCl. The mixture was extracted with EtOAc (3 × 80 mL) and the combined organic extract was dried over Na_2SO_4, filtered, and evaporated to give methyl 2-bromo-5-cyanobenzoate **(2)** (2.7 g, crude).

Synthesis of methyl 2-bromo-5-carbamimidoylbenzoate

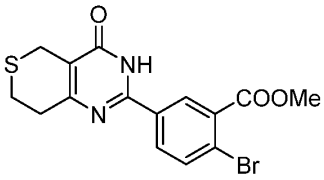


[00326] *Scheme 3B*

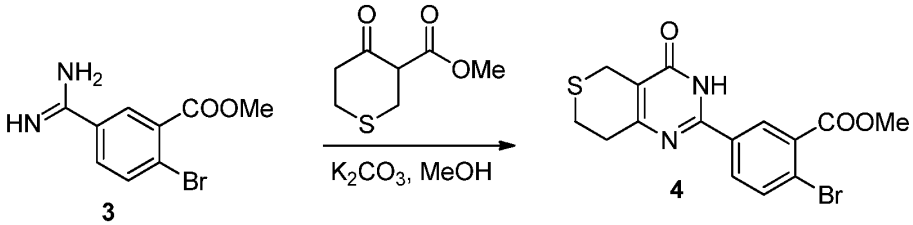


[00327] A mixture of methyl 2-bromo-5-cyanobenzoate (**2**) (2.50 g, 10.4 mmol, 1.0 eq), ammonium chloride (0.38 g, 5.4 mmol, 2.5 eq), and sodium methoxide (0.22 g, 5.4 mmol, 2.5 eq) in MeOH (50 mL) was stirred at 40°C for 16 h. The mixture was concentrated to afford methyl 2-bromo-5-carbamimidoylbenzoate (**3**) (crude). LC-MS (ESI) m/z calculated for $C_9H_9BrN_2O_2+H^+$: 257.1; found: 256.9.

Synthesis of methyl 2-bromo-5-(4-oxo-4,5,7,8-tetrahydro-3H-thiopyrano[4,3-d]pyrimidin-2-yl) benzoate



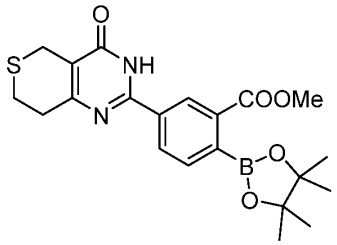
[00328] *Scheme 3C*



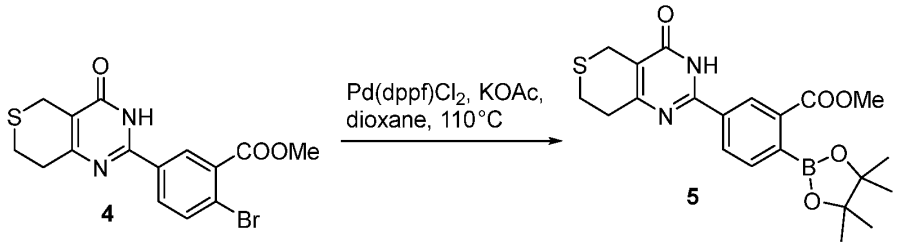
[00329] A mixture of methyl 2-bromo-5-carbamimidoylbenzoate (**3**) (2.5 g, 9.7 mmol, 1.0 eq), methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (1.7 g, 9.7 mmol, 1.0 eq), and K_2CO_3 (4.0 g, 29.2 mmol, 3.0 eq) in MeOH (50 mL) was stirred at 70°C for 16 h. The mixture

was cooled and filtered. The filtrate was added into water (300 mL). The solid was filtered and dried to afford methyl 2-bromo-5-(4-oxo-4,5,7,8-tetrahydro-3H-thiopyrano[4,3-d]pyrimidin-2-yl) benzoate (**4**) (1.5 g, 3.9 mmol). LC-MS (ESI) m/z calculated for $C_{15}H_{13}BrN_2O_3S+H^+$: 382.2; found: 383.0.

Synthesis of methyl 5-(4-oxo-4,5,7,8-tetrahydro-3H-thiopyrano[4,3-d]pyrimidin-2-yl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate

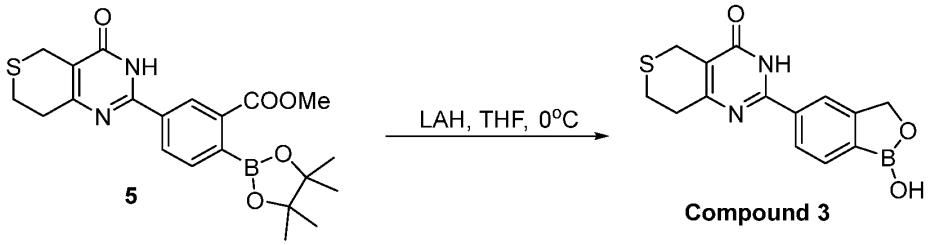


[00330] *Scheme 3D*



[00331] The mixture of methyl 2-bromo-5-(4-oxo-4,5,7,8-tetrahydro-3H-thiopyrano[4,3-d]pyrimidin-2-yl) benzoate (**4**) (0.60 g, 1.57 mmol, 1.0 eq), $(Bpin)_2$ (0.80 g, 3.15 mmol, 2.0 eq), $Pd(dppf)Cl_2$ (115 mg, 0.16 mmol, 0.1 eq), and KOAc (462 mg, 4.72 mmol, 3.0 eq) in dioxane (15 mL) was stirred at 110°C for 16 h. The mixture was diluted with EtOAc (30 mL), washed with water (15 mL), dried over Na_2SO_4, and concentrated. The crude product was purified on a silica gel column (PE:EtOAc = 5:1 to 1:1) to afford methyl 5-(4-oxo-4,5,7,8-tetrahydro-3H-thiopyrano[4,3-d]pyrimidin-2-yl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (**5**) (340 mg, 0.79 mmol). LC-MS (ESI) m/z calculated for $C_{21}H_{25}BN_2O_5S+H^+$: 429.3; found: 429.1.

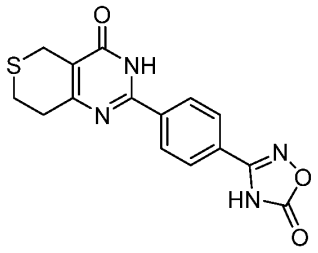
[00332] *Scheme 3E*



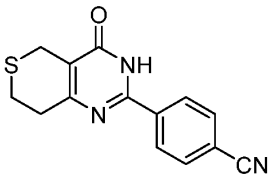
[00333] To a solution of methyl 5-(4-oxo-4,5,7,8-tetrahydro-3H-thiopyrano[4,3-d]pyrimidin-2-yl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (**5**) (0.34 g, 0.79 mmol, 1.0 eq) in THF (15 mL) was added LAH (60 mg, 1.60 mmol, 2.0 eq). The mixture was stirred at 0°C for 1 h. The mixture was added to H_2O, filtered, and concentrated under vacuum to afford a crude product which was purified by flash chromatography (H_2O:CH_3CN = 90:10 to 50:50) to provide 2-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 3**). LC-MS (ESI) m/z calculated for $C_{14}H_{13}BN_2O_3S$ $+H^+$: 301.1; found: 301.0. ^{1}H NMR (400 MHz, MeOD): δ 8.09 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 3.54 (s, 2H), 5.07 (s, 2H), 2.95-2.85 (m, 4H).

Compound 4 and Compound 43

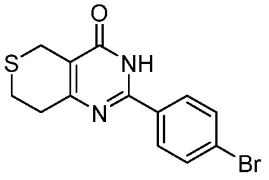
Synthesis of 3-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)-1,2,4-oxadiazol-5(4H)-one (Compound 4)



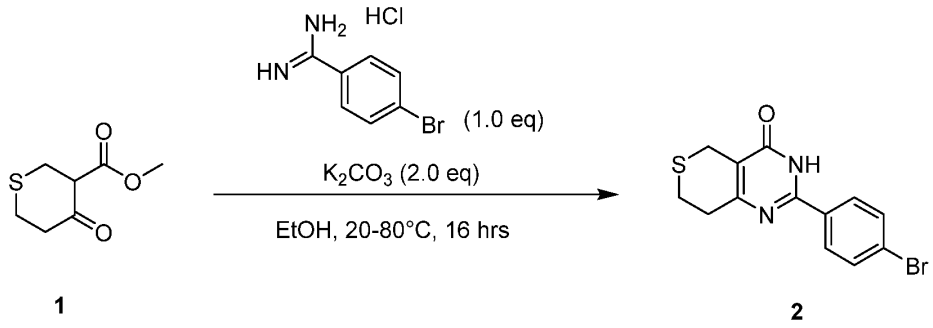
Synthesis of 4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzonitrile (Compound 43)



Synthesis of 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



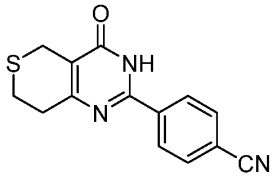
[00334] *Scheme 4A*



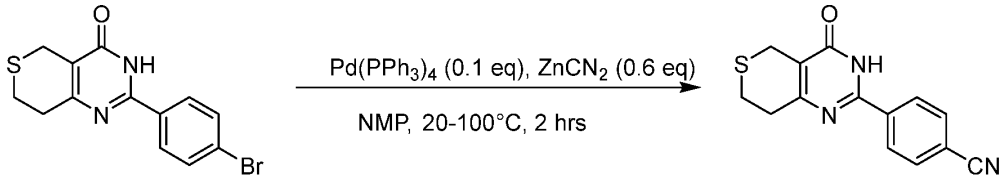
1 **2**

[00335] To a solution of methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**1**) (5.00g, 28.7 mmol, 1.00 eq) and 4-bromobenzamidine hydrochloride (6.76g, 28.7 mmol, 1.00 eq) in EtOH (50 mL) was added K_2CO_3 (7.93g, 57.4 mmol, 2 eq) at 20°C. The mixture was stirred at 80°C for 16h. LC-MS showed that (**1**) was consumed completely. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give a residue that was suspended in water (100 mL), then stirred at 20°C for 4h. The mixture was filtered and the filter cake was dried under reduced pressure to give 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**2**) (6.60 g, crude) which was used directly in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.08 - 8.02 (m, 2H), 7.72 - 7.67 (m, 2H), 3.51 (s, 2H), 2.87 (qd, J = 4.4, 8.4 Hz, 4H). LC-MS: 324.3 + bromo isomers (M+1).

Synthesis of 4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzonitrile (Compound 43)



[00336] *Scheme 4B*

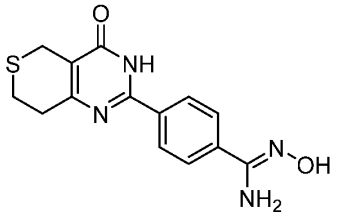


2 **Compound 43**

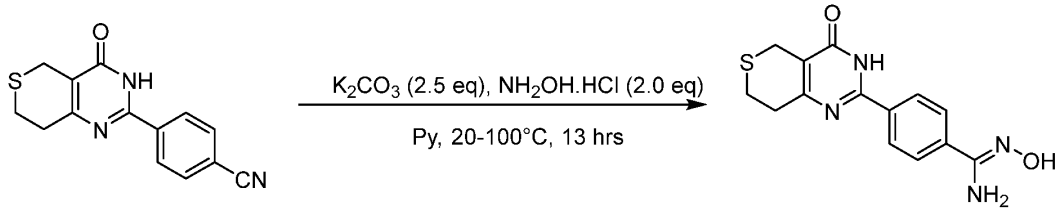
[00337] To a solution of 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**2**) (4.50g, 13.9 mmol, 1 eq) in NMP (45 mL) were added, portion-wise, $Zn(CN)_2$ (981mg, 8.35 mmol, 530 µL, 0.6 eq) and $Pd(PPh_3)_4$ (1.61g, 1.39 mmol, 0.1 eq) at

20°C under N_2. The mixture was stirred at 100°C for 2h. LC-MS showed that (**2**) was consumed completely. The resulting mixture was cooled to 20°C and diluted with saturated Na_2CO_3 (120 mL) at 20°C. Then the mixture was extracted with EtOAc (40 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1). 4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzonitrile (**Compound 43**) (1.80 g, 6.68 mmol) was obtained. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.07 – 12.77 (m, 1H), 8.25 (br d, J = 7.5 Hz, 2H), 8.00 (d, J = 8.4 Hz, 2H), 3.55 (s, 2H), 2.91 (s, 4H). LC-MS: 270.1 (M+1).

Synthesis of (Z)-N'-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzimidamide



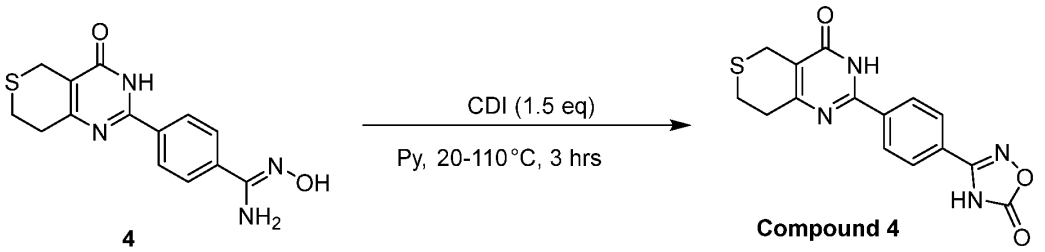
[00338] *Scheme 4C*



Compound 43 **4**

[00339] To a solution of 4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzonitrile (**Compound 43**) (500mg, 1.86 mmol, 1 eq) in pyridine (5 mL) was added $NH_2OH.HCl$ (258mg, 3.71 mmol, 2 eq) and K_2CO_3 (641mg, 4.64 mmol, 2.50 eq) at 20°C. The mixture was stirred at 100°C for 13h. LC-MS showed that (**Compound 43**) was consumed. After cooling to 20°C, the reaction mixture was filtered and the filter cake dried and concentrated under reduced pressure to give a residue that was suspended in water (5.00 mL) and was stirred at 20°C for 4 h. The mixture was filtered and the filter cake dried and concentrated under reduced pressure to give (Z)-N'-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzimidamide (**4**) (333mg, crude), which was used in the next step without further purification. LC-MS: 302.9 (M+1).

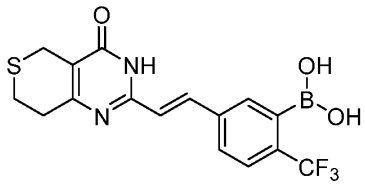
[00340] *Scheme 4D*



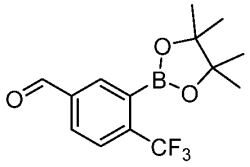
[00341] To a solution of (Z)-N'-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzimidamide (**4**) (333mg, 1.10 mmol, 1 eq) in pyridine (3.30 mL) was added CDI (268mg, 1.65 mmol, 1.50 eq) at 20°C. The mixture was stirred at 110°C for 3h. LC-MS showed that (**4**) was consumed completely. After cooling to 20°C, the mixture was filtered and the filter cake dried and concentrated under reduced pressure to give a residue that was stirred in DCM (2 mL) at RT for 6h. The mixture was filtered and the filter cake dried and concentrated under reduced pressure to give 3-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)-1,2,4-oxadiazol-5(4H)-one (**Compound 4**). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.26 – 12.46 (m, 1H), 8.23 (d, J = 8.5 Hz, 2H), 7.94 (d, J = 8.5 Hz, 2H), 7.18 (s, 1H), 3.55 (s, 2H), 2.91 (s, 4H). LC-MS: 329.1 (M+1).

Compound 5

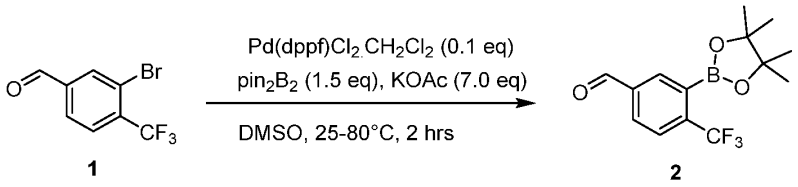
Synthesis of (E)-(5-(2-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)vinyl)-2-(trifluoromethyl)phenyl)boronic acid (Compound 5)



Synthesis of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)benzaldehyde

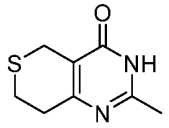


[00342] *Scheme 5A*

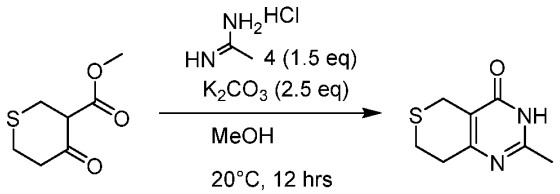


[00343] To a solution of 3-bromo-4-(trifluoromethyl)benzaldehyde (**1**) (0.50 g, 1.98 mmol, 1.00 eq) and Pin_2B_2 (753 mg, 2.96 mmol, 1.50 eq) in DMSO (10.0 mL) was added KOAc (1.36 g, 13.8 mmol, 7.00 eq) and $Pd(dppf)Cl_2.CH_2Cl_2$ (161 mg, 198 μmol, 0.10 eq) at 25°C under N_2. The suspension was degassed *in vacuo* and purged with N_2 several times. The mixture was then warmed to 80°C and stirred at 80°C for 2 h. Thin-layer chromatography (TLC; petroleum ether/ethyl acetate = 20/1) showed that 3-bromo-4-(trifluoromethyl)benzaldehyde (**1**) was consumed completely. The mixture was cooled to 25°C and then diluted with EtOAc (5.00 mL). The mixture was filtered, and the filtrate was washed with water (5.00 mL × 2) and brine (5.00 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by Prep-TLC (SiO_2, Petroleum ether/Ethyl acetate = 10/1). 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)benzaldehyde (**2**) (0.20 g, crude) was obtained and used in the next step without further purification.

Synthesis of 2-methyl-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



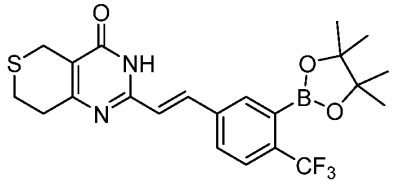
[00344] *Scheme 5B*



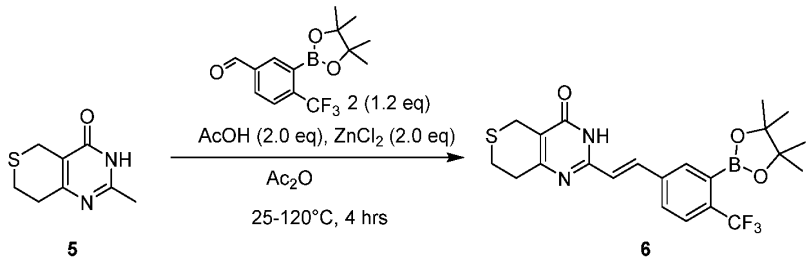
3 **5**

[00345] To a solution of acetamidine:hydrochloride (**4**) (814 mg, 8.61 mmol, 1.50 eq) in MeOH (10.0 mL) was added K_2CO_3 (1.98 g, 14.4 mmol, 2.50 eq) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**3**) (1.00 g, 5.74 mmol, 1.00 eq). The mixture was stirred at 20°C for 12 h. TLC (petroleum ether/ethyl acetate = 8/1) indicated methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**3**) was consumed completely. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. 2-methyl-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (1.00 g, crude) was obtained and used in the next step without further purification. The obtained compound was checked by ^1H NMR.

Synthesis of (E)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)styryl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one

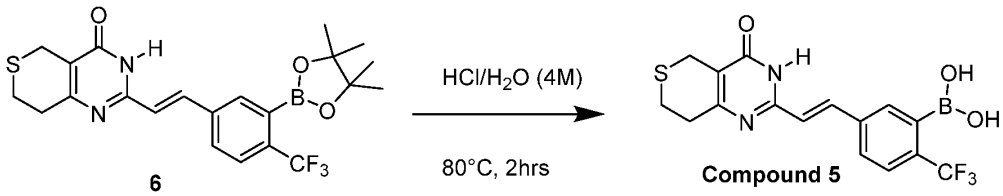


[00346] *Scheme 5C*



[00347] To a solution of 2-methyl-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (0.20 g, 666 μmol, 1.21 eq) in Ac_2O (1.00 mL) was added 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)benzaldehyde (**2**) (0.10 g, 549 μmol, 1.00 eq), $ZnCl_2$ (150 mg, 1.10 mmol, 51.4 uL, 2.00 eq), and AcOH (65.9 mg, 1.10 mmol, 62.8 uL, 2.00 eq) at 25°C under N_2. The mixture was stirred at 120°C for 4 h. LC-MS (ET48116-9-P1A) showed 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)-benzaldehyde (**2**) was consumed completely and one main peak with the desired m/z was detected. The mixture was cooled to 25°C and then concentrated under reduced pressure to remove most of the Ac_2O. The resulting residue was dissolved in EtOAc (5.00 mL) and then washed with saturated $NaHCO_3$ (2.00 mL × 2). The organic layer was separated and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO_2, petroleum ether/ethyl acetate = 1/1). (E)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)styryl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**6**) (0.25 g, crude) was obtained.

[00348] *Scheme 5D*

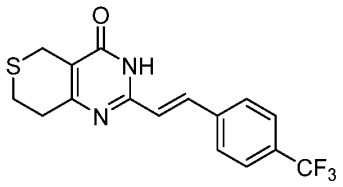


[00349] The mixture of (E)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)styryl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**6**) (0.25 g,

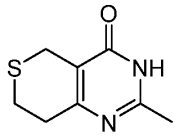
538.44 μmol, 1 eq) in HCl/H_2O (4 M, 2.50 mL) was stirred at 80°C for 2 h. LC-MS showed starting material (**6**) was consumed completely. The reaction mixture was concentrated under reduced pressure to give a residue that was purified by prep-HPLC (column: Phenomenex luna C18 80 * 40 mm * 3 μm; mobile phase: [water (HCl) – ACN]; B%: 18%-48%, 7 min). (E)-(5-(2-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)vinyl)-2-(trifluoromethyl)phenyl)boronic acid (**Compound 5**) was obtained. LC-MS: 383.0 (M+1). ^{1}H NMR (400MHz, DMSO-d_6): δ = 7.89 (br d, *J*=16.4 Hz, 1H), 7.80 – 7.66 (m, 3H), 7.04 (br d, *J*=16.3 Hz, 1H), 3.51 (br s, 2H), 2.93 – 2.81 (m, 4H).

Compound 6

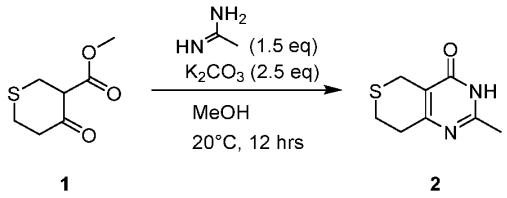
Synthesis of (E)-2-(4-(trifluoromethyl)styryl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 6)



Synthesis of 2-methyl-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one

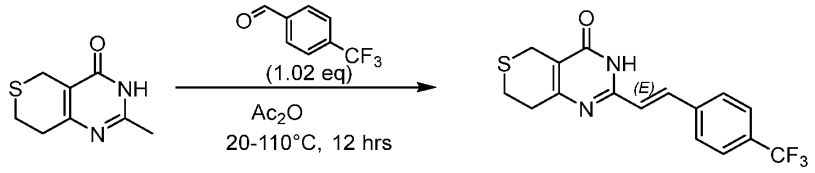


[00350] *Scheme 6A*



[00351] To a solution of acetamidine/hydrochloride (814 mg, 8.61 mmol, 1.50 eq) in MeOH (10.0 mL) was added K_2CO_3 (1.98 g, 14.4 mmol, 2.50 eq) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**1**) (1.00 g, 5.74 mmol, 1.00 eq). The mixture was stirred at 20°C for 12 h. TLC (petroleum ether/ethyl acetate = 8/1) indicated starting material (**1**) was consumed completely. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. 2-methyl-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**2**) (1.00 g, crude) was obtained and used in the next step without further purification.

[00352] *Scheme 6B*

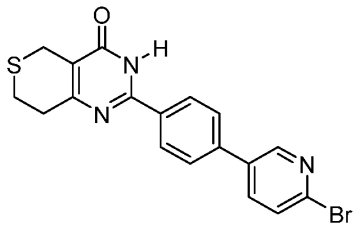


2 **Compound 6**

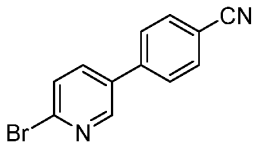
[00353] To a solution of 2-methyl-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**2**) (0.25 g, 1.37 mmol, 1.00 eq) in Ac_2O (2.50 mL) was added 4-(trifluoromethyl) benzaldehyde (244 mg, 1.40 mmol, 187 μL, 1.02 eq) at 20°C. The mixture was stirred at 110°C for 12 h. The mixture was cooled to 20°C and concentrated under reduced pressure to give a residue. The mixture was diluted with H_2O (2.00 mL) and extracted with EtOAc (2.00 mL × 3). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. Part of the residue was purified by prep-HPLC (column: Waters Xbridge BEH C18 100 * 30 mm * 10 μm; mobile phase: [water (10mM NH_4HCO_3)-ACN]; B%: 40%-65%, 10 min). (E)-2-(4-(trifluoromethyl)styryl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 6**) was obtained. LCMS: 339.1 (M+1). ^1H NMR (400MHz, DMSO-d_6): δ = 12.62 – 12.51 (m, 1H), 7.91 – 7.73 (m, 5H), 7.04 – 6.92 (m, 1H), 3.52 – 3.45 (m, 2H), 2.89 – 2.79 (m, 4H).

Compound 7

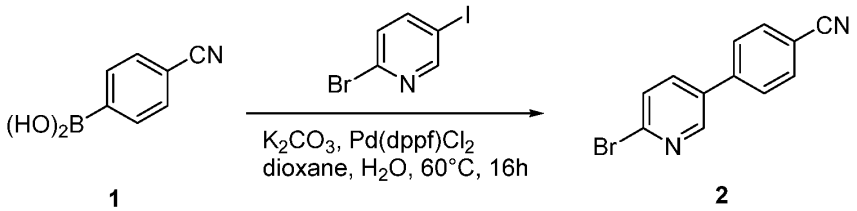
Synthesis of 2-(4-(6-bromopyridin-3-yl) phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano [4,3-d] pyrimidin-4-one (Compound 7)



Synthesis of 4-(6-bromopyridin-3-yl)benzonitrile



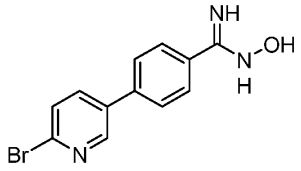
[00354] *Scheme 7A*



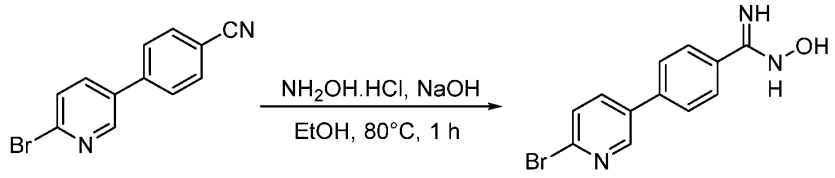
1 **2**

[00355] A mixture of (4-cyanophenyl)boronic acid (**1**) (50.0 g, 340.0 mmol, 1.0 eq), 2-bromo-5-iodopyridine (96.6 g, 340.2 mmol,1.0 eq), and K_2CO_3 (141.0 g,1.02 mol, 3.0 eq) in dioxane (3.2 L) and H_2O (800 mL) was stirred at 60°C for 16 h. The mixture was filtered and concentrated. The crude residue was purified through a silica gel column (PE:EtOAc = 10:1 to 1:1) to afford 4-(6-bromopyridin-3-yl)benzonitrile (**2**) (63.0 g). LC-MS (ESI) m/z calculated for $C_{12}H_7BrN_2+H^+$: 261.0; found: 261.1 (isotope of ^{81}Br). This reaction is presented as Scheme 7A.

Synthesis of 4-(6-bromopyridin-3-yl)-N-hydroxybenzimidamide



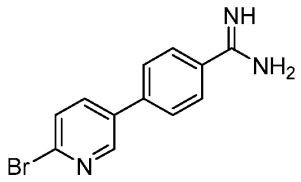
[00356] *Scheme 7B*



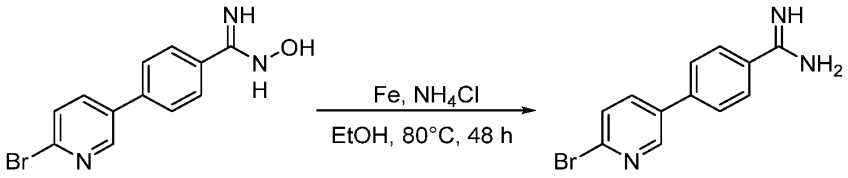
2 **3**

[00357] A mixture of 4-(6-bromopyridin-3-yl)benzonitrile (**2**) (60 g, 231.6 mmol, 1.0 eq), $NH_2OH{\cdot}HCl$ (40.2 g, 579 mmol, 2.5 eq), and NaOH (23.16 g, 579 mmol, 2.5 eq) in EtOH (500 mL) was stirred at 80°C for 3 h. The mixture was diluted with EtOH (100 mL) and filtered. The filtrate was concentrated to afford 4-(6-bromopyridin-3-yl)-N-hydroxybenzimidamide (**3**) (40 g, crude). LC-MS (ESI) m/z calculated for $C_{12}H_{10}BrN_3O+H^+$: 294.0; found: 294.1 (isotope of ^{81}Br). This reaction is presented as Scheme 7B.

Synthesis of 4-(6-bromopyridin-3-yl) benzimidamide



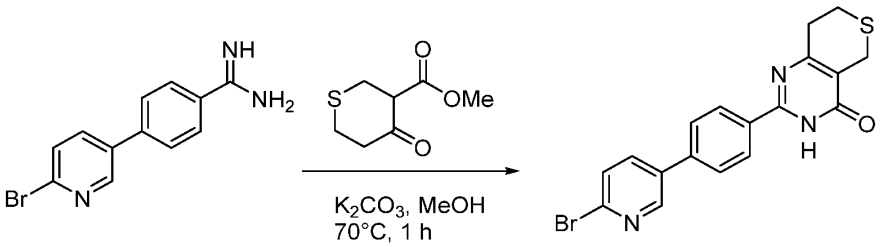
[00358] *Scheme 7C*



3 **4**

[00359] A mixture of 4-(6-bromopyridin-3-yl)-N-hydroxybenzimidamide (**3**) (40.0 g, 136.8 mmol, 1.0 eq), NH_4Cl (146.48 g, 3.460 mol, 20 eq), and Fe (230.04 g, 2.74 mol, 20 eq) in EtOH (600 mL) was stirred at 80°C for 48 h. The mixture was cooled and filtered. The solvent was removed *in vacuo* to afford 4-(6-bromopyridin-3-yl) benzimidamide (**4**) (30. 0 g, crude). LC-MS (ESI) m/z calculated for $C_{12}H_{10}BrN_3+H^+$: 276.0; found: 276.1 (isotope of ^{79}Br). This reaction is presented as Scheme 7C.

[00360] *Scheme 7D*



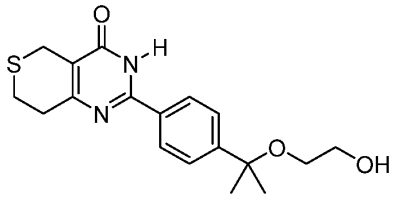
4 **Compound 7**

[00361] A mixture of 4-(6-bromopyridin-3-yl) benzimidamide (**4**) (30.0 g, 108.6 mmol, 1.0 eq), methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (37.8 g, 217.2 mmol, 2 eq), and K_2CO_3 (45.0 g, 325.8 mmol, 3.0 eq) in MeOH (500 mL) was stirred at 70°C for 4 h. The mixture was concentrated under reduced pressure. The residue was washed with H_2O (200 mL) and filtered. The solid was triturated in MeOH (150 mL), EtOAc (150 mL), and acetone (100 mL), respectively, to remove most of the impurities. The mixture was filtered off. The crude solid was triturated in aqueous NaOH (1% wt, 20 mL) and filtered. The solid was washed with water (50 mL) and acetone (100 mL) and dried under vacuum to afford 2-(4-(6-bromopyridin-3-yl) phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano [4,3-d]pyrimidin-4-one

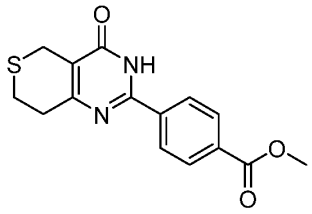
(**Compound 7**). LC-MS (ESI) m/z calculated for $C_{18}H_{14}BrN_3OS+H^+$: 402.0; found: 401.9 (isotope of ^{81}Br). ^1H NMR (400 MHz, DMSO): δ 12.82 (brs, 1H), 8.81 (d, J = 2.4 Hz, 1H), 8.22 (d, J = 8.3 Hz, 2H), 8.14 (dd, J = 8.4, 2.4 Hz, 1H), 7.91 (d, J = 8.3 Hz, 2H), 7.77 (d, J = 8.4 Hz, 1H), 3.54 (s, 2H), 2.91 (brs, 4H). This reaction is presented as Scheme 7D.

Compound 8

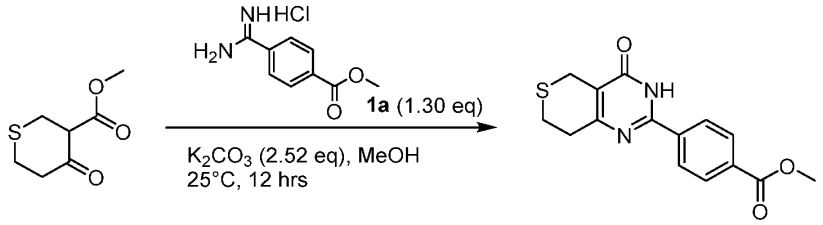
Synthesis of 2-(4-(2-(2-hydroxyethoxy)propan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 8)



Synthesis of methyl 4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate



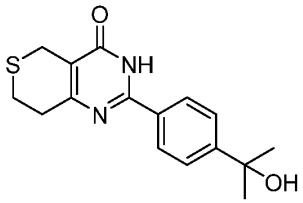
[00362] *Scheme 8A*



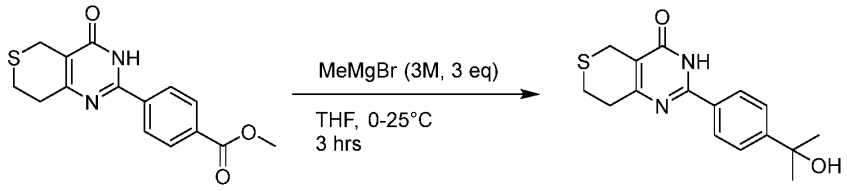
1 **2**

[00363] To the solution of methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**1**) (2 g, 11.48 mmol, 1 eq) in MeOH (20 mL) was added methyl 4-carbamimidoylbenzoate hydrochloride (**1a**) (3.20 g, 14.91 mmol, 1.30 eq) and K_2CO_3 (4.00 g, 28.94 mmol, 2.52 eq) at 25°C. The mixture was stirred at 25°C for 12 h. The mixture was filtered, and the filter cake was concentrated under reduced pressure to give a residue. The residue was suspended in H_2O (40 mL) and stirred at 25°C for 12 h. The mixture was filtered, and the filter cake was dried *in vacuo* to give methyl 4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**2**) (1.5 g, crude). The crude product was used in the next step directly without further purification. This reaction is presented as Scheme 8A.

Synthesis of 2-(4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



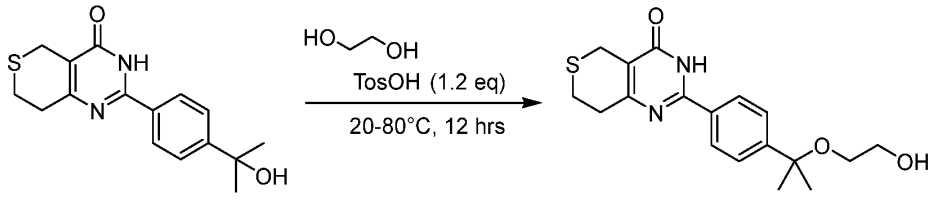
[00364] *Scheme 8B*



2 **3**

[00365] To a solution of methyl 4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**2**) (0.3 g, 992.24 μmol, 1 eq) in THF (3 mL), MeMgBr (3 M, 992.24 μL, 3 eq) was added dropwise at 0°C under N_2. The mixture was stirred at 25°C for 3 h. The reaction mixture was quenched by the addition of NH_4Cl (5 mL), and the aqueous phase was extracted with 10 mL of DCM (5 mL × 2). The combined organic layers were concentrated under reduced pressure to give a residue. 2-(4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (2 g, crude) was obtained, which was used in the next step directly without further purification. ^{1}H NMR: ET47629-1-P1A (400 MHz, $CDCl_3$): δ 12.54 – 12.27 (m, 1H), 8.07 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8.5 Hz, 2H), 3.62 (br s, 2H), 3.03 – 2.94 (m, 2H), 2.91 – 2.82 (m, 2H), 1.55 (s, 6H). This reaction is presented as Scheme 8B.

[00366] *Scheme 8C*



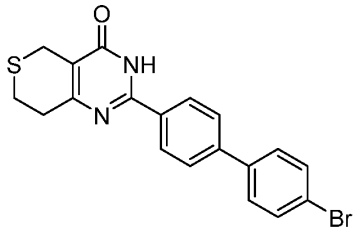
3 **Compound 8**

[00367] To a solution of 2-(4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (0.12 g, 396.84 μmol, 1 eq) in ethylene glycol (5 mL) was added TosOH (82.00 mg, 476.21 μmol, 1.2 eq) at 20°C. The mixture was stirred at 20°C for 1 h, then warmed to 80°C and stirred at 80°C for 12 h. The mixture was purified directly

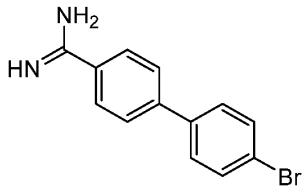
by prep-HPLC (neutral condition, column: Waters Xbridge BEH C18 100 * 30 mm * 10 μm; mobile phase: [water (NH_4HCO_3)-ACN]; B%: 10%-50%, 10 min). 2-(4-(2-(2-hydroxyethoxy)propan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 8**) was obtained. LC-MS: 347.2 (M +1). ^{1}H NMR: ET47430-4-P1A1 (400 MHz, $CDCl_3$): δ 8.14 (d, J = 8.2 Hz, 2H), 7.60 (d, J = 8.3 Hz, 2H), 3.80 – 3.69 (m, 4H), 3.34 (t, J = 4.6 Hz, 2H), 3.14 – 3.06 (m, 2H), 3.01 – 2.93 (m, 2H), 1.62 (s, 6H). This reaction is presented as Scheme 8C.

Compound 9

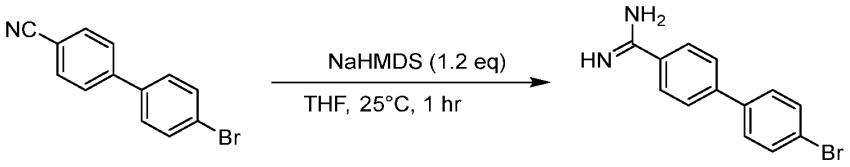
Synthesis of 2-(4'-bromo-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 9)



Synthesis of 4'-bromo-[1,1'-biphenyl]-4-carboximidamide



[00368] *Scheme 9A*

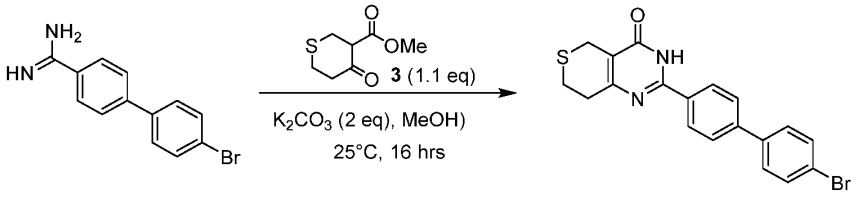


1 **2**

[00369] To a mixture of 4'-bromo-[1,1'-biphenyl]-4-carbonitrile (**1**) (4 g, 15.5 mmol, 1 eq) in THF (40 mL) was added NaHMDS (1 M, 18.60 mL, 1.2 eq) dropwise at 25°C under N_2. The mixture was stirred at 25°C for 1 h, and a liquid was obtained. The reaction mixture was quenched by the addition of water (30 mL) at 25°C. The pH of the mixture was adjusted to 2~3 by addition of 1 M HCl. The aqueous layer was separated and evaporated to about 15% of its original volume, resulting in crystallization of the desired HCl-salts. The mixture was filtered, and the filter cake was dried *in vacuo*. 4'-bromo-[1,1'-biphenyl]-4-carboximidamide (**2**)

(4.53 g, 14.5 mmol, HCl) was obtained. The crude product was used in the next step without further purification. ^{1}H NMR: (400 MHz, DMSO-d_6): δ 9.44 (s, 2H), 9.19 (s, 2H), 7.94 (s, 4H), 7.81 – 7.65 (m, 4H).

[00370] *Scheme 9B*

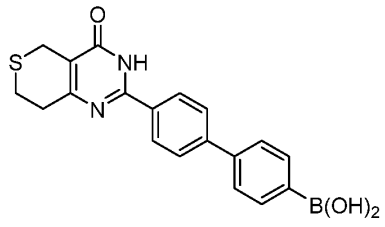


2 **Compound 9**

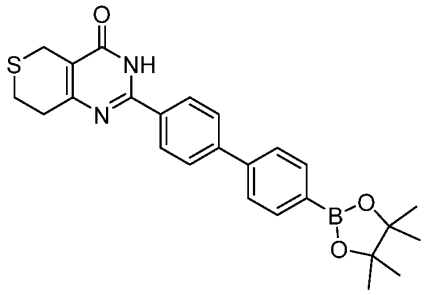
[00371] To a solution of 4'-bromo-[1,1'-biphenyl]-4-carboximidamide (**2**) (0.066 g, 378.8 μmol, 1.1 eq) in MeOH (1.8 mL) was added methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**3**) (107.3 mg, 344.4 μmol, 1 eq, HCl) and K_2CO_3 (95.2 mg, 688.8 μmol, 2 eq) at 25°C. The mixture was stirred at 25°C for 16 h. The reaction mixture was filtered, and the filter cake was dried *in vacuo* to give a residue. The crude product was purified by precipitation from DMSO (3 mL). 2-(4'-bromo-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 9**) was obtained. LC-MS: 399.19 (M+1). ^{1}H NMR: ET48394-6-P1A (400 MHz, DMSO-d_6): δ 12.86 – 12.62 (m, 1H), 8.21 (d, J = 8.3 Hz, 2H), 7.86 – 7.81 (m, 2H), 7.75 – 7.68 (m, 4H), 3.55 (s, 2H), 2.91 (s, 4H).

Compound 10

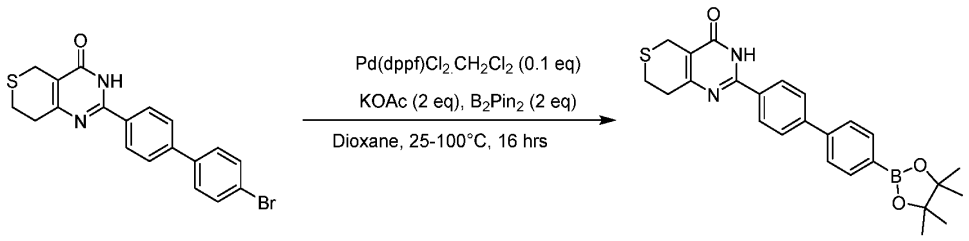
Synthesis of (4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (Compound 10)



Synthesis of 2-(4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



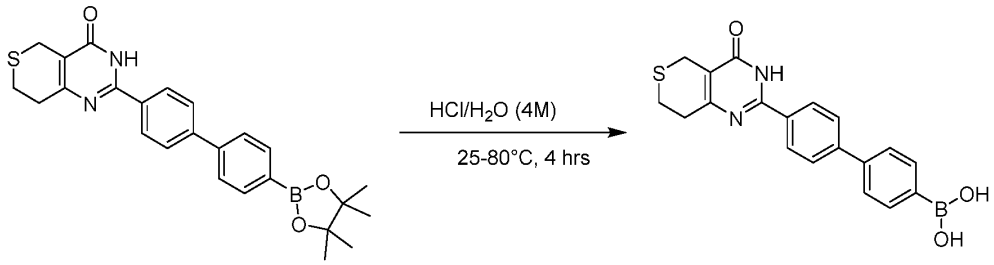
[00372] *Scheme 10A*



Compound 9 **5**

[00373] To a mixture of 2-(4'-bromo-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 9**) (0.31 g, 776.4 μmol, 1 eq) and B_2Pin_2 (394.3 mg, 1.55 mmol, 2 eq) in dioxane (6 mL) was added KOAc (152.4 mg, 1.55 mmol, 2 eq) and $Pd(dppf)Cl_2.CH_2Cl_2$ (63.4 mg, 77.6 μmol, 0.1 eq) in portions at 25°C under N_2. The mixture was degassed under vacuum and purged three times with N_2. Then the reaction mixture was heated to 100 °C and stirred for 16 h. A suspension liquid was obtained. The crude product was triturated with H_2O (10 mL) at 25°C for 2 h. The mixture was filtered, and the filter cake was washed with DCM (5 mL) and MeOH (5 mL). The filter cake was dried under reduced pressure to give 2-(4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (0.18 g) which was used in the next step without further purification.

[00374] *Scheme 10B*

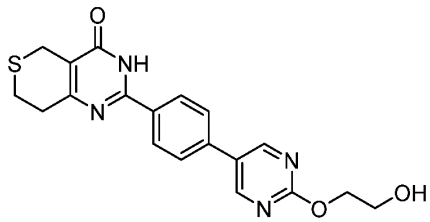


5 Compound 10

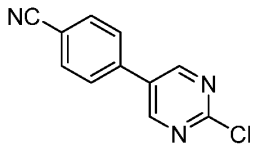
[00375] To a round-bottom flask was added 2-(4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (0.18 g, 403.2 μmol, 1 eq) and HCl (4 M, 4 mL, 39.7 eq) at 25°C. The reaction mixture was stirred at 80°C for 4 h. The mixture was filtered, and the filter cake was washed with DCM (5 mL) and MeOH (5 mL). The filter cake was dried *in vacuo*. (4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**Compound 10**) was obtained. LC-MS: 365.1 (M+1). ^{1}H NMR: ET48394-20 (400 MHz, DMSO-d_6): δ 8.20 (d, J = 8.5 Hz, 2H), 7.89 (dd, J = 8.3, 19.4 Hz, 4H), 7.74 (d, J = 8.2 Hz, 2H), 3.55 (s, 2H), 2.92 (s, 4H).

Compound 11

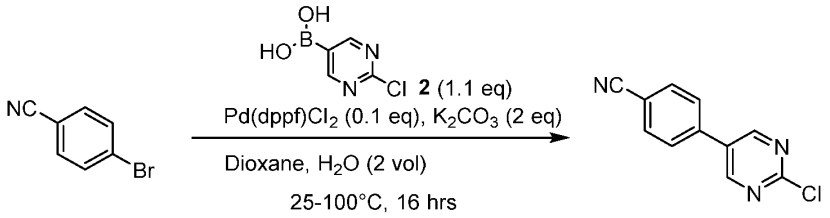
Synthesis of 2-(4-(2-(2-hydroxyethoxy)pyrimidin-5-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 11)



Synthesis of 4-(2-chloropyrimidin-5-yl)benzonitrile



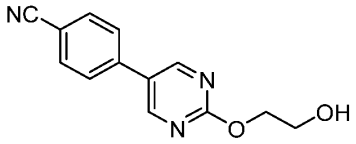
[00376] *Scheme 11A*



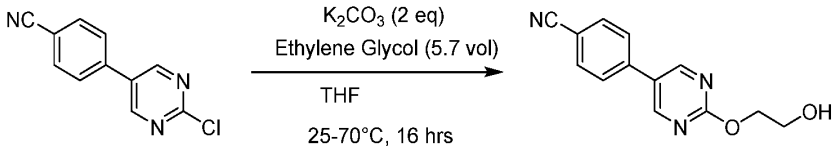
1 3

[00377] To a mixture of (2-chloropyrimidin-5-yl)boronic acid (**2**) (9.57 g, 60.43 mmol, 1.10 eq) and 4-bromobenzonitrile (**1**) (10.0 g, 54.9 mmol, 1.00 eq) in dioxane (200 mL) and H_2O (20.0 mL) was added K_2CO_3 (15.2 g, 110 mmol, 2.00 eq) and $Pd(dppf)Cl_2.CH_2Cl_2$ (4.49 g, 5.49 mmol, 0.10 eq) in one portion at 25°C under N_2. The mixture was degassed with N_2 and then stirred at 100°C for 16 h. A liquid was obtained. The reaction mixture was diluted with water (200 mL) and extracted with EtOAc 600 mL (200 mL × 3). The combined organic layers were concentrated under reduced pressure to give a residue that was purified by flash silica gel chromatography (ISCO®; 200 g SepaFlash® Silica Flash Column, eluent of 0 ~ 20% ethyl acetate/petroleum ether gradient @ 100 mL / min, R_f = 0.25). 4-(2-chloropyrimidin-5-yl)benzonitrile (**3**) (1.1 g) was obtained and used as a crude in the next step.

Synthesis of 4-(2-(2-hydroxyethoxy)pyrimidin-5-yl)benzonitrile



[00378] *Scheme 11B*

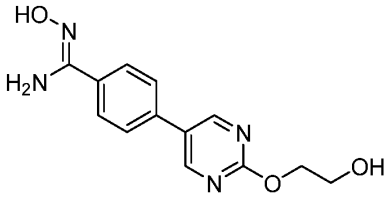


3 4

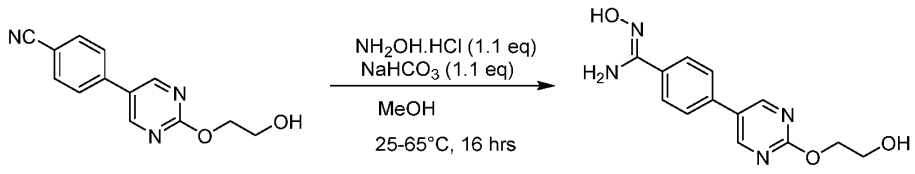
[00379] To a mixture of 4-(2-chloropyrimidin-5-yl)benzonitrile (**3**) (0.70 g, 3.25 mmol, 1.00 eq) in THF (4.00 mL) and ethylene glycol (4.00 mL) was added K_2CO_3 (897 mg, 6.49 mmol, 2.00 eq) in one portion at 25°C under N_2. The mixture was stirred at 70°C for 16 h. A suspension liquid was obtained. The reaction mixture was diluted with water (20 mL) and extracted with EtOAc 30 mL (10 mL × 3). The combined organic layers were concentrated under reduced pressure to give a residue that was purified by flash silica gel chromatography

(ISCO®; 40 g SepaFlash® Silica Flash Column, eluent of 0 ~ 50% ethyl acetate/petroleum ether gradient @ 40 mL/min, R_f = 0.27). 4-(2-(2-hydroxyethoxy)pyrimidin-5-yl)benzonitrile (**4**) (540 mg, 2.24 mmol) was obtained and used crude in the next step.

Synthesis of (Z)-N'-hydroxy-4-(2-(2-hydroxyethoxy)pyrimidin-5-yl)benzimidamide



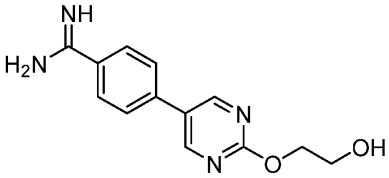
[00380] *Scheme 11C*



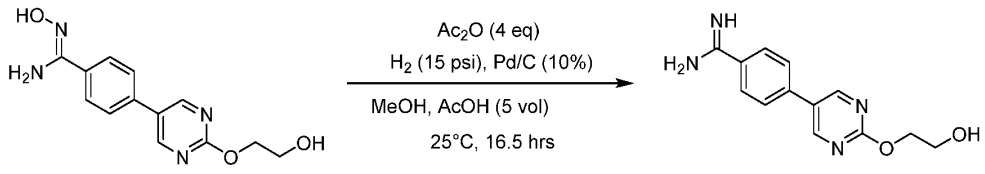
4 **5**

[00381] To a mixture of 4-(2-(2-hydroxyethoxy)pyrimidin-5-yl)benzonitrile (**4**) (0.54 g, 2.24 mmol, 1.00 eq) in MeOH (5.00 mL) was added $NH_2OH.HCl$ (171 mg, 2.46 mmol, 1.10 eq) and $NaHCO_3$ (207 mg, 2.46 mmol, 95.8 μL, 1.10 eq) in one portion at 25°C under N_2. The mixture was stirred at 65°C for 16 h. A liquid was obtained. The reaction mixture was filtered and concentrated under reduced pressure to give a (Z)-N'-hydroxy-4-(2-(2-hydroxyethoxy)pyrimidin-5-yl)benzimidamide (**5**) (500 mg, crude) and used in the next step without further purification.

Synthesis of 4-(2-(2-hydroxyethoxy)pyrimidin-5-yl)benzimidamide



[00382] *Scheme 11D*

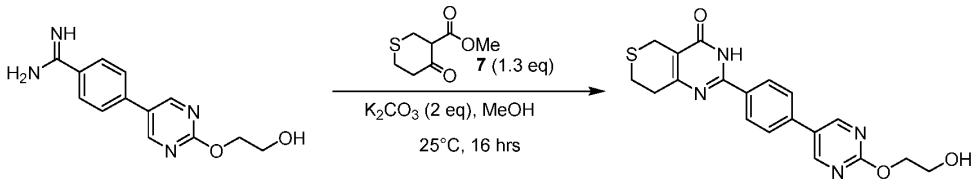


5 **6**

[00383] To a mixture of (Z)-N'-hydroxy-4-(2-(2-hydroxyethoxy)pyrimidin-5-yl)benzimidamide (**5**) (0.50 g, 1.82 mmol, 1.00 eq) in AcOH (2.62 g, 43.7 mmol, 2.50 mL) was

added Ac_2O (744 mg, 7.29 mmol, 683 µL, 4.00 eq) in one portion at 25°C under N_2. The mixture was stirred at 25°C for 30 min. MeOH (20 mL) and Pd/C (0.2 g, 218.76 µmol) were then added. The resulting mixture was degassed three times with H_2 and stirred under H_2 (15 psi) at 25°C for 16 h. The reaction mixture was filtered and concentrated under reduced pressure to give 4-(2-(2-hydroxyethoxy)pyrimidin-5-yl)benzimidamide (**6**) (500 mg, crude) and used in the next step without further purification.

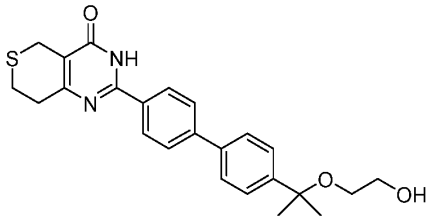
[00384] *Scheme 11E*



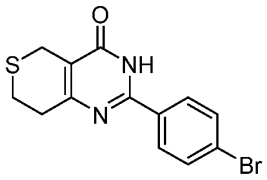
[00385] To a mixture of 4-(2-(2-hydroxyethoxy)pyrimidin-5-yl)benzimidamide (**6**) (500 mg, 1.94 mmol, 1.00 eq) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**7**) (438 mg, 2.52 mmol, 1.30 eq) in MeOH (4.00 mL) was added K_2CO_3 (535 mg, 3.87 mmol, 2.00 eq) in one portion at 25°C under N_2. The mixture was stirred at 25°C for 16 h. The reaction mixture was filtered and concentrated under reduced pressure to give a residue that was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150 * 40 mm * 10 µm; mobile phase: [water (NH_4HCO_3) – ACN]; B%: 10% - 40%, 8 min). 2-(4-(2-(2-hydroxyethoxy)pyrimidin-5-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 11**) was obtained. ^{1}H NMR (400 MHz, DMSO-d6) δ = 13.15 – 12.49 (m, 1H), 9.03 (s, 2H), 8.21 (br d, J = 8.2 Hz, 2H), 7.91 (d, J = 8.6 Hz, 2H), 4.93 (t, J = 5.5 Hz, 1H), 4.42 – 4.36 (m, 2H), 3.76 (q, J = 5.4 Hz, 2H), 3.54 (s, 2H), 2.95 – 2.86 (m, 4H). LCMS: 383 (M+1).

Compound 12

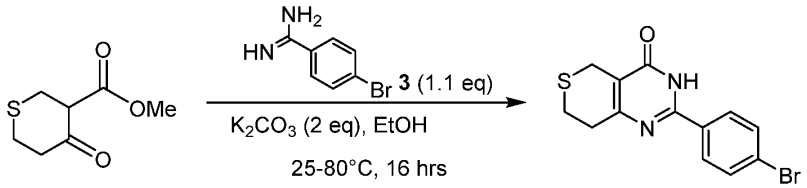
Synthesis of 2-(4'-(2-(2-hydroxyethoxy)propan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 12)



Synthesis of 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



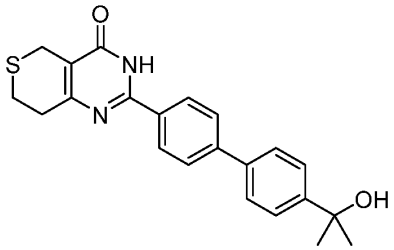
[00386] *Scheme 12A*



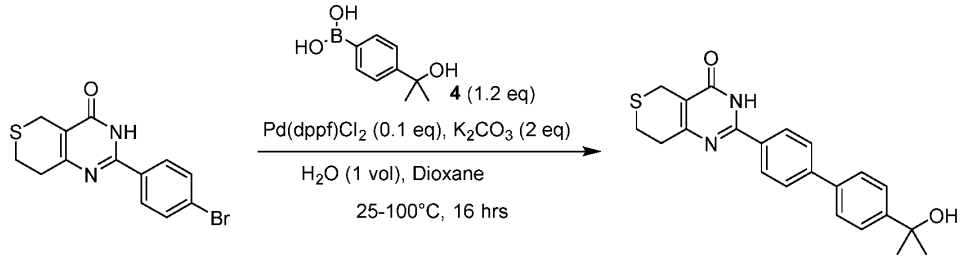
1 **2**

[00387] To a mixture of methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**1**) (8.00 g, 45.9 mmol, 1.00 eq) and 4-bromobenzimidamide (**3**) (11.9 g, 50.5 mmol, 1.10 eq, HCl) in EtOH (100 mL) was added K_2CO_3 (12.7 g, 91.8 mmol, 2.00 eq) in one portion at 25°C under N_2. The mixture was stirred at 80°C for 16 h. The reaction mixture was filtered and concentrated under reduced pressure to give 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**2**) (15.6 g, crude) that was used in the next step without further purification. ^{1}H NMR: (400 MHz, DMSO-d6): δ 8.19 – 8.13 (m, 2H), 7.53 – 7.46 (m, 2H), 3.42 (s, 2H), 2.81 – 2.75 (m, 2H), 2.74 – 2.67 (m, 2H).

Synthesis of 2-(4'-(2-hydroxypropan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



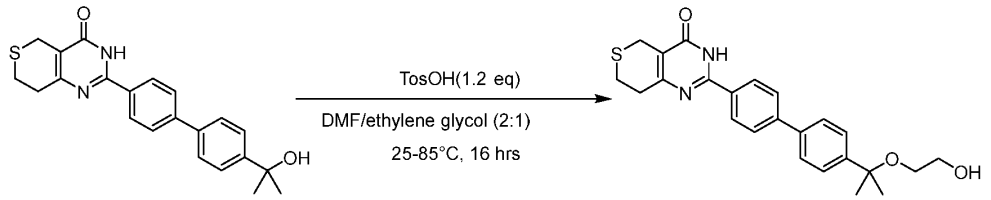
[00388] *Scheme 12B*



2 **5**

[00389] To a mixture of 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**2**) (4.00 g, 12.4 mmol, 1.00 *eq*) and (4-(2-hydroxypropan-2-yl)phenyl)boronic acid (**4**) (2.67 g, 14.9 mmol, 1.20 *eq*) in dioxane (40.0 mL) and H_2O (5.00 mL) was added K_2CO_3 (3.42 g, 24.8 mmol, 2.00 *eq*) and $Pd(dppf)Cl_2.CH_2Cl_2$ (1.01 g, 1.24 mmol, 0.10 *eq*) in one portion at 25°C under N_2. The mixture was degassed under vacuum, purged three times with N_2, and then heated to 100°C and stirred for 16 h. The reaction mixture was diluted with EtOAc (20.0 mL) and then filtered. The filter cake was washed with water (10 mL) and MeOH (10 mL) to give 2-(4'-(2-hydroxypropan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (2.10 g, 5.55 mmol, crude) and used in the next step without further purification. ^1H NMR: (400 MHz, DMSO-d6): δ = 13.13 – 12.31 (m, 1H), 8.51 – 7.75 (m, 4H), 7.75 – 7.15 (m, 4H), 5.07 (br d, J = 3.2 Hz, 1H), 3.54 (br s, 2H), 2.90 (br s, 4H), 1.78 – 0.88 (m, 6H).

[00390] *Scheme 12C*



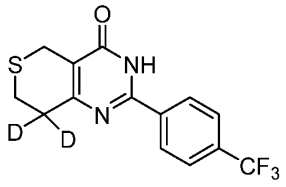
5 **Compound 12**

[00391] To a mixture of 2-(4'-(2-hydroxypropan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (1.50 g, 3.96 mmol, 1.00 eq) in DMF (10.0 mL) was added TosOH (819 mg, 4.76 mmol, 1.20 eq) and ethylene glycol (5.55 g, 89.4 mmol, 5.00 mL, 22.6 eq) in one portion at 25°C under N_2. The mixture was stirred at 85°C for 16 h. The mixture was cooled to RT and filtered. The filter cake was dissolved in DMSO and then purified directly by prep-HPLC (column: Phenomenex Luna 80 * 30 mm * 3 μm; mobile phase: [water (HCl) - MEOH]; B%: 40% - 65%, 8 min) to give 2-(4'-(2-(2-hydroxyethoxy)propan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-

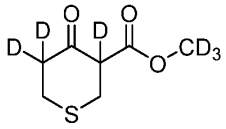
d]pyrimidin-4-one (**Compound 12**). LCMS: 423.2 (M+1). ^{1}H NMR: (400 MHz, CHLOROFORM-d): δ 11.47 – 10.94 (m, 1H), 8.14 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 2H), 7.67 – 7.61 (m, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 3.80 – 3.69 (m, 4H), 3.41 - 3.31 (m, 2H), 3.10 – 3.03 (m, 2H), 2.99 – 2.91 (m, 2H), 1.66 – 1.62 (m, 6H).

Compound 13

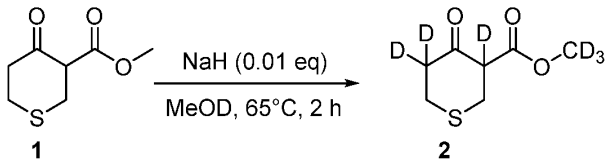
Synthesis of 2-(4-(trifluoromethyl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one-8,8-d2 (Compound 13)



Synthesis of methyl-d3 4-oxotetrahydro-2H-thiopyran-3-carboxylate-3,5,5-d3



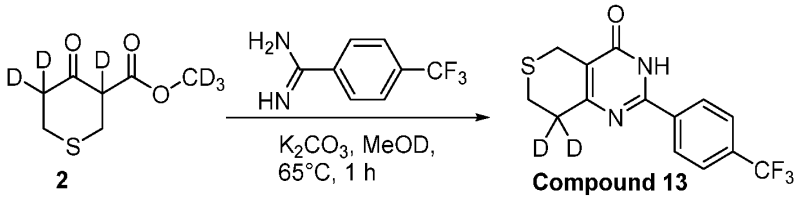
[00392] *Scheme 13A*



1 **2**

[00393] A mixture of methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**1**) (0.9 g, 5.16 mmol, 1.0 eq) and NaH (2.1 mg, 0.051 mmol, 0.01 eq) in MeOD (13.5 mL) was stirred at 65°C for 2 h. Methyl-d3 4-oxotetrahydro-2H-thiopyran-3-carboxylate-3,5,5-d3 (**2**) was obtained as a crude product in MeOD solution. ^{1}H NMR (400 MHz, MeOD): δ 3.23-3.07 (m, 1H), 2.95 (s, 1H), 2.76 (s, 1H), 2.73 (s, 2H).

[00394] *Scheme 13B*



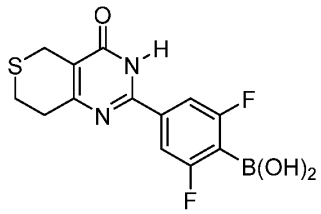
2 **Compound 13**

[00395] A mixture of (**2**) (0.90 g, 5.07 mmol, 1.11 eq), and 4-(trifluoromethyl)benzimidamide (0.861 g, 4.56 mmol, 1.0 eq) in MeOD (12 mL) was stirred at 65°C for 1 h. The mixture was concentrated to a half volume, then added to H_2O (50 mL).

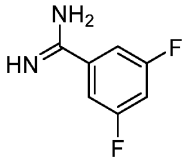
The precipitate was filtered and washed with H_2O (30 mL) and MeOH/H_2O (20 mL, 1:1, v/v), and dried *in vacuo* to afford 2-(4-(trifluoromethyl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimi-din-4-one-8,8-d2 (**Compound 13**). LC-MS (ESI) m/z calculated for $C_{14}H_9D_2F_3N_2OS+H^+$: 315.1; found: 315.1. ^1H NMR (400 MHz, DMSO-d_6): δ 8.29 (d, J = 8 Hz, 2H), 7.88 (d, J = 8 Hz, 2H), 3.54 (s, 2H), 2.89 (s, 2H). ^{19}F NMR (375 MHz, DMSO-d_6): δ 61.33 (s, 3F).

Compound 14

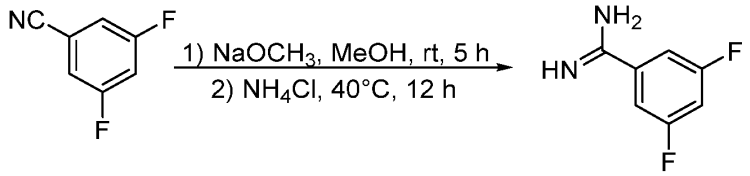
Synthesis of (2,6-difluoro-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (Compound 14)



Synthesis of 3,5-difluorobenzimidamide



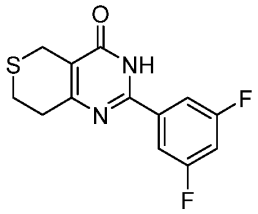
[00396] *Scheme 14A*



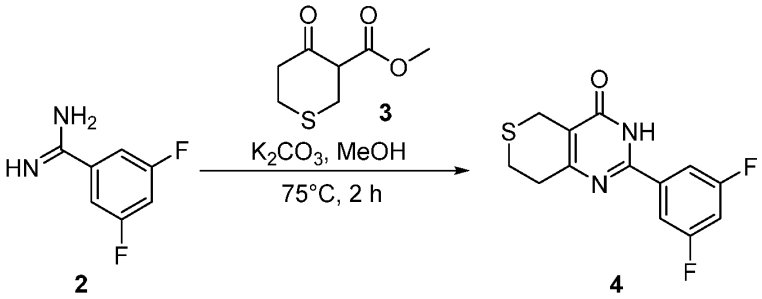
1 **2**

[00397] To a solution of 3,5-difluorobenzonitrile (**1**) (5.0 g, 36.0 mmol, 1.0 eq) in MeOH (50 mL) was added MeONa (3.9 g, 71.9 mmol, 2.0 eq); the mixture was stirred at 20°C for 4 h. To the mixture was added NH_4Cl (3.9 g, 71.9 mmol, 2.0 eq), and then the mixture was stirred at 40°C for 16 h. It was cooled to RT, filtered, and then the filtrate was concentrated to give 3,5-difluorobenzimidamide (**2**) (7 g, crude). LC-MS 157.4 $[M+H]^+$.

Synthesis of 2-(3,5-difluorophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one

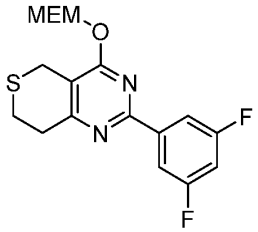


[00398] *Scheme 14B*

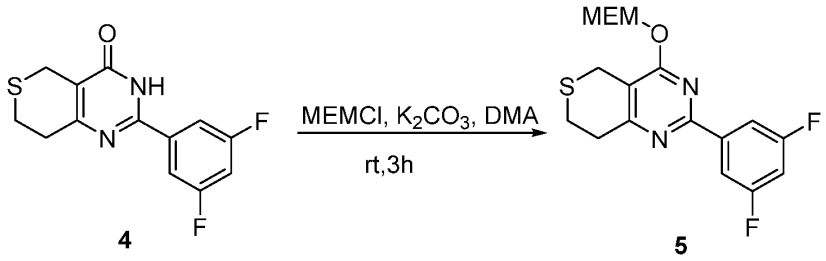


[00399] To a mixture of 3,5-difluorobenzimidamide (**2**) (7 g, 44.9 mmol, 1.0 eq) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**3**) (6.2 g, 35.9 mmol, 0.8 eq) in MeOH (50 mL) was added K_2CO_3 (12.4 g, 89.7 mmol, 2.0 eq) under N_2. The mixture was stirred at 75 °C for 2 h. The reaction mixture was concentrated to give 2-(3,5-difluorophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**), which was used directly for the next step. LC-MS 281.1 $[M+H]^+$.

Synthesis of 2-(3,5-difluorophenyl)-4-((2-methoxyethoxy)methoxy)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidine

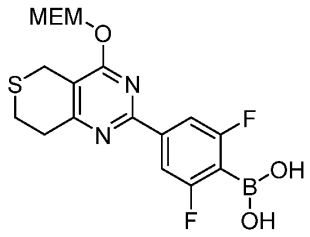


[00400] *Scheme 14C*

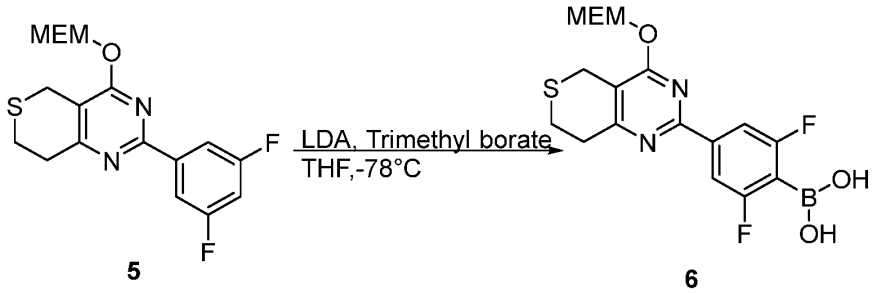


[00401] To a mixture of 2-(3,5-difluorophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (crude, 1.0 eq) in dimethylacetamide (DMA) (50 mL) was added 1-(chloromethoxy)-2-methoxyethane (11.2 g, 89.7 mmol, 2.0 eq). The reaction mixture was stirred at RT for 3 h and then poured into H_2O (300 mL) and extracted with EtOAc (2 × 100 mL). The combined organic layer was washed with brine (300 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel eluted with 0-30% EtOAc/hexane to afford 2-(3,5-difluorophenyl)-4-((2-methoxyethoxy)methoxy)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidine (**5**) (6 g). LC-MS: 369.2 $[M+H]^+$.

Synthesis of (2,6-difluoro-4-(4-((2-methoxyethoxy)methoxy)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid



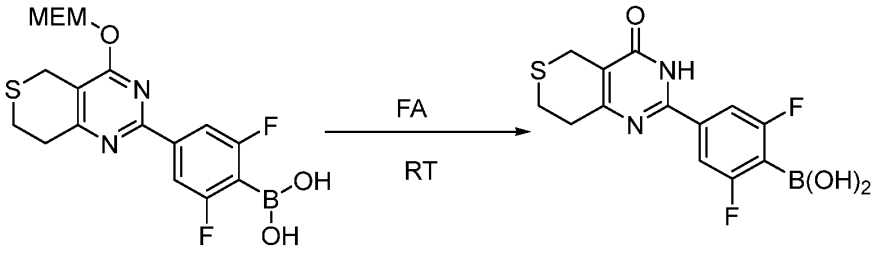
[00402] *Scheme 14D*



5 **6**

[00403] To a mixture of 2-(3,5-difluorophenyl)-4-((2-methoxyethoxy)methoxy)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidine (**5**) (400 mg, 1.4 mmol, 1.0 eq) in dry THF (10 mL) cooled at -78°C was added lithium diisopropylamide (LDA) (2 M, 1.1 mL, 1.5 eq) dropwise; the mixture was stirred at -78°C for 1 h. Trimethyl borate was added (191 mg, 1.9 mmol, 1.3 eq) and then the mixture was stirred at -78°C for 3 h. The mixture was quenched with NH_4Cl solution (30 mL) and extracted with EtOAc (2x20 mL). The combined organic layer was washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by reverse-phase chromatography (0-50% acetonitrile/0.05% formic acid) to afford (2,6-difluoro-4-(4-((2-methoxyethoxy)methoxy)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**6**) (200 mg). LC-MS: 413.4 $[M+H]^+$.

[00404] *Scheme 14E*

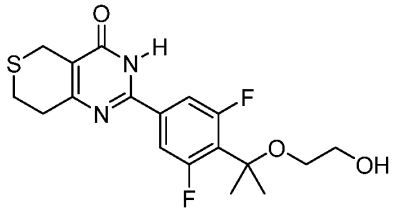


6 **Compound 14**

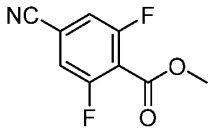
[00405] A mixture of (2,6-difluoro-4-(4-((2-methoxyethoxy)methoxy)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**6**) (200 mg, 0.48 mmol, 1.0 eq) in formaldehyde (FA) (3 mL) was stirred at room temperature (RT) for 2 h. It was then concentrated and purified by Prep-HPLC to afford (2,6-difluoro-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**Compound 14**). LC-MS 325.1 $[M+H]^+$. ^1H NMR (400MHz, DMSO-d_6): δ 12.85 (s, 1H), 8.86 (s, 2H), 7.72 (d, J = 7.2 Hz, 2H), 3.54 (s, 2H), 2.90 (s, 4H).

Compound 15

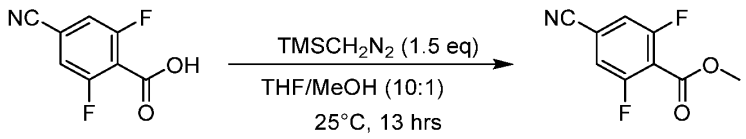
Synthesis of 2-(3,5-difluoro-4-(2-(2-hydroxyethoxy)propan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 15)



Synthesis of methyl 4-cyano-2,6-difluorobenzoate



[00406] *Scheme 15A*

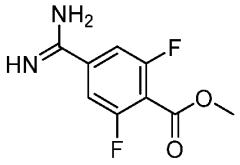


1 **2**

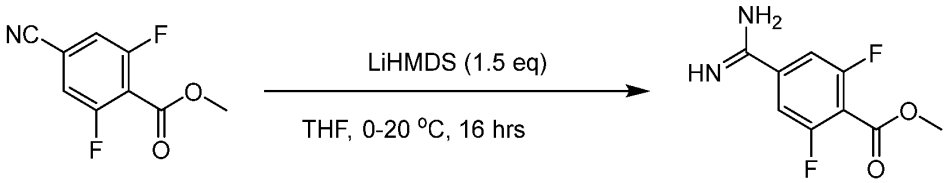
[00407] To a solution of 4-cyano-2,6-difluorobenzoic acid (**1**) (5.00 g, 27.31 mmol, 1.0 eq) in THF, (50 mL)/MeOH (50 mL) $TMSCHN_2$ (2 M, 20.48 mL, 1.5 eq) was added dropwise at

25°C. The mixture was stirred at 25°C for 13 h. TLC indicated (petroleum ether/ethyl acetate = 5/1, R_f(Cpd.1) = 0.33) (**1**) was consumed completely. The reaction mixture was concentrated under reduced pressure to give a residue. Methyl 4-cyano-2,6-difluorobenzoate (**2**) (4.1 g, crude) was obtained.

Synthesis of methyl 4-carbamimidoyl-2,6-difluorobenzoate



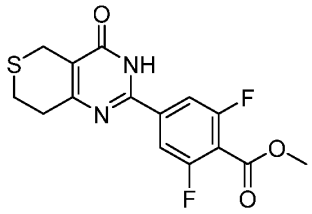
[00408] *Scheme 15B*



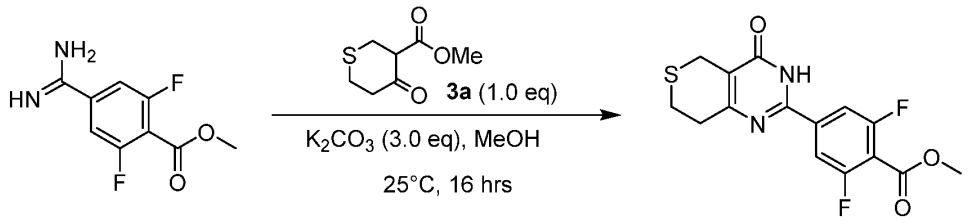
2 **3**

[00409] To a solution of methyl 4-cyano-2,6-difluorobenzoate (**2**) (2.00 g, 10.15 mmol, 1.0 eq) in THF (20 mL) was added LiHMDS (1 M, 15.22 mL, 1.5 eq) at 0°C. The mixture was stirred at 20°C for 16 h. LC-MS (ET68120-10-P1A1) showed that (**2**) was consumed completely, and the desired mass was detected. The reaction mixture was quenched by addition of HCl/dioxane (4 mol/L, 8 mL) at 0°C, and then concentrated under reduced pressure to give a residue. The residue was triturated with MeOH (40 mL) and the mixture was stirred at 20°C for 1 h. Then the mixture was filtered, and the filter cake was dried under reduced pressure. Methyl 4-carbamimidoyl-2,6-difluorobenzoate (**3**) (2.6 g, crude, HCl) was used directly in the next step without further purification.

Synthesis of methyl 2,6-difluoro-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate



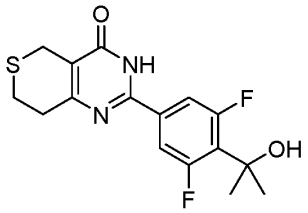
[00410] *Scheme 15C*



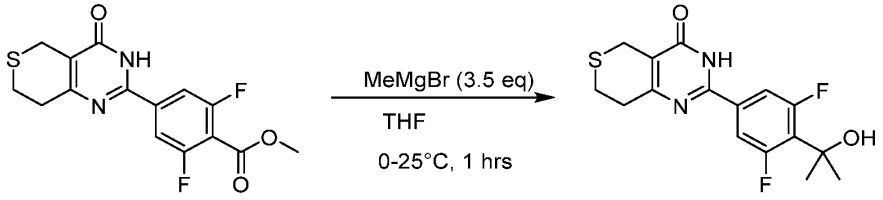
3 4

[00411] To a solution of methyl 4-carbamimidoyl-2,6-difluorobenzoate (**3**) (470 mg, 1.88 mmol, 1.0 eq, HCl) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**3a**) (327 mg, 1.88 mmol, 1.0 eq) in MeOH (4.7 mL) was added K_2CO_3 (778 mg, 5.63 mmol, 3.0 eq) at 25°C. The mixture was stirred at 25°C for 16 h. LC-MS showed that (**3**) was consumed completely, and one peak with the desired mass was detected. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (neutral condition; column: Waters Xbridge Prep OBD C18 150 × 40 mm × 10 μm; mobile phase: [water (NH_4HCO_3) - ACN]; B%: 10% - 40%, 8 mins). Methyl 2,6-difluoro-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**4**) (180 mg) was obtained.

Synthesis of 2-(3,5-difluoro-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



[00412] *Scheme 15D*

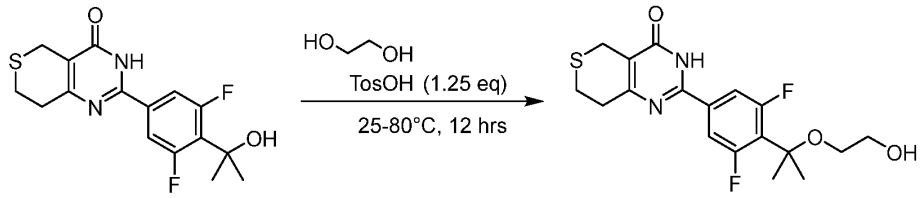


4 5

[00413] To a solution of methyl 2,6-difluoro-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**4**) (300 mg, 887 μmol, 1.0 eq) in THF (5.0 mL) was added MeMgBr (3 M, 1.03 mL, 3.5 eq) at 0°C. The mixture was stirred at 25°C for 1 h.

TLC (petroleum ether/ethyl acetate =1/1, R_f (Cpd.4) = 0.60) indicated that (**4**) was consumed completely, and two new spots formed. LC-MS (ET65158-9-P1A1) showed that (**4**) was consumed completely and one main peak with the desired MS was detected. The reaction mixture was quenched by the addition of NH_4Cl (15 ml) at 0°C, and then extracted with 45 mL of EtOAc (15 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by Prep-TLC (petroleum ether/ethyl acetate = 1/1). 2-(3,5-difluoro-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (150 mg, crude) was obtained and used immediately in the next step. LC-MS (ET65158-9-P1A1, product: R_t = 0.575 mins).

[00414] *Scheme 15E*

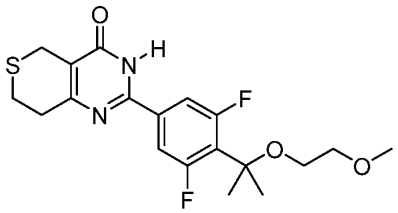


5 **Compound 15**

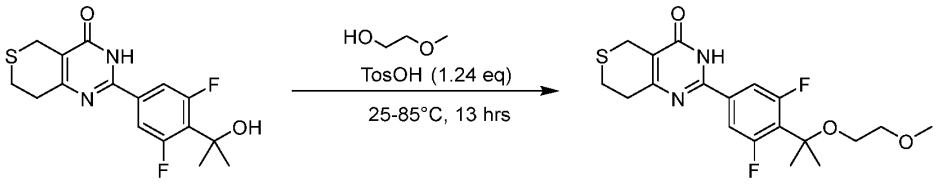
[00415] To a solution of 2-(3,5-difluoro-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (20.0 mg, 59.11 μmol, 1.0 eq) in ethylene glycol (1.59 g, 25.62 mmol, 1.43 mL, 433.41 eq) was added TosOH (12.7 mg, 73.88 μmol, 1.25 eq) at 25°C. The mixture was stirred at 80°C for 12 h. TLC (petroleum ether/ethyl acetate = 0/1, R_f(Cpd.5) = 0.53) indicated that (**5**) was consumed completely, and two new spots formed. After cooling to 25°C, the mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO_2, petroleum ether/ethyl acetate = 0/1) to provide 2-(3,5-difluoro-4-(2-(2-hydroxyethoxy)propan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 15**) was obtained. LC-MS: 383.1 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.78 - 7.70 (m, 2H), 4.49 - 4.45 (m, 1H) 3.47 - 3.43 (m, 4H), 3.21 - 3.17 (m, 2H), 2.85~2.75 (m, 4H), 1.637 (s, 6H).

Compound 16

Synthesis of 2-(3,5-difluoro-4-(2-(2-methoxyethoxy)propan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 16)



[00416] *Scheme 16A*

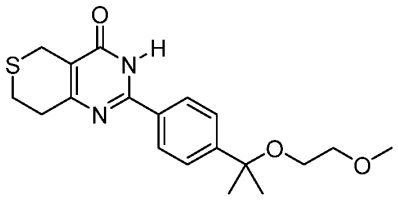


[00417] Synthesis of 2-(3,5-difluoro-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) is shown in Schemes 15A-15D.

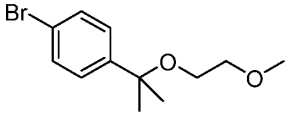
[00418] To a solution of 2-(3,5-difluoro-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (50.0 mg, 147 μmol, 1.0 eq) in 2-methoxyethanol (0.5 mL) was added TosOH (31.6 mg, 183 μmol, 1.24 eq) at 25°C and then heated to 85°C and stirred for 13 h. LC-MS showed ~17% of (**5**) remained. Several new peaks were shown on LC-MS, and the desired compound was detected. After cooling to 25°C, the reaction mixture was purified by prep-HPLC (neutral condition; column: Waters Xbridge BEH C18 100 × 30mm × 10um; mobile phase: [water (NH_4HCO_3) – can]; B%: 25%-55%, 8 mins) to provide 2-(3,5-difluoro-4-(2-(2-methoxyethoxy)propan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 16**). LC-MS: 397.1 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.81~7.75 (m, 2H), 3.549 (s, 2H) 3.53~3.38 (m, 2H), 3.35~3.25 (m, 2H), 3.22 (s, 3H), 2.906 (m, 4H), 1.653 (s, 6H).

Compound 17

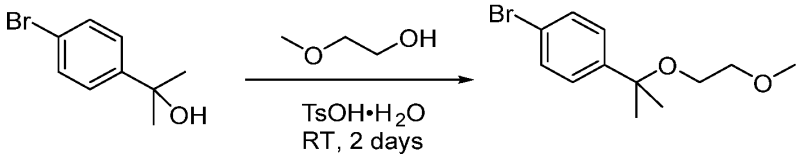
Synthesis of 2-(4-(2-(2-methoxyethoxy)propan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 17)



Synthesis of 1-bromo-4-(2-(2-methoxyethoxy)propan-2-yl)benzene



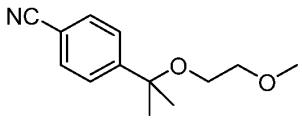
[00419] *Scheme 17A*



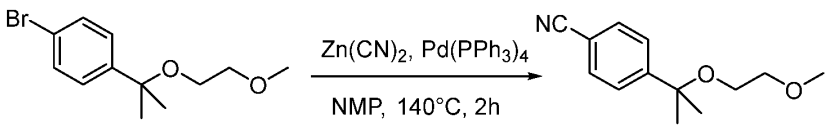
1 **2**

[00420] To solution of 2-(4-bromophenyl)propan-2-ol (**1**) (2.6 g, 12.2 mmol, 1.0 eq) in 2-methoxyethan-1-ol (20 mL) was added TsOH·H_2O (2.3 g, 12.2 mmol, 1 eq). The mixture was stirred at room temperature (RT) for 2 days, then quenched with water (100 mL) and extracted with EtOAc (3 × 60 mL). The organic layers were concentrated and the residue was purified by column chromatography on silica gel eluted with 0-30% EtOAc/hexane to afford 1-bromo-4-(2-(2-methoxyethoxy)propan-2-yl)benzene (**2**) (2.7 g). ^{1}H NMR (400MHz, DMSO-d_6): δ 7.54-7.51 (m, 2H), 7.38-7.35 (m, 2H), 3.42-3.40 (m, 2H), 3.24-3.22 (m, 5H), 1.44 (s, 6H).

Synthesis of 4-(2-(2-methoxyethoxy)propan-2-yl)benzonitrile



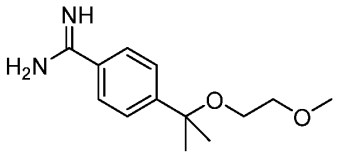
[00421] *Scheme 17B*



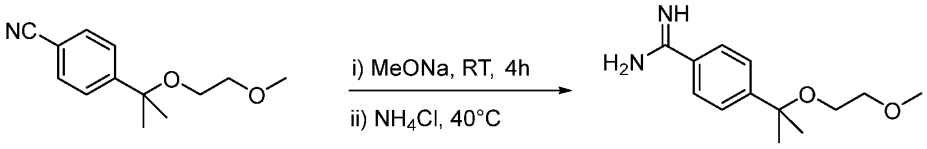
2 **3**

[00422] To a solution of 1-bromo-4-(2-(2-methoxyethoxy)propan-2-yl)benzene (**2**) (1.5 g, 3.7 mmol, 1 eq) in NMP (10 mL) was added $Zn(CN)_2$ (260 mg, 2.2 mmol, 0.6 eq) and $Pd(PPh_3)_4$ (430 mg, 0.37 mmol, 0.1 eq). The resulting solution was stirred for 2 h at 140°C under Ar. The resulting mixture was cooled to RT, diluted with water (50 mL), and extracted with EtOAc (3x60 mL). The organic layers were combined, washed with brine (100 mL), dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel eluted with 0-40% EtOAc/hexane to give 4-(2-(2-methoxyethoxy)propan-2-yl)benzonitrile (**3**) (600 mg). ^{1}H NMR (400MHz, DMSO-d_6): δ 7.81 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 3.44 (t, J = 5.2 Hz, 2H), 3.28-3.25 (m, 5H), 1.47 (s, 6H).

Synthesis of 4-(2-(2-methoxyethoxy)propan-2-yl)benzimidamide



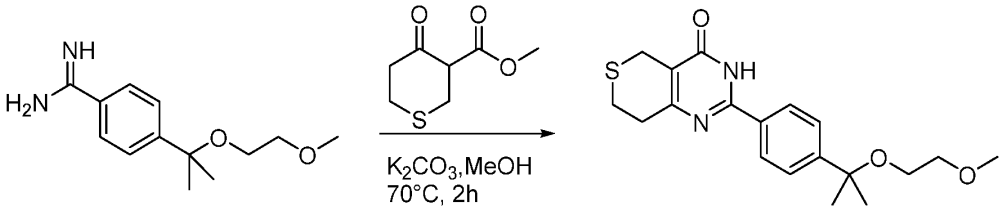
[00423] *Scheme 17C*



3 **4**

[00424] To a solution of 4-(2-(2-methoxyethoxy)propan-2-yl)benzonitrile (**3**) (0.6 g, 2.7 mmol, 1.0 eq) in MeOH (5 mL) was added MeONa (295 mg, 5.5 mmol, 2.0 eq); the mixture was stirred at RT for 4 h. NH_4Cl (287 mg, 5.5 mmol, 2.0 eq) was added and then the mixture was stirred at 40°C for 16 h. The mixture was cooled to RT, filtered, and then the filtrate was concentrated to give 4-(2-(2-methoxyethoxy)propan-2-yl)benzimidamide (**4**) (285 mg, crude). LC-MS: 237.2 $[M+H]^+$.

[00425] *Scheme 17D*

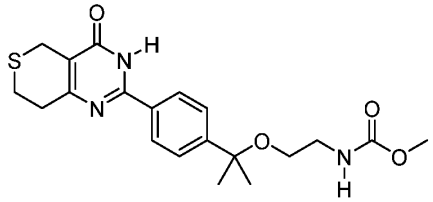


4 **Compound 17**

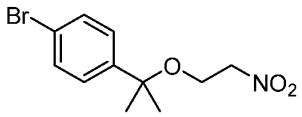
[00426] To a solution of 4-(2-(2-methoxyethoxy)propan-2-yl)benzimidamide (**4**) (150 mg, 0.7 mmol, 1.0 eq) in MeOH (5 mL) was added K_2CO_3 (180 mg, 1.3 mmol, 2.0 eq) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (80 mg, 0.5 mmol, 0.7 eq) under N_2. The mixture was stirred at 70°C for 2 h. It was then concentrated and purified by Prep-HPLC to give 2-(4-(2-(2-methoxyethoxy)propan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 17**). LC-MS: 361.1 $[M+H]^+$. ^1H NMR (400MHz, DMSO-d_6): δ 12.70 (s, 1H), 8.06 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.6 Hz, 2H), 3.52 (s, 2H), 3.46-3.41 (m, 2H), 3.25 (d, J = 5.0 Hz, 5H), 2.88 (dd, J = 7.6, 4.0 Hz, 4H), 1.48 (s, 6H).

Compound 18

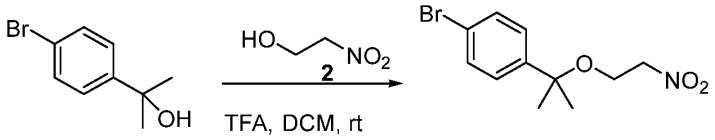
Synthesis of methyl (2-((2-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (Compound 18)



Synthesis of 1-bromo-4-(2-(2-nitroethoxy)propan-2-yl)benzene



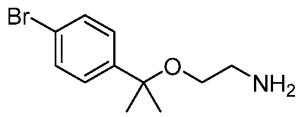
[00427] *Scheme 18A*



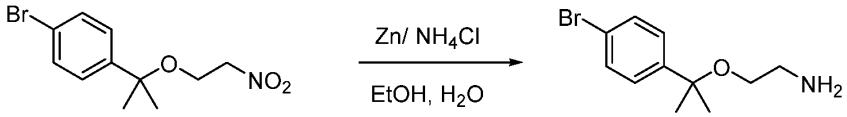
1 **3**

[00428] To a solution of 2-(4-bromophenyl)propan-2-ol **(1)** (2.6 g, 12.2 mmol, 1.0 eq) in DCM (20 mL) was added TFA (2.3 g, 12.2 mmol, 1.0 eq) and 2-nitroethan-1-ol (**2**) (10 mL). The mixture was stirred at RT for 3 days. The reaction was quenched with water (100 mL) and extracted with EtOAc (3 × 60 mL). The organic layers were concentrated, and the residue was purified by column chromatography on silica gel eluted with 0-30% EtOAc/hexane to afford 1-bromo-4-(2-(2-nitroethoxy)propan-2-yl)benzene (**3**) (2.7 g). ^1H NMR (400 MHz, CDCl3): δ 7.80 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 4.55 (t, J = 4.8 Hz, 2H), 3.69 (t, J = 4.8 Hz, 2H), 1.51 (s, 6H).

Synthesis of 2-((2-(4-bromophenyl)propan-2-yl)oxy)ethan-1-amine



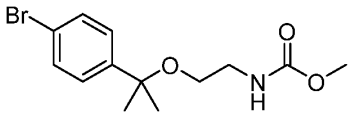
[00429] *Scheme 18B*



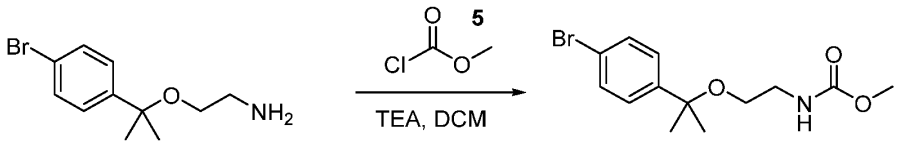
3 **4**

[00430] To a solution of 1-bromo-4-(2-(2-nitroethoxy)propan-2-yl)benzene (**3**) (1.5 g, 3.7 mmol, 1.0 eq) in EtOH/H_2O (10 mL/2 mL) was added Zn (1.2 g, 18.5 mmol, 5.0 eq) and NH_4Cl (1.0 g, 18.5 mmol, 5.0 eq). The resulting mixture was stirred for 12 h at 80°C under Ar. The reaction mixture was cooled to RT, diluted with water (50 mL), and extracted with EtOAc (3 × 60 mL). The organic layers were combined, washed with brine (100 mL), dried over anhydrous sodium sulfate, and concentrated to give 2-((2-(4-bromophenyl)propan-2-yl)oxy)ethan-1-amine (**4**) (1.2 g, crude) LC-MS: 258.0, 260.0 $[M+H]^+$.

Synthesis of methyl (2-((2-(4-bromophenyl)propan-2-yl)oxy)ethyl)carbamate



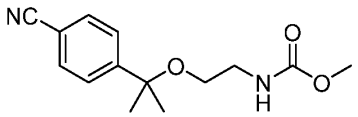
[00431] *Scheme 18C*



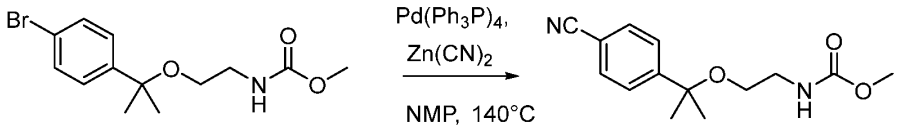
4 **6**

[00432] To a solution of 2-((2-(4-bromophenyl)propan-2-yl)oxy)ethan-1-amine (**4**) (1.2 g, 4.7 mmol, 1.0 eq) in DCM (10 mL) was added TEA (707 mg, 7.0 mmol, 1.5 eq), followed by the addition of methyl carbonochloridate (**5**) (395 mg, 4.2 mmol, 0.9 eq). The mixture was stirred at RT for one day. The reaction was concentrated and the residue was purified by column chromatography on silica gel eluted with 0-30% EtOAc/hexane to afford methyl (2-((2-(4-bromophenyl)propan-2-yl)oxy)ethyl)carbamate (**6**) (1.0 g). LC-MS: 357.0, 359.0 $[M+H+MeCN]^+$.

Synthesis of methyl (2-((2-(4-cyanophenyl)propan-2-yl)oxy)ethyl)carbamate



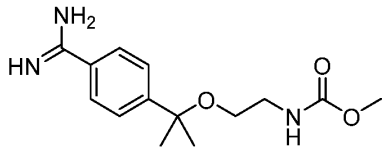
[00433] *Scheme 18D*



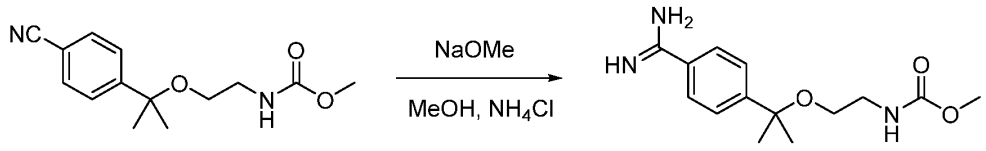
6 **7**

[00434] To a solution of methyl (2-((2-(4-bromophenyl)propan-2-yl)oxy)ethyl)carbamate (**6**) (1.0 g, 3.7 mmol, 1 eq) in NMP (10 mL) was added $Zn(CN)_2$ (260 mg, 2.2 mmol, 0.6 eq), followed by $Pd(PPh_3)_4$ (430 mg, 0.37 mmol, 0.1 eq). The resulting solution was stirred for 2 h at 140°C under Ar. The resulting mixture was cooled to RT, diluted with water (50 mL), and extracted with EtOAc (3 × 20 mL). The organic layers were combined, washed with brine (100mL), dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel eluted with 0-40% EtOAc/hexane to give methyl (2-((2-(4-cyanophenyl)propan-2-yl)oxy)ethyl)carbamate (**7**) (600 mg). ^{1}H NMR (400 MHz, DMSO): δ 7.86 – 7.76 (m, 2H), 7.64 – 7.58 (m, 2H), 7.25 – 7.11 (m, 1H), 3.33 (s, 3H), 3.15 – 3.04 (m, 4H), 1.46 (s, 6H).

Synthesis of methyl (2-((2-(4-carbamimidoylphenyl)propan-2-yl)oxy)ethyl)carbamate



[00435] *Scheme 18E*

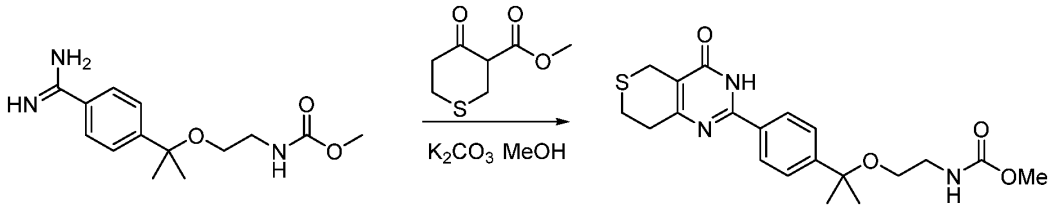


7 **8**

[00436] To a solution of methyl (2-((2-(4-cyanophenyl)propan-2-yl)oxy)ethyl)carbamate (**7**) (0.6 g, 2.7 mmol, 1.0 eq) in MeOH (5 mL) was added MeONa (295 mg, 5.5 mmol, 2.0 eq); the mixture was stirred at RT for 4 h. NH_4Cl (287 mg, 5.5 mmol, 2.0 eq) was added and the mixture was stirred at 40°C for 16 h. The mixture was cooled to RT, filtered, and the filtrate

was concentrated to give methyl (2-((2-(4-carbamimidoylphenyl)propan-2-yl)oxy)ethyl)carbamate (**8**) (285 mg, crude). LC-MS: 280.2 $[M+H]^+$.

[00437] *Scheme 18F*

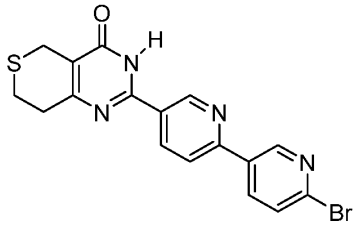


8 **Compound 18**

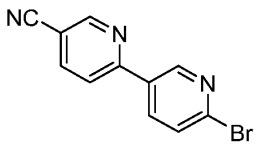
[00438] To a solution of methyl (2-((2-(4-carbamimidoylphenyl)propan-2-yl)oxy)ethyl)carbamate (**8**) (285 mg, 0.7 mmol, 1.0 eq) in MeOH (5 mL) was added K_2CO_3 (180 mg, 1.3 mmol, 2.0 eq) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (80 mg, 0.5 mmol, 0.7 eq) under N_2. The mixture was stirred at 70°C for 2 h, and then concentrated and purified by Prep-HPLC to give methyl (2-((2-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**Compound 18**). LC-MS: 404.2 $[M+H]^+$. ^1H NMR (400 MHz, DMSO): δ 12.71 (s, 1H), 8.05 (d, J = 8.2 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.16 (s, 1H), 3.51 (m, 5H), 3.12 (dd, J = 12.5, 5.0 Hz, 4H), 2.89 (d, J = 4.0 Hz, 4H), 1.47 (s, 6H).

Compound 19

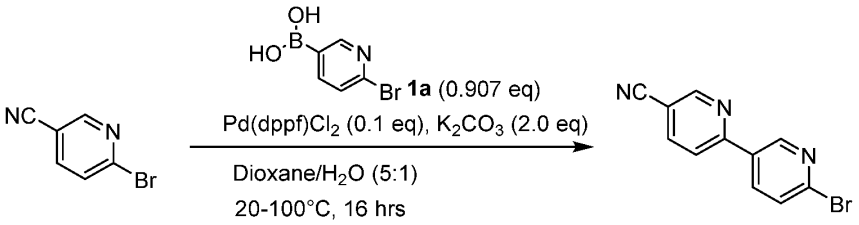
Synthesis of 2-(6'-bromo-[2,3'-bipyridin]-5-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 19)



Synthesis of 6'-bromo-[2,3'-bipyridine]-5-carbonitrile



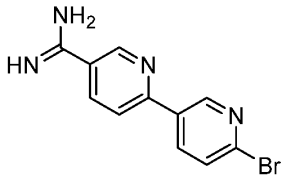
[00439] *Scheme 19A*



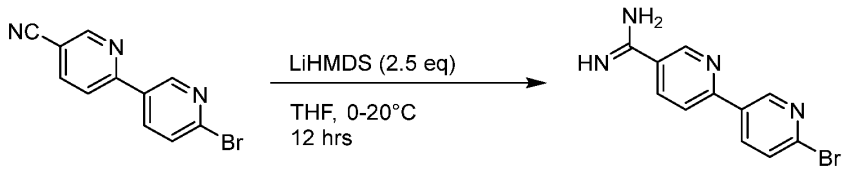
1 2

[00440] To a solution of 6-bromonicotinonitrile (**1**) (2.00 g, 10.93 mmol, 1.0 eq) and (6-bromopyridin-3-yl)boronic acid (**1a**) (2.00 g, 9.91 mmol, 9.07e-1 eq) in dioxane (20 mL)/H_2O (4 mL) was added K_2CO_3 (3.02 g, 21.86 mmol, 2.0 eq) and $Pd(dppf)Cl_2$ (799.65 mg, 1.09 mmol, 0.1 eq) at 20°C under N_2. The mixture was stirred at 100°C for 16 h under N_2. After cooling to 25°C, the mixture was filtered and then the filtrate was concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50/1 to 0/1). 6'-bromo-[2,3'-bipyridine]-5-carbonitrile (**2**) (730 mg) was obtained. ^1H NMR: (400 MHz, $CDCl_3$): δ = 8.92 (dd, J = 1.8, 12.3 Hz, 2H), 8.19 (dd, J = 2.5, 8.3 Hz, 1H), 8.00 (dd, J = 2.0, 8.3 Hz, 1H), 7.81 (d, J = 8.3 Hz, 1H), 7.58 (d, J = 8.3 Hz, 1H).

Synthesis of 6'-bromo-[2,3'-bipyridine]-5-carboximidamide



[00441] *Scheme 19B*

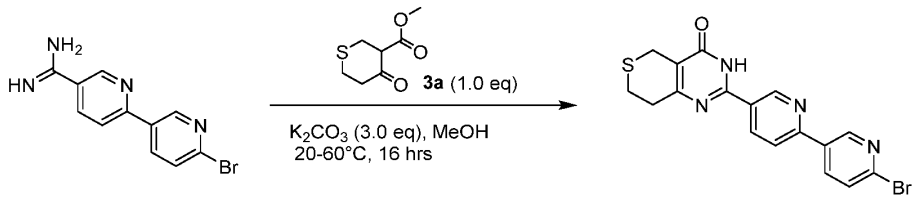


2 3

[00442] To a solution of 6'-bromo-[2,3'-bipyridine]-5-carbonitrile (**2**) (280 mg, 1.08 mmol, 1.0 eq) in THF (3 mL) was added lithium bis(trimethylsilyl)amide (LiHMDS) (1 M, 2.69 mL, 2.5 eq) at 0°C under N_2. The mixture was warmed to 20°C and stirred at 20°C for 12 h. The reaction mixture was quenched by addition of HCl/dioxane (4 mol/L, 1 mL) at 0°C and then concentrated under reduced pressure to give a residue. The residue was suspended in MeOH

(7 mL), and the mixture stirred at 20°C for 1 hr. Then the mixture was filtered, and the filter cake was dried under reduced pressure to give a 6'-bromo-[2,3'-bipyridine]-5-carboximidamide (**3**) (350 mg, crude, HCl) and used immediately in the next step.

[00443] *Scheme 19C*

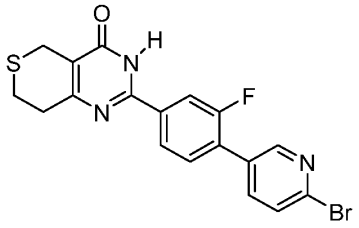


3 **Compound 19**

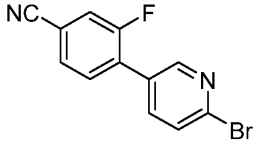
[00444] To a solution of 6'-bromo-[2,3'-bipyridine]-5-carboximidamide (**3**) (350 mg, 1.12 mmol, 1.0 eq, HCl) in MeOH (3.5 mL) was added methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**3a**) (194.45 mg, 1.12 mmol, 1.0 eq) and K_2CO_3 (462.79 mg, 3.35 mmol, 3.0 eq) at 20°C. The mixture was stirred at 60°C for 16 h. The mixture was filtered, and concentrated under reduced pressure to give a residue that was purified by prep-HPLC (neutral condition; column: Phenomenex C18 75 * 30mm * 3 μm; mobile phase: [water (NH_4HCO_3)-ACN]; B%: 25%-40%, 10 mins). 2-(6'-bromo-[2,3'-bipyridin]-5-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 19**) was obtained. LC-MS: 401 (M+1). ^{1}H NMR: (400 MHz, DMSO-d_6): δ = 9.27 (d, J = 1.3 Hz, 1H), 8.94 (d, J = 2.2 Hz, 1H), 8.43 (dd, J = 1.9, 8.3 Hz, 1H), 8.27 (dd, J = 2.3, 8.4 Hz, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 3.28 (s, 2H), 2.61 (br dd, J = 5.0, 15.4 Hz, 4H).

Compound 20

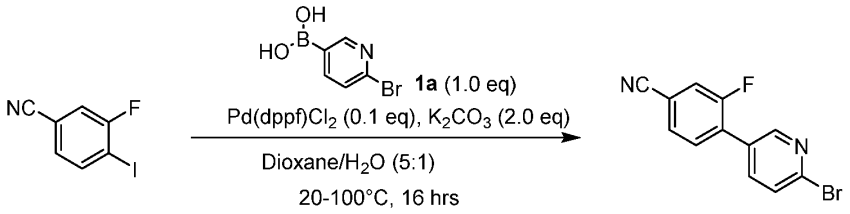
Synthesis of 2-(4-(6-bromopyridin-3-yl)-3-fluorophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 20)



Synthesis of 4-(6-bromopyridin-3-yl)-3-fluorobenzonitrile



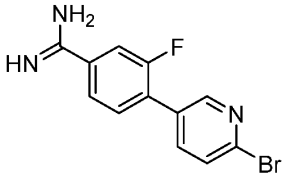
[00445] *Scheme 20A*



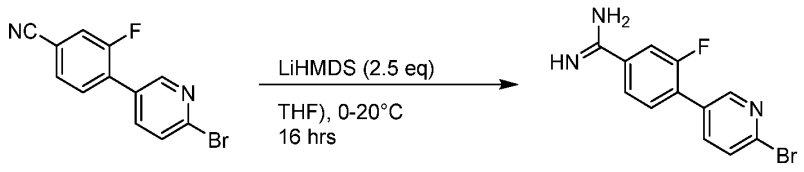
1 2

[00446] To a solution of (6-bromopyridin-3-yl)boronic acid (**1a**) (408.52 mg, 2.02 mmol, 1.0 *eq*) in dioxane (5 mL)/H_2O (1 mL) was added 3-fluoro-4-iodobenzonitrile (**1**) (0.5 g, 2.02 mmol, 1.0 *eq*), K_2CO_3 (559.52 mg, 4.05 mmol, 2.0 *eq*), and Pd(dppf)Cl_2 (148.11 mg, 202.42 μmol, 0.1 *eq*) at 20 °C under N_2. The mixture was stirred at 100 °C for 16 h under N_2. After cooling to 20 °C, the mixture was filtered, and concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 20/1 to 0/1). 4-(6-bromopyridin-3-yl)-3-fluorobenzonitrile (**2**) (200 mg, crude) was obtained and used as is in the next step. ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 8.48 (s, 1H), 7.68 (td, J = 1.9, 8.3 Hz, 1H), 7.56 (s, 1H), 7.50 (br d, J = 7.5 Hz, 2H), 7.47 - 7.45 (m, 1H).

Synthesis of 4-(6-bromopyridin-3-yl)-3-fluorobenzimidamide



[00447] *Scheme 20B*

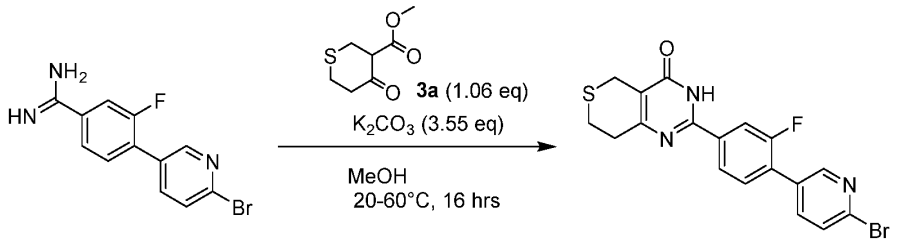


2 3

[00448] To a solution of 4-(6-bromopyridin-3-yl)-3-fluorobenzonitrile (**2**) (200 mg, 721.78 μmol, 1.0 eq) in THF (2 mL) was added LiHMDS (1 M, 1.80 mL, 2.5 eq) at 0 °C under N_2. The mixture was warmed to 20 °C and stirred at 20 °C for 16 h. The reaction mixture was quenched by addition of HCl/dioxane (4 mol/L, 0.6 mL) at 0 °C and then concentrated under reduced pressure to give a residue that was suspended in MeOH (3 ml), and the mixture stirred at 20°C for 1 h. Then the mixture was filtered, and the filter cake was dried under reduced

pressure to give 4-(6-bromopyridin-3-yl)-3-fluorobenzimidamide (**3**) (0.11 g, crude) that was used in the next step without further purification.

[00449] *Scheme 20C*

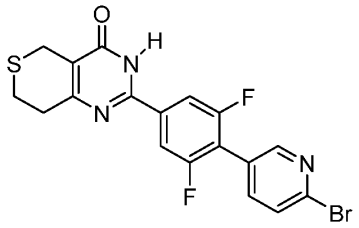


3 **Compound 20**

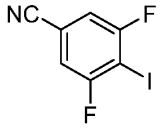
[00450] To a solution of methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**3a**) (68.75 mg, 394.62 μmol, 1.06 eq) and 4-(6-bromopyridin-3-yl)-3-fluorobenzimidamide (**3**) (0.11 g, 373.99 μmol, 1.0 eq) in MeOH (2 mL) was added K_2CO_3 (183.33 mg, 1.33 mmol, 3.55 eq) at 20°C. The mixture was stirred at 60°C for 16 h. After cooling to 20°C, the mixture was filtered, and then the filtrate was concentrated under reduced pressure to give a residue that was purified by prep-HPLC (neutral condition; column: Waters Xbridge Prep OBD C18 150 * 40 mm * 10 μm; mobile phase: [water (NH_3H_2O+NH_4HCO_3)-ACN]; B%: 30%-60%, 8 mins). 2-(4-(6-bromopyridin-3-yl)-3-fluorophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 20**) was obtained. LC-MS: 417 + bromo isomers (M+1). ^{1}H NMR: (400 MHz, DMSO-d_6): δ 8.66 (s, 1H), 8.13 - 8.06 (m, 2H), 8.06 - 8.00 (m, 1H), 7.86 - 7.77 (m, 2H), 3.55 (s, 2H), 2.91 (s, 4H).

Compound 21

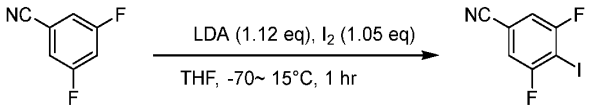
Synthesis of 2-(4-(6-bromopyridin-3-yl)-3,5-difluorophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 21)



Synthesis of 3,5-difluoro-4-iodobenzonitrile



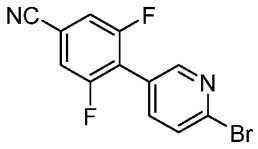
[00451] *Scheme 21A*



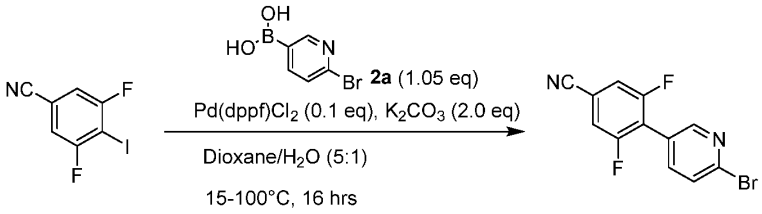
1 2

[00452] To a solution of 3,5-difluorobenzonitrile (**1**) (2.00 g, 14.07 mmol, 1.0 eq) in THF (20 mL), lithium diisopropylamide (LDA) (2 M, 7.86 mL, 1.12 eq) was added dropwise at -70°C. Then I_2 (3.76 g, 14.80 mmol, 2.98 mL, 1.05 eq) in THF (10 mL) at -70°C was added at -70°C. The reaction mixture was slowly warmed to 15°C then stirred at 15°C for 1 hr. The mixture was quenched with 10% sodium thiosulfite solution (15 ml). The reaction mixture was extracted with a 1/1 mixture of ethyl acetate/hexanes (7 mL × 3). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by prep-HPLC (neutral condition; column: Welch Xtimate C18 250 * 70 mm # 10 μm; mobile phase: [water (NH_4HCO_3)-ACN]; B%: 40%-70%, 20 mins). 3,5-difluoro-4-iodobenzonitrile (**2**) (2.00 g) was obtained. ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 7.23 - 7.18 (m, 2H).

Synthesis of 4-(6-bromopyridin-3-yl)-3,5-difluorobenzonitrile



[00453] *Scheme 21B*

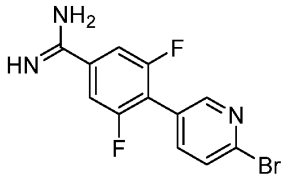


2 3

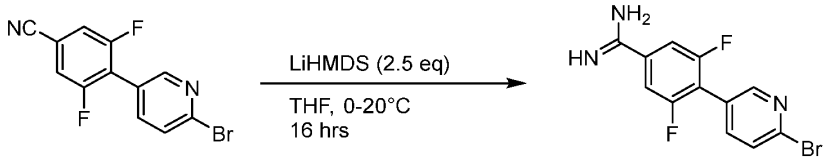
[00454] To a solution of (6-bromopyridin-3-yl)boronic acid (**2a**) (1.60 g, 7.93 mmol, 1.05 eq) and 3,5-difluoro-4-iodobenzonitrile (**2**) (2.00 g, 11.32 mmol, 1.0 eq) in dioxane (20 mL)/H_2O (4 mL) was added K_2CO_3 (2.09 g, 15.09 mmol, 2.0 eq) and $Pd(dppf)Cl_2$ (552.23 mg, 0.75 mmol, 0.1 eq) at 15°C under N_2. The mixture heated to 100°C for 16 h under N_2. After cooling to 15°C, the mixture was filtered, and then the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50/1 to 0/1). 4-(6-bromopyridin-3-yl)-3,5-difluorobenzonitrile

(**3**) (620 mg, 210 mmol) was obtained. ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 8.43 (s, 1H), 7.63 - 7.55 (m, 2H), 7.34 - 7.26 (m, 2H).

Synthesis of 4-(6-bromopyridin-3-yl)-3,5-difluorobenzimidamide



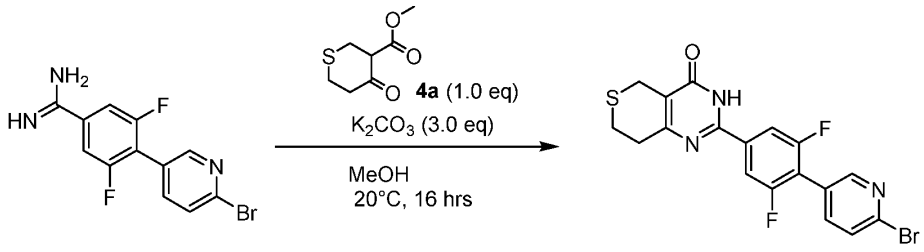
[00455] *Scheme 21C*



3 **4**

[00456] To a solution of 4-(6-bromopyridin-3-yl)-3,5-difluorobenzonitrile (**3**) (500 mg, 1.69 mmol, 1.0 eq) in THF (5 mL) was added LiHMDS (1 M, 4.24 mL, 2.5 eq) at 0°C under N_2. The mixture was warmed to 20°C and stirred for 16 h. The reaction mixture was quenched by addition of HCl/dioxane (4 mol/L, 2.0 mL) at 0°C, and then concentrated under reduced pressure to give a residue. The residue was suspended in MeOH (8 ml), and the mixture was stirred at 20°C for 1 hr. Then the mixture was filtered, and the filtrate was concentrated under reduced pressure to give 4-(6-bromopyridin-3-yl)-3,5-difluorobenzimidamide (**4**) (540 mg, crude, HCl). The compound was used immediately in the next step.

[00457] *Scheme 21D*



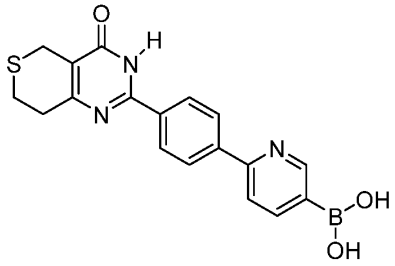
4 **Compound 21**

[00458] To a solution of 4-(6-bromopyridin-3-yl)-3,5-difluorobenzimidamide (**4**) (440 mg, 1.41 mmol, 1.0 eq) in MeOH (4.4 mL) was added methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**4a**) (245.60 mg, 1.41 mmol, 1.0 eq) and K_2CO_3 (584.52 mg, 4.23 mmol, 3.0 eq) at 20°C. The mixture was stirred at 20°C for 16 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by

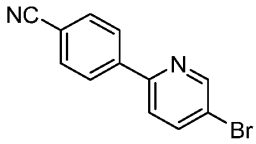
prep-HPLC (neutral condition; column: NP-1; mobile phase: [Heptane-EtOH]; B%: 10%-70%, 10 mins). 2-(4-(6-bromopyridin-3-yl)-3,5-difluorophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 21**) was obtained. LC-MS: 438 + bromo isomers (M+1). ^{1}H NMR: (400 MHz, DMSO-d_6): δ = 13.06 - 12.89 (m, 1H), 8.64 (s, 1H), 8.10 - 8.00 (m, 3H), 7.93 (d, J = 8.4 Hz, 1H), 3.62 (s, 2H), 2.98 (s, 4H).

Compound 22

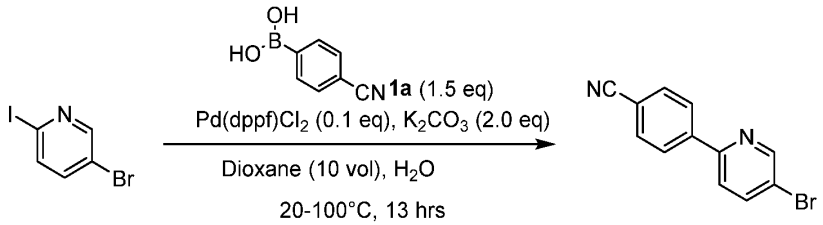
Synthesis of (6-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)pyridin-3-yl)boronic acid (Compound 22)



Synthesis of 4-(5-bromopyridin-2-yl)benzonitrile



[00459] Scheme *22A*

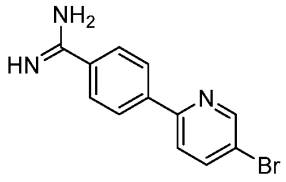


1 2

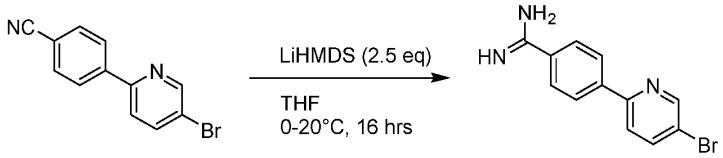
[00460] To a solution of 5-bromo-2-iodopyridine (**1**) (0.5 g, 1.76 mmol, 1.0 eq) and (4-cyanophenyl)boronic acid (**1a**) (388.19 mg, 2.64 mmol, 1.5 eq) in dioxane (5 mL)/H_2O (1 mL) was added Pd(dppf)Cl_2 (128.87 mg, 176.12 μmol, 0.1 eq) and K_2CO_3 (486.83 mg, 3.52 mmol, 2.0 eq) at 20°C under N_2. The mixture was stirred at 100°C for 13 h. After cooling to 20°C, the mixture was filtered, and then the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50/1 to 0/1). 4-(5-bromopyridin-2-yl)benzonitrile (**2**) (400 mg, crude) was obtained

and used immediately in the next step. ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 8.71 (d, J = 2.1 Hz, 1H), 8.06 - 8.00 (m, 2H), 7.87.

Synthesis of 4-(5-bromopyridin-2-yl)benzimidamide



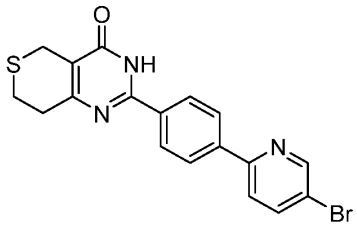
[00461] *Scheme 22B*



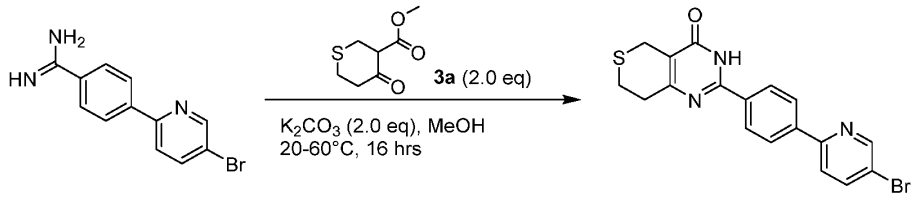
2 **3**

[00462] To a solution of 4-(5-bromopyridin-2-yl)benzonitrile (**2**) (100 mg, 385.95 µmol, 1.0 eq) in THF (1 mL) was added LiHMDS (1 M, 964.87 uL, 2.5 eq) at 0°C under N_2. The mixture was warmed to 20°C and stirred for 16 h. The reaction mixture was quenched by addition of HCl/dioxane (4 mol/L, 0.3 mL) at 0°C, and then concentrated under reduced pressure to give a residue. The residue was suspended in MeOH (2 ml), and the mixture was stirred at 20°C for 1 h. Then the mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. 4-(5-bromopyridin-2-yl)benzimidamide (**3**) (110 mg, crude, HCl salt) was obtained and used in the next step without further purification.

Synthesis of 2-(4-(5-bromopyridin-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one

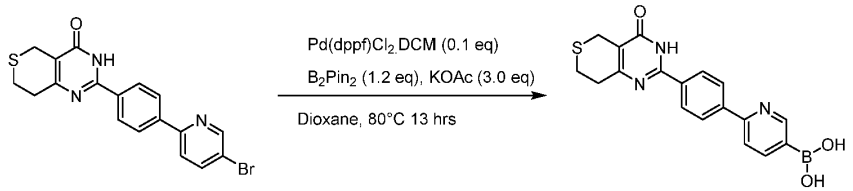


[00463] *Scheme 22C*



[00464] To a solution of 4-(5-bromopyridin-2-yl)benzimidamide (**3**) (100 mg, 362.15 µmol, 1.0 eq) in MeOH (1 mL) was added methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**3a**) (126.18 mg, 724.29 µmol, 2.0 eq) and K_2CO_3 (100.10 mg, 724.29 µmol, 2.0 eq) at 20°C. The mixture was stirred at 60°C for 16 h. The mixture was filtered, and the filter cake was dried under reduced pressure to give 2-(4-(5-bromopyridin-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (238 mg, crude) that was used immediately in the next step. ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 8.75 - 8.69 (m, 1H), 8.14 - 8.04 (m, 4H), 7.90 - 7.83 (m, 1H), 7.68 - 7.61 (m, 1H), 3.65 - 3.62 (m, 2H), 3.04 - 2.97 (m, 2H), 2.92 - 2.86 (m, 2H).

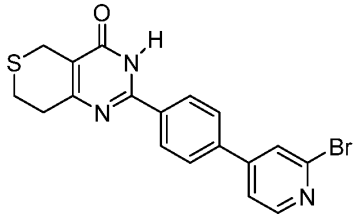
[00465] *Scheme 22D*



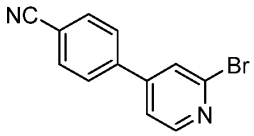
[00466] To a solution of 2-(4-(5-bromopyridin-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (238 mg, 594.57 µmol, 1.0 eq) in dioxane (3 mL) was added B_2Pin_2 (181.18 mg, 713.48 µmol, 1.2 eq), KOAc (175.06 mg, 1.78 mmol, 3.0 eq), and $Pd(dppf)Cl_2$.DCM (48.55 mg, 59.46 µmol, 0.1 eq) under N_2. The mixture was stirred at 80°C for 13 h. After cooling to 25°C, the mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (neutral condition; column: Waters Xbridge Prep OBD C18 150 * 40mm * 10µm; mobile phase: [water (NH_4HCO_3)-ACN]; B%: 15%-45%, 8 mins). (6-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)pyridin-3-yl)boronic acid (**Compound 22**) was obtained. LC-MS: 366.1 (M+1). ^{1}H NMR: (400 MHz, DMSO-d_6): δ = 12.83 (br s, 1H), 9.01 (s, 1H), 8.41 (s, 2H), 8.30 - 8.20 (m, 5H), 8.06 (d, J = 8.0 Hz, 1H), 3.55 (s, 2H), 2.92 (s, 4H).

Compound 23

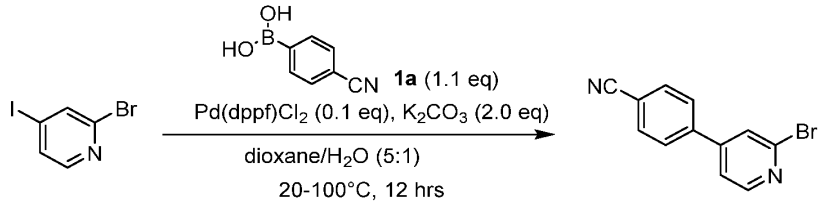
Synthesis of 2-(4-(2-bromopyridin-4-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 23)



Synthesis of 4-(2-bromopyridin-4-yl)benzonitrile



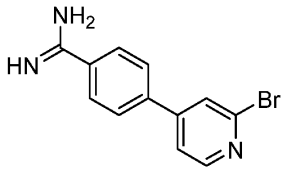
[00467] *Scheme 23A*



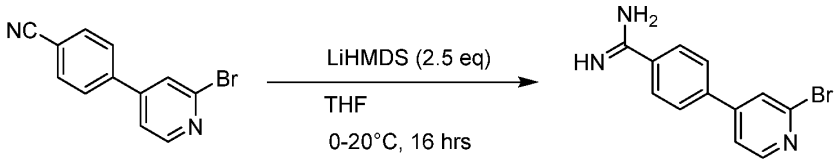
1 **2**

[00468] To a solution of 2-bromo-4-iodopyridine (**1**) (2.50 g, 8.81 mmol, 1.00 eq) and (4-cyanophenyl)boronic acid (**1a**) (1.42 g, 9.69 mmol, 1.10 eq) in dioxane (25.0 mL) and H_2O (5.00 mL) was added K_2CO_3 (2.43 g, 17.6 mmol, 2.00 eq) at 20°C. The suspension was degassed and purged three times with N_2. Then to the mixture was added $Pd(dppf)Cl_2$ (644 mg, 881 μmol, 0.10 eq) at 20°C under N_2. The suspension was degassed and purged three times with N_2. The mixture was stirred under N_2 at 100°C for 12 h. The reaction mixture was diluted with H_2O (10.0 mL) and extracted with EtOAc 60.0 mL (20.0 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 10/ 1 to 1/ 1). 4-(2-bromopyridin-4-yl)benzonitrile (**2**) (950 mg) was obtained. ^1H NMR: (400 MHz, DMSO-d6): δ = 8.57 (d, J = 5.3 Hz, 1H), 8.17 - 8.03 (m, 5H), 7.91 (dd, J = 1.7, 5.2 Hz, 1H).

Synthesis of 4-(2-bromopyridin-4-yl)benzimidamide



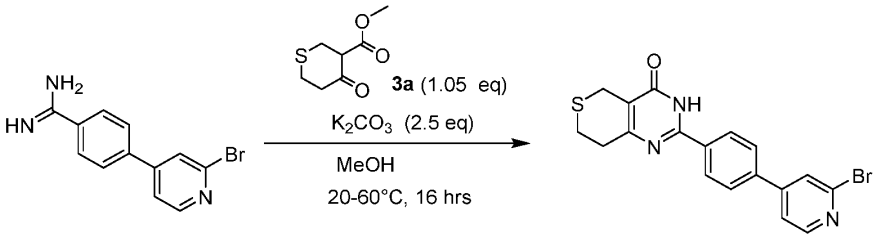
[00469] *Scheme 23B*



2 **3**

[00470] To a solution of 4-(2-bromopyridin-4-yl)benzonitrile (**2**) (400 mg, 1.54 mmol, 1.00 eq) in THF (4.00 mL), LiHMDS (1 M, 3.09 mL, 2.00 eq) was added dropwise at 0°C. The mixture was stirred at 20°C for 16 h. The reaction mixture was diluted with 4 N HCl (4.00 mL). The combined organic layers were concentrated under reduced pressure to give a residue. The residue was suspended in MeOH (20.0 mL) and stirred at 20°C for 1 hr. Then the mixture was filtered. The filtrate was concentrated under reduced pressure to give a residue. 4-(2-bromopyridin-4-yl)benzimidamide (**3**) (500 mg, crude) was obtained and used in the next step without further purification.

[00471] *Scheme 23C*



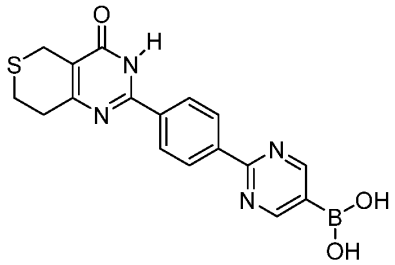
3 **Compound 23**

[00472] To a solution of 4-(2-bromopyridin-4-yl)benzimidamide (**3**) (250 mg, 905 μmol, 1.00 eq) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**3a**) (166 mg, 951 μmol, 1.05 eq) in MeOH (2.50 mL) was added K_2CO_3 (313 mg, 2.26 mmol, 2.50 eq) at 20°C. The mixture was stirred at 60°C for 16 h. The reaction mixture was filtered. The filter cake was slurried by MeOH (0.50 mL) and H_2O (1.00 mL) and then filtered. The filter cake was dried *in vacuo* to give a residue. The residue was purified by prep-HPLC (column: Phenomenex luna C18 80 * 40 mm * 3 μm; mobile phase: [water (HCl)-ACN]; B%: 35%-65%, 7 min). 2-(4-(2-

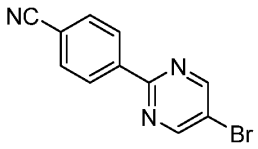
bromopyridin-4-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 23**) was obtained. ^{1}H NMR (400 MHz, DMSO-d6): δ = 8.48 (d, J = 5.3 Hz, 1H), 8.27 (d, J = 8.4 Hz, 2H), 8.07 (s, 1H), 7.99 (d, J = 8.4 Hz, 2H), 7.87 (dd, J = 1.2, 5.2 Hz, 1H), 3.54 (s, 2H), 2.89 (s, 4H). LC-MS: 404.1 (M+1).

Compound 24

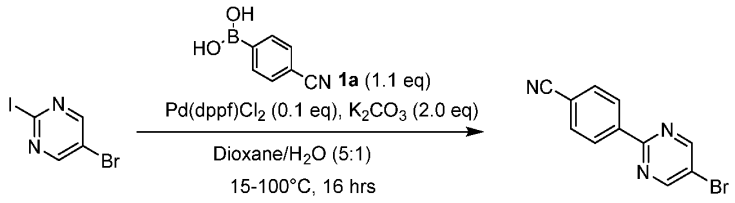
Synthesis of (2-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)pyrimidin-5-yl)boronic acid (Compound 24)



Synthesis of 4-(5-bromopyrimidin-2-yl)benzonitrile



[00473] *Scheme 24A*

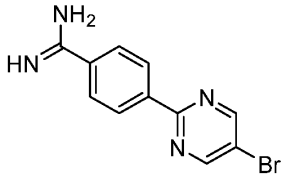


1 2

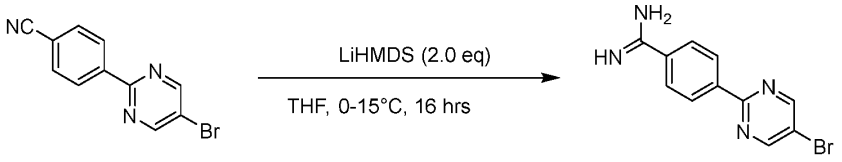
[00474] To a solution of 5-bromo-2-iodopyrimidine (**1**) (2.50 g, 8.78 mmol, 1.00 eq) and (4-cyanophenyl)boronic acid (**1a**) (1.42 g, 9.65 mmol, 1.10 eq) in dioxane (25.0 mL) and H_2O (5.00 mL) was added K_2CO_3 (2.43 g, 17.6 mmol, 2.00 eq) at 15°C. The suspension was degassed and purged three times with N_2. Then to the mixture was added Pd(dppf)Cl_2 (642 mg, 878 μmol, 0.10 eq) at 15°C under N_2. The suspension was degassed and purged three times with N_2. The mixture was stirred under N_2 at 100°C for 16 h. The reaction mixture was diluted with H_2O (10.0 mL) and filtered. The filtrate was extracted with EtOAc (10 mL × 2). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was suspended in MTBE (10.0 mL), stirred at 15°C for

16 h, then filtered. The filter cake was dried under reduced pressure to give a residue. 4-(5-bromopyrimidin-2-yl)benzonitrile (**2**) (2.00 g, crude) was obtained and used immediately in the next step. ^{1}H NMR (400 MHz, DMSO-d6): δ = 9.16 (s, 2H), 8.49 (br d, J = 8.3 Hz, 2H), 8.01 (br d, J = 8.3 Hz, 2H).

Synthesis of 4-(5-bromopyrimidin-2-yl)benzimidamide



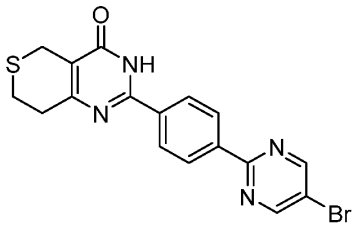
[00475] *Scheme 24B*



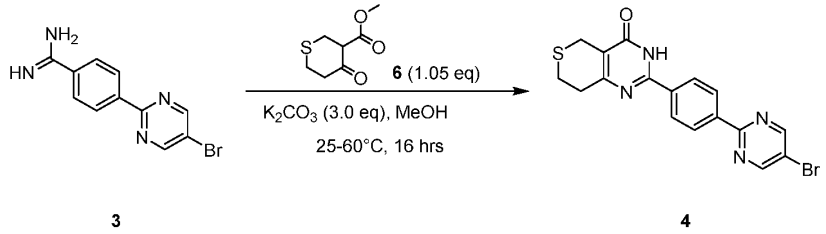
2 **3**

[00476] To a solution of 4-(5-bromopyrimidin-2-yl)benzonitrile (**2**) (0.80 g, 3.08 mmol, 1.00 eq) in THF (8.00 mL) was added LiHMDS (1 M, 6.15 mL, 2.00 eq) at 0 °C. The mixture was stirred at 15°C for 16 h. The reaction mixture was diluted with 4 N HCl/dioxane (6.00 mL). The combined organic layers were concentrated under reduced pressure to give a residue. The residue was suspended in MeOH (8.00 mL) and stirred at 15°C for 2 h, then filtered. The filtrate was concentrated under reduced pressure to give a residue. 4-(5-bromopyrimidin-2-yl)benzimidamide (**3**) (0.8 g, crude) was obtained which was used in the next step without further purification.

Synthesis of 2-(4-(5-bromopyrimidin-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



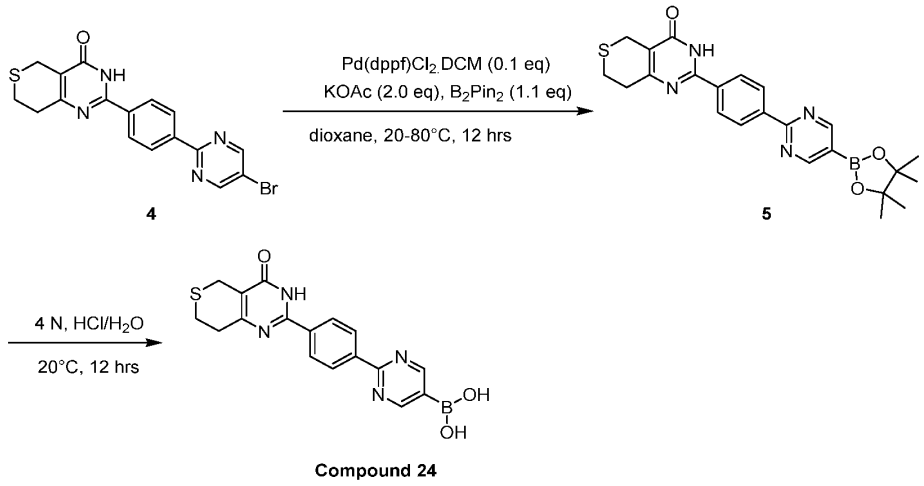
[00477] *Scheme 24C*



3 4

[00478] To a solution of 4-(5-bromopyrimidin-2-yl)benzimidamide (**3**) (0.80 g, 2.89 mmol, 1.00 eq) and methyl 4-oxotetrahydrothiopyran-3-carboxylate (**6**) (528 mg, 3.03 mmol, 1.05 eq) in MeOH (16.0 mL) was added K_2CO_3 (1.20 g, 8.66 mmol, 3.00 eq) at 25°C. The mixture was stirred at 60°C for 16 h. The reaction mixture was filtered. The filtrate was concentrated under reduced pressure. The resulting solid was suspended in water (5 mL) and stirred at 20°C for 3 h. Then the mixture was filtered, and the filter cake was dried *in vacuo* to give 2-(4-(5-bromopyrimidin-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (300 mg, crude). The crude product was used in the next step directly without further purification.

[00479] *Scheme 24D*



4 5

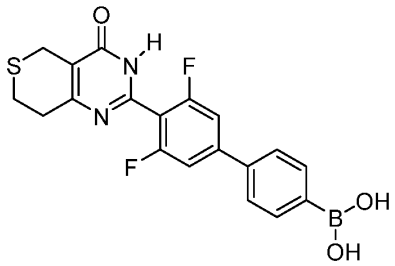
Compound 24

[00480] To a solution of 2-(4-(5-bromopyrimidin-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (0.1 g, 249.20 μmol, 1 eq) in dioxane (2 mL) was added 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (69.61 mg, 274.12 μmol, 1.1 eq), KOAc (48.91 mg, 498.41 μmol, 2 eq), and $Pd(dppf)Cl_2.CH_2Cl_2$ (20.35 mg, 24.92 μmol, 0.1 eq) at 20°C under N_2. The mixture was stirred at 80°C for 12 h under N_2. The mixture was concentrated under reduced pressure to give a residue. The residue was suspended in $MeCN/H_2O$ (1:1, 3 mL) and stirred at 20°C for 2 h.

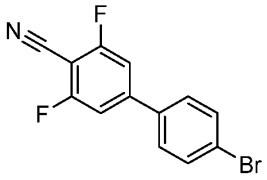
Then the mixture was filtered, and the filter cake was dried *in vacuo* to give 2-(4-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (50 mg, crude). The crude product was suspended in HCl/H_2O (4 N, 3 mL) and stirred at 20°C for 12 h. Then the mixture was diluted with DMSO (5 mL) and purified by prep-HPLC (column: Phenomenex luna C18 250 * 50 mm * 10 μm; mobile phase: [water (HCl)-ACN]; B%: 15%-45%, 10 min). (2-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)pyrimidin-5-yl)boronic acid (**Compound 24**) was obtained. LCMS: 367 (M+1). ^{1}H NMR: (400 MHz, DMSO-d6): δ = 9.16 (s, 2H), 8.54 (d, J = 8.5 Hz, 2H), 8.25 (d, J = 8.5 Hz, 2H), 3.55 (s, 2H), 2.92 (s, 4H).

Compound 25

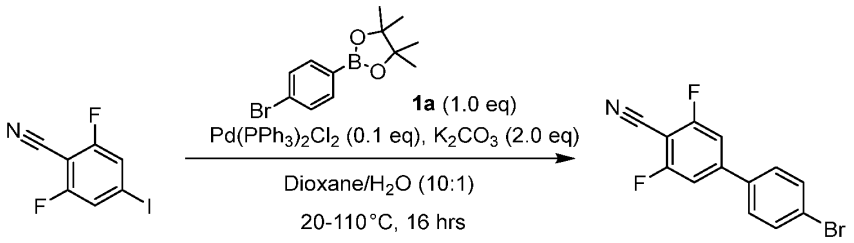
Synthesis of (3',5'-difluoro-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (Compound 25)



Synthesis of 4'-bromo-3,5-difluoro-[1,1'-biphenyl]-4-carbonitrile



[00481] *Scheme 25A*

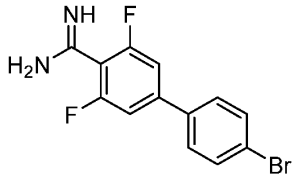


1 **2**

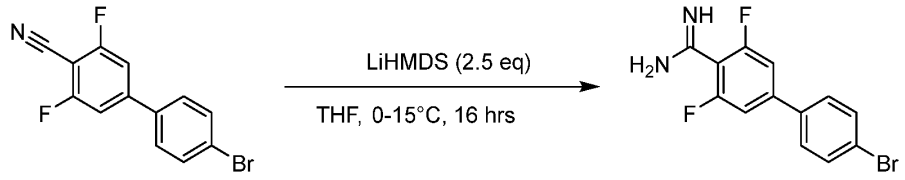
[00482] To a mixture of 2,6-difluoro-4-iodobenzonitrile (**1**) (5 g, 22.94 mmol, 1 eq) and 2-(4-bromophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**1a**) (6.49 g, 22.94 mmol, 1 eq) in dioxane (100 mL) and H_2O (10 mL) was added K_2CO_3 (6.34 g, 45.87 mmol, 2 eq) and

$Pd(PPh_3)_2Cl_2$ (1.61 g, 2.29 mmol, 0.1 eq) at 20°C under N_2. The mixture was degassed and purged three times with N_2, and then the mixture was stirred at 110°C for 16 h under an N_2 atmosphere. The mixture was cooled to 20°C and then diluted with water (100 mL). Then the mixture was extracted with EtOAc 300 mL (100 mL × 3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50/1 to 0/1). 4'-bromo-3,5-difluoro-[1,1'-biphenyl]-4-carbonitrile (**2**) (1.8g) was obtained. ^1H NMR: (400 MHz, $CDCl_3$): δ = 7.57 (d, J = 8.6 Hz, 2H), 7.39 - 7.33 (m, 2H), 7.20 - 7.17 (m, 2H).

Synthesis of 4'-bromo-3,5-difluoro-[1,1'-biphenyl]-4-carboximidamide



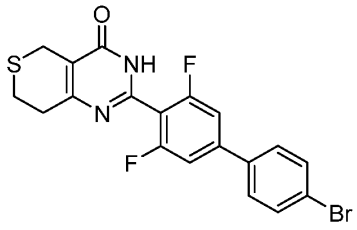
[00483] *Scheme 25B*



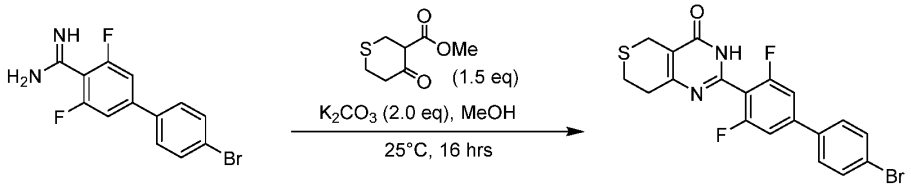
2 **3**

[00484] To a solution of 4'-bromo-3,5-difluoro-[1,1'-biphenyl]-4-carbonitrile (**2**) (0.5 g, 1.70 mmol, 1 eq) in THF (5 mL), LiHMDS (1 M, 4.25 mL, 2.5 eq) was added dropwise at 0°C. The mixture was stirred at 15°C for 16 h. The reaction mixture was quenched by dropwise addition of 4 N HCl/dioxane (10 mL). The mixture was concentrated under reduced pressure to give a residue. The residue was suspended in MeOH (10 mL) and stirred at 20°C for 1 hr. Then the mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. 4'-bromo-3,5-difluoro-[1,1'-biphenyl]-4-carboximidamide (**3**) (528 mg, crude) was obtained which was used in the next step without further purification.

Synthesis of 2-(4'-bromo-3,5-difluoro-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



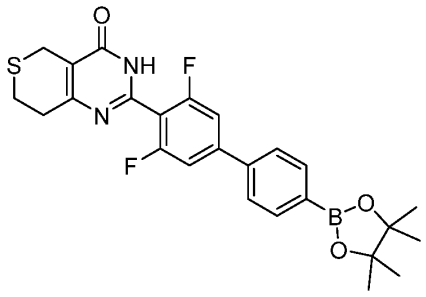
[00485] *Scheme 25C*



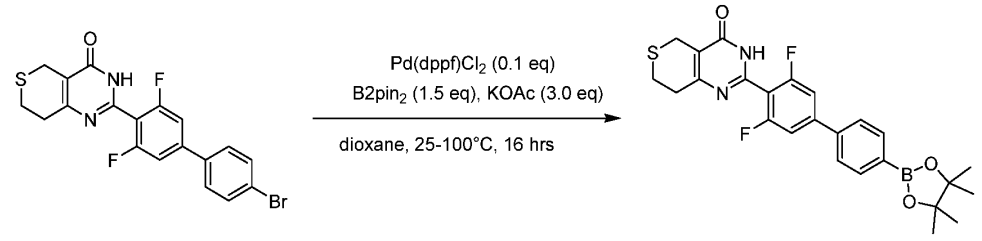
3 4

[00486] To a mixture of 4'-bromo-3,5-difluoro-[1,1'-biphenyl]-4-carboximidamide-HCl (**3**) (528 mg, 1.52 mmol, 1 eq) in MeOH (10 mL) was added K_2CO_3 (419.88 mg, 3.04 mmol, 2 eq) and methyl 4-oxotetrahydrothiopyran-3-carboxylate (396.97 mg, 2.28 mmol, 1.5 eq) at 25°C under N_2. Then the mixture was stirred at 25°C for 16 h under an N_2 atmosphere. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was triturated with water (10 mL) at 25°C for 1 hr and then filtered. The filter cake was dried *in vacuo* to give 2-(4'-bromo-3,5-difluoro-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (0.9 g, crude) which was used directly in the next step.

Synthesis of 2-(3,5-difluoro-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one

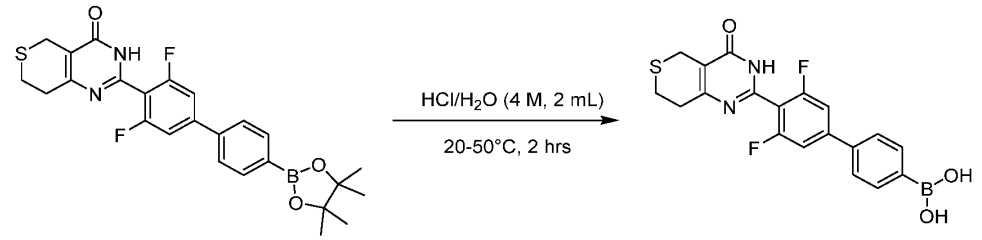


[00487] *Scheme 25D*



[00488] To a mixture of 2-(4'-bromo-3,5-difluoro-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (150 mg, 344.60 μmol, 1 eq) in dioxane (3 mL) was added B_2Pin_2 (131.26 mg, 516.90 μmol, 1.5 eq) and KOAc (101.46 mg, 1.03 mmol, 3 eq) at 25°C. The mixture was degassed and purged three times with N_2 and then $Pd(dppf)Cl_2$ (28.14 mg, 34.46 μmol, 0.1 *eq*) was added at 25°C under N_2. The mixture was stirred at 100°C for 16 h under a N_2 atmosphere. After cooling to 20°C, the reaction mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was triturated with water (2 mL) and acetonitrile (2 ml) at 25°C for 1 hr. The mixture was filtered, and the filter cake was dried *in vacuo*. 2-(3,5-difluoro-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (120 mg) was obtained.

[00489] *Scheme 25E*

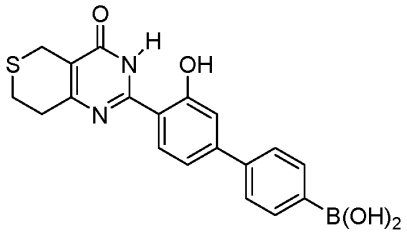


[00490] To a stirred solution of HCl/H_2O (4 M, 2 mL), 2-(3,5-difluoro-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (120 mg, 248.78 μmol, 1 eq) was added portion-wise at 20°C under N_2. Then the mixture was stirred at 50°C for 2 h under an N_2 atmosphere. The mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Waters Xbridge BEH C18 100 * 30 mm * 10 μm; mobile phase: [water (NH_4HCO_3)-ACN]; B%: 25%-55%, 8 min). (3',5'-difluoro-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**Compound 25**) was obtained. LCMS: 401 (M+1). ^1H NMR: (400 MHz, DMSO-d6): δ = 13.30 - 12.89 (m, 1H),

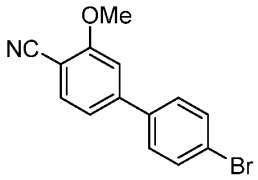
8.19 (s, 2H), 7.98 - 7.86 (m, 2H), 7.84 - 7.77 (m, 2H), 7.73 - 7.65 (m, 2H), 3.55 (br s, 2H), 2.97 - 2.81 (m, 4H).

Compound 26

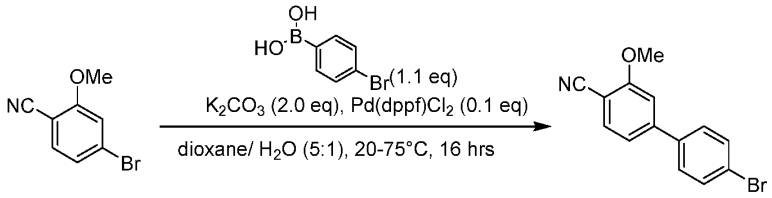
Synthesis of (3'-hydroxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (Compound 26)



Synthesis of 4'-bromo-3-methoxy-[1,1'-biphenyl]-4-carbonitrile



[00491] *Scheme 26A*

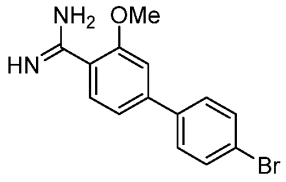


1 2

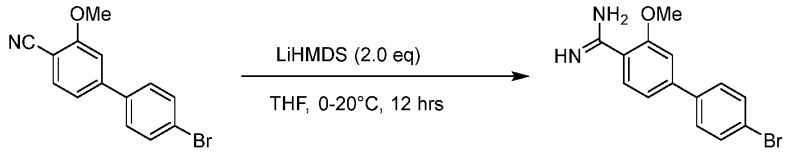
[00492] To a solution of 4-bromo-2-methoxybenzonitrile (**1**) (10.0 g, 47.2 mmol, 1.00 eq) and (4-bromophenyl)boronic acid (10.4 g, 51.9 mmol, 1.10 eq) in dioxane (50.0 mL) and H_2O (10.0 mL) was added K_2CO_3 (13.0 g, 94.3 mmol, 2.00 eq) and $Pd(dppf)Cl_2$ (3.45 g, 4.72 mmol, 0.10 eq) at 20°C. The mixture was heated to 75°C for 16 h. TLC (petroleum ether/ethyl acetate = 5/ 1, R_f = 0.53) indicated that (**1**) was consumed completely. The reaction mixture was diluted with EtOAc (50.0 mL) and H_2O (20.0 mL). The organic layer was separated from the mixture and the aqueous phase was extracted with EtOAc (50.0 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethylacetate = 10/1 to 0/1) to provide 4'-bromo-3-methoxy-[1,1'-biphenyl]-4-carbonitrile (**2**) (3.30 g, 8.02 mmol). The compound was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.86 (d,

J = 8.1 Hz, 1H), 7.84 - 7.72 (m, 4H), 7.52 (d, *J* = 1.1 Hz, 1H), 7.45 (dd, *J* = 1.4, 8.0 Hz, 1H), 4.08 (s, 3H).

Synthesis of 4'-bromo-3-methoxy-[1,1'-biphenyl]-4-carboximidamide



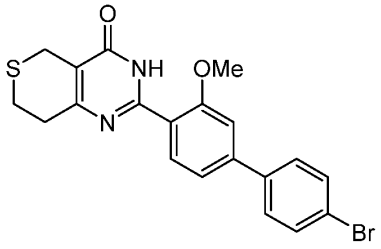
[00493] *Scheme 26B*



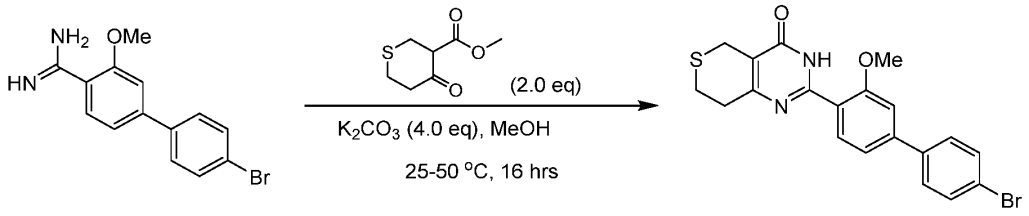
2 **3**

[00494] To a solution of 4'-bromo-3-methoxy-[1,1'-biphenyl]-4-carbonitrile (**2**) (2.00 g, 6.94 mmol, 1.00 eq) in THF (20.0 mL), LiHMDS (1 M, 13.9 mL, 2.00 eq) was added dropwise at 0°C and then allowed to warm to 20°C and stirred for 12 h. The reaction mixture was quenched with HCl/dioxane (13.0 mL) at 0 °C, and then concentrated under reduced pressure to give a residue. The residue was triturated with MeOH (10 mL) and filtered. The filter cake was dried *in vacuo* to give a residue. 4'-bromo-3-methoxy-[1,1'-biphenyl]-4-carboximidamide (**3**) (3.00 g, crude) was obtained. The crude product was used in the next step without further purification. LC-MS: 306 (M+1).

Synthesis of 2-(4'-bromo-3-methoxy-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



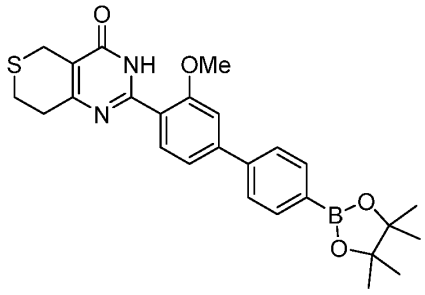
[00495] *Scheme 26C*



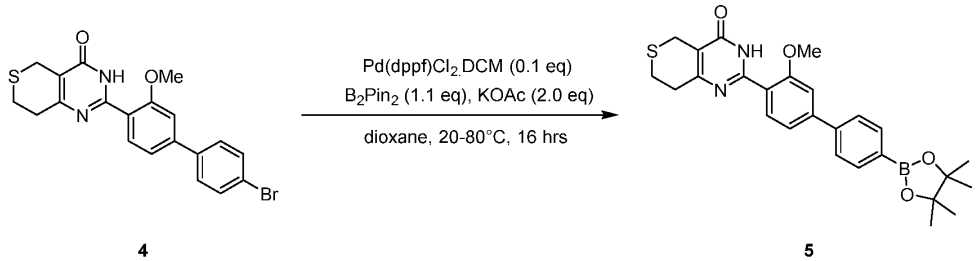
3 4

[00496] To a solution of 4'-bromo-3-methoxy-[1,1'-biphenyl]-4-carboximidamide (**3**) (3.00 g, 9.83 mmol, 1.00 eq) and methyl 4-oxotetrahydrothiopyran-3-carboxylate (3.43 g, 19.6 mmol, 2.00 eq) in MeOH (45.0 mL) was added K_2CO_3 (5.43 g, 39.3 mmol, 4.00 eq) at 25°C and then heated to 50°C and stirred for 16 h. LC-MS showed that (**3**) was consumed. The reaction mixture was filtered, and the filter cake was dried under reduced pressure to give a residue. The residue was triturated with H_2O (30.0 mL) and stirred at 20°C for 16 h. Then the mixture was filtered, and the filter cake was dried under reduced pressure to give 2-(4'-bromo-3-methoxy-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (1.5 g, crude), which was used in the next step without further purification. LC-MS: 430.2 (and bromo isomers) (M+1).

Synthesis of 2-(3-methoxy-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



[00497] *Scheme 26D*

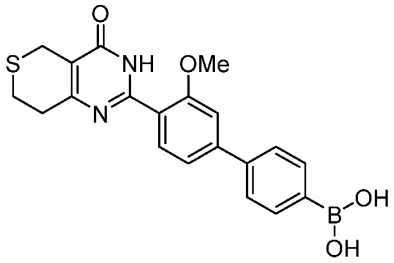


4 5

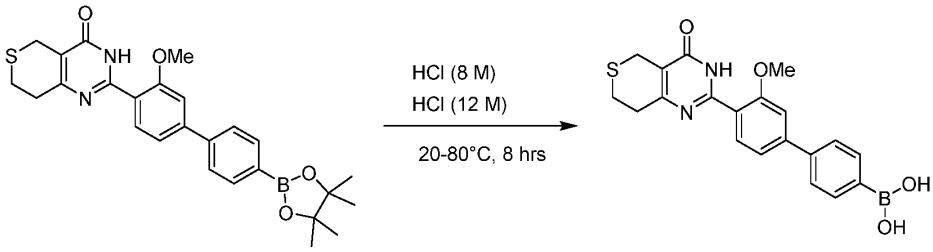
[00498] To a solution of 2-(4'-bromo-3-methoxy-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (1.00 g, 2.33 mmol, 1.00 eq) and Pin_2B_2 (650 mg,

2.56 mmol, 1.10 eq) in dioxane (50.0 mL) was added KOAc (457 mg, 4.66 mmol, 2.00 eq) and Pd(dppf)Cl_2.CH_2Cl_2 (190 mg, 233 μmol, 0.10 eq) at 20°C. The mixture was heated to 80°C and stirred for 16 h. LC-MS showed (**4**) was consumed completely and one main peak with the desired mass was detected. The mixture was filtered, and the filtrate was concentrated under reduced pressure to provide 2-(3-methoxy-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (1.60 g, crude). The crude product was used in the next step without further purification. LC-MS: 477.2 (M+1).

Synthesis of (3'-methoxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid



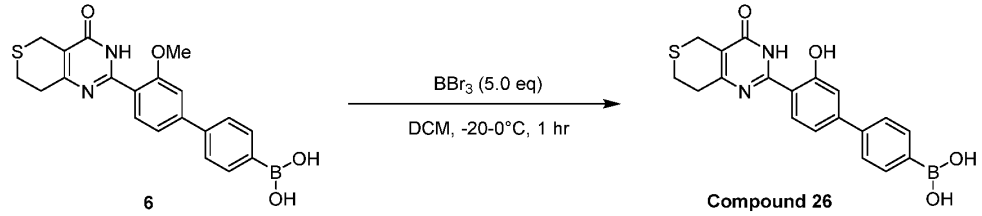
[00499] *Scheme 26E*



5 **6**

[00500] A mixture of 2-(3-methoxy-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (1.60 g, 3.36 mmol, 1.00 eq) in HCl (8 M, 32.0 mL, 76.2 eq) was heated to 80°C and stirred for 16 h. HCl (12 M, 8.00 mL, 28.6 eq) was added to the mixture at 20°C, and then heated to 80°C for 4 h. LC-MS showed (**5**) was consumed completely and one main peak with the desired mass was detected. The reaction mixture was filtered, then the filter cake was dried under reduced pressure to give (3'-methoxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**6**) (710 mg, crude). The crude product was used in the next step without further purification. LC-MS: 395.2 (M+1).

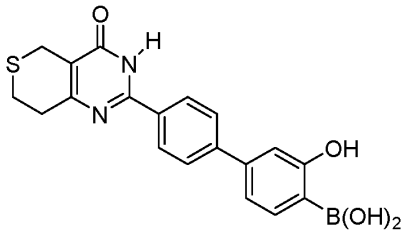
[00501] *Scheme 26F*



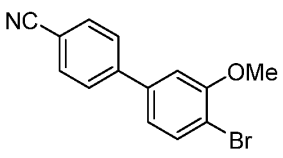
[00502] To a solution of (3'-methoxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**6**) (710 mg, 1.80 mmol, 1.00 eq) in DCM (21.6 mL), BBr_3 (1 M in DCM, 9.00 mL, 5.00 eq) was added dropwise at -20°C. The mixture was heated to 20°C and stirred for 1h. LC-MS showed that (**6**) was consumed completely and one peak with the desired mass was detected. The reaction mixture was quenched by the addition of ice water (20.0 mL) at 0°C. The mixture was filtered, and the filter cake was dried under reduced pressure to give a residue that was purified by prep-HPLC (column: Phenomenex Gemini NXC18 (75 × 30 mm × 3 μm); mobile phase: [H_2O (0.05% NH_3H_2O + 10 mM NH_4HCO_3)-ACN]; gradient: 15%-65% B over 8.0 min) to give (3'-hydroxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**Compound 26**). LC-MS: 381.2 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.27 (d, J = 8.1 Hz, 1H), 8.13 (s, 2H), 7.90 (d, J = 8.0 Hz, 2H), 7.71 (d, J = 8.0 Hz, 2H), 7.32 - 7.19 (m, 2H), 3.55 (s, 2H), 2.93 (s, 4H).

Compound 27

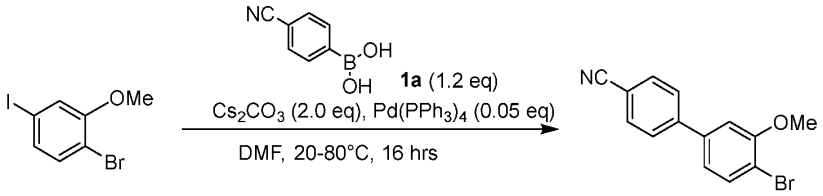
Synthesis of (3-hydroxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (Compound 27)



Synthesis of 4'-bromo-3'-methoxy-[1,1'-biphenyl]-4-carbonitrile



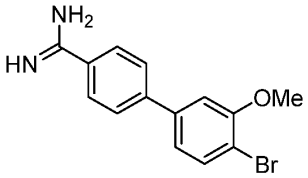
[00503] *Scheme 27A*



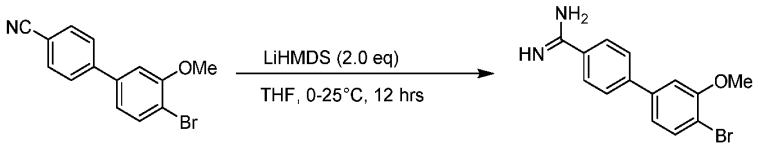
1 2

[00504] To a solution of 1-bromo-4-iodo-2-methoxybenzene (**1**) (8 g, 25.56 mmol, 1 eq) and (4-cyanophenyl)boronic acid (**1a**) (4.51 g, 30.7 mmol, 1.20 eq) in dimethylformamide (DMF) (160 mL) was added Cs_2CO_3 (16.7 g, 51.1 mmol, 2.00 eq) and $Pd(PPh_3)_4$ (1.48 g, 1.28 mmol, 0.05 eq) at 25°C. The mixture was heated to 80°C and stirred for 16 h under N_2. LC-MS showed that (**1**) was consumed completely. The reaction mixture was diluted with EtOAc and H_2O. The organic layer was separated from the mixture and the aqueous phase was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 10/ 1 to 0/ 1) to provide 4'-bromo-3'-methoxy-[1,1'-biphenyl]-4-carbonitrile (**2**) (4.50 g, 15.6 mmol). ^1H NMR (400 MHz, DMSO-d6): δ = 8.00 (s, 4H), 7.74 (d, J = 8.2 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.31 (dd, J = 2.1, 8.2 Hz, 1H), 4.02 (s, 3H).

Synthesis of 4'-bromo-3'-methoxy-[1,1'-biphenyl]-4-carboximidamide



[00505] *Scheme 27B*

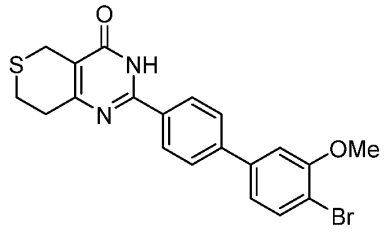


2 3

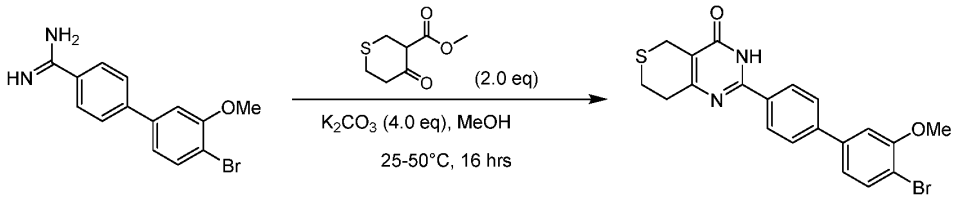
[00506] To a solution of 4'-bromo-3'-methoxy-[1,1'-biphenyl]-4-carbonitrile (**2**) (2.00 g, 6.94 mmol, 1.00 eq) in THF (20.0 mL), was added dropwise LiHMDS (1 M, 13.9 mL, 2.00 eq) at 0°C. The mixture was warmed to 25°C and stirred for 12h. LC-MS showed that (**2**) was consumed completely. The reaction mixture was quenched by dropwise addition of

HCl/dioxane (4M, 8.00 mL) at 0°C, then concentrated under reduced pressure to give a residue. The residue was suspended in MeOH (20.0 mL), and the mixture stirred at 25°C for 1 h. Then the mixture was filtered, and the filtrate was concentrated under reduced pressure to give 4'-bromo-3'-methoxy-[1,1'-biphenyl]-4-carboximidamide (**3**) (3.00 g, crude) and used directly in the next step without further purification. LC-MS: 306.1 + bromo isomers (M+1).

Synthesis of 2-(4'-bromo-3'-methoxy-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



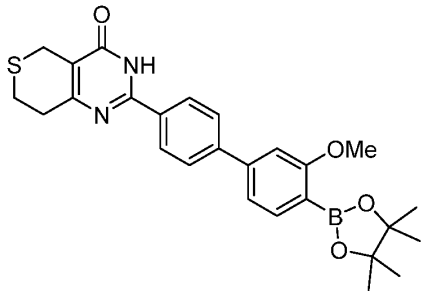
[00507] *Scheme 27C*



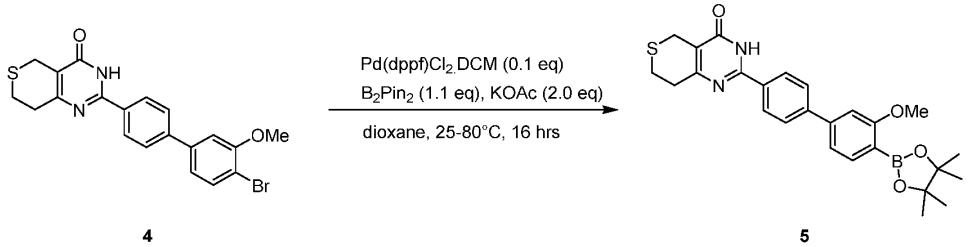
3 **4**

[00508] To a solution of 4'-bromo-3'-methoxy-[1,1'-biphenyl]-4-carboximidamide (**3**) (1.00 g, 3.28 mmol, 1.00 eq) and methyl 4-oxotetrahydrothiopyran-3-carboxylate (1.14 g, 6.55 mmol, 2 eq) in MeOH (15.0 mL) was added K_2CO_3 (1.81 g, 13.1 mmol, 4.00 eq) at 25°C. The mixture was heated to 50°C and stirred for 16 h. LC-MS showed (**3**) was consumed completely. The reaction mixture was filtered, and the filter cake was dried under reduced pressure to give a residue. H_2O (10.0 mL) was added to the residue to form a slurry that was stirred at 20°C for 16h. Then the mixture was filtered, and the filter cake was dried under reduced pressure to give 2-(4'-bromo-3'-methoxy-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (1.50 g, crude). LC-MS: 430.2 and bromo isomers (M+1). ^{1}H NMR (400 MHz, DMSO-d6): δ = 12.76 (br s, 1H), 8.26 - 8.20 (m, J = 8.4 Hz, 2H), 7.90 - 7.82 (m, J = 8.4 Hz, 2H), 7.68 (d, J = 8.3 Hz, 1H), 7.42 (d, J = 1.9 Hz, 1H), 7.27 (dd, J = 2.0, 8.3 Hz, 1H), 3.98 (s, 3H), 3.53 (s, 2H), 2.89 (s, 4H).

Synthesis of 2-(3'-methoxy-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one

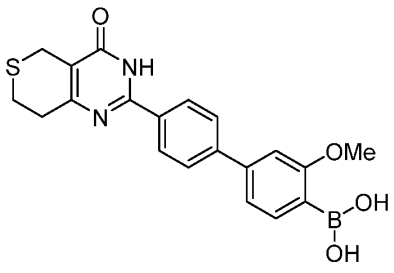


[00509] *Scheme 27D*

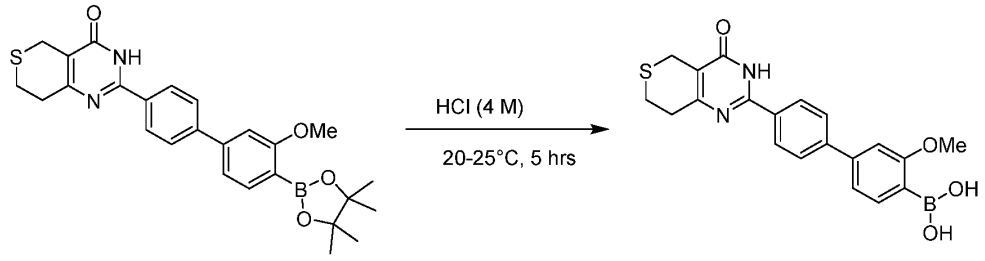


[00510] To a solution of 2-(4'-bromo-3'-methoxy-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (1.00 g, 2.33 mmol, 1.00 eq) and B_2Pin_2 (651 mg, 2.56 mmol, 1.10 eq) in dioxane (30.0 mL) was added KOAc (457 mg, 4.66 mmol, 2.00 eq) and Pd(dppf)Cl_2.DCM (190 mg, 233 μmol, 0.10 eq) at 25°C. The mixture was heated to 80°C and stirred under N_2 for 16h. LC-MS showed that (**4**) was consumed completely. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. 2-(3'-methoxy-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (1.00 g, crude) was obtained, which was used directly in the next step without further purification. LC-MS: 477.1 (M+1).

Synthesis of (3-methoxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid



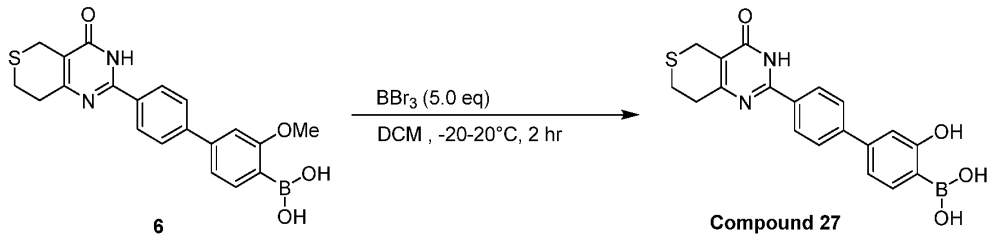
[00511] *Scheme 27E*



5 6

[00512] 2-(3'-methoxy-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (1.00 g, 2.10 mmol, 1.00 eq) was added portion-wise to a stirred solution of HCl/H_2O (4 M, 20.0 mL, 38.1 eq) at 20°C. The mixture was stirred at 25°C for 5h. LC-MS showed that (**5**) was consumed. The reaction mixture was filtered and the filter cake dried under reduced pressure to give (3-methoxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**6**) (250 mg, crude), which was used as is in the next step. LC-MS: 395.2 (M+1).

[00513] *Scheme 27F*

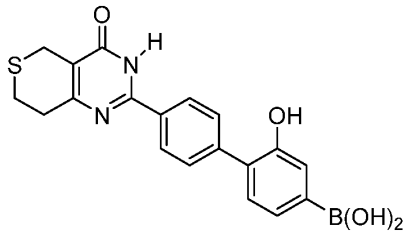


6 Compound 27

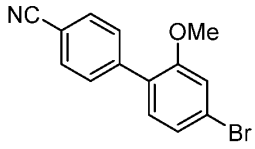
[00514] To a solution of (3-methoxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**6**) (250 mg, 634 μmol, 1.00 eq) in DCM (7.50 mL), was added BBr_3 (1 M, 3.17 mL, 5.00 eq) dropwise at -20°C. The mixture was stirred at 20°C for 2h. LC-MS showed that (**6**) was consumed completely. The reaction mixture was cooled to 0°C and quenched with ice water (10.0 mL) and stirred at 0 °C for 10min. The mixture was filtered and the filter cake dried under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Phenomenex luna C18 100 × 40 mm × 5 μm; mobile phase: [H_2O (0.04% HCl)-ACN]; gradient: 10%-50% B over 8.0 min) to provide (3-hydroxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**Compound 27**). LC-MS: 381.2 (M+1). ^{1}H NMR (400 MHz, DMSO-d6): δ = 8.24 - 8.07 (m, 2H), 7.94 - 7.84 (m, 1H), 7.83 - 7.74 (m, 2H), 7.37 - 7.04 (m, 2H), 3.55 (s, 2H), 2.92 (s, 4H).

Compound 28

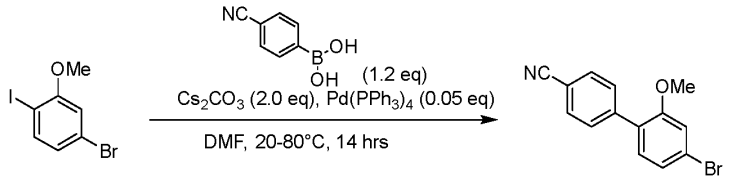
Synthesis of (2-hydroxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (Compound 28)



Synthesis of 4'-bromo-2'-methoxy-[1,1'-biphenyl]-4-carbonitrile



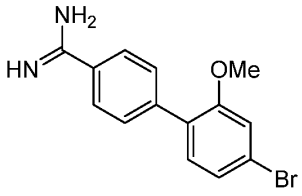
[00515] *Scheme 28A*



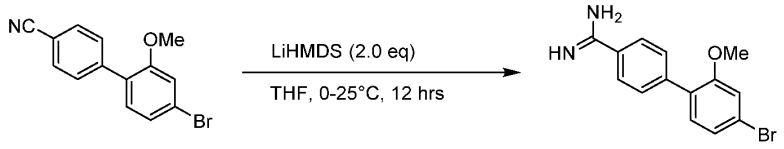
1 2

[00516] To a solution of 4-bromo-1-iodo-2-methoxybenzene (**1**) (8.00g, 25.6 mmol, 1.00 eq) and (4-cyanophenyl)boronic acid (4.51g, 30.7 mmol, 1.20 eq) in DMF (80.0 mL) was added Cs_2CO_3 (16.7g, 51.1 mmol, 2.00 eq) and $Pd(PPh_3)_4$ (1.48g, 1.28 mmol, 0.05 eq) at 20°C under N_2. The mixture was stirred at 80°C for 14 h under N_2. TLC (petroleum ether/ethyl acetate = 8/1, R_f = 0.54) indicated that (**1**) was consumed completely. The reaction mixture was diluted with EtOAc (50.0 mL) and H_2O (150 mL). The layers were separated and the aqueous phase was extracted with EtOAc (50.0 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 10/1 to 0/1) to give 4'-bromo-2'-methoxy-[1,1'-biphenyl]-4-carbonitrile (**2**) (6.10 g, 21.2 mmol). ^1H NMR (400 MHz, DMSO-d6): δ = 7.93 (d, J = 8.4 Hz, 2H), 7.73 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 1.4 Hz, 1H), 7.38 - 7.30 (m, 2H), 3.92 - 3.83 (m, 3H).

Synthesis of 4'-bromo-2'-methoxy-[1,1'-biphenyl]-4-carboximidamide



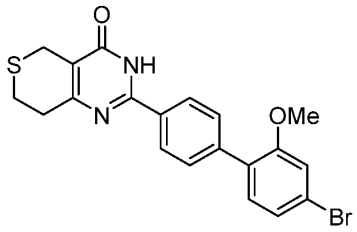
[00517] *Scheme 28B*



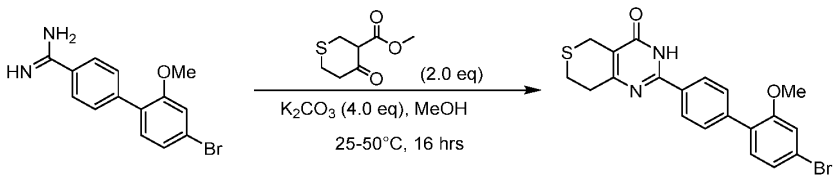
2 **3**

[00518] To a solution of 4'-bromo-2'-methoxy-[1,1'-biphenyl]-4-carbonitrile (**2**) (1.00g, 3.47 mmol, 1.00 eq) in THF (10.0 mL), LiHMDS (1 M, 6.94 mL, 2.00 eq) was added dropwise at 0°C. The mixture was stirred at 25°C for 12 h. LC-MS showed that (**2**) was consumed completely. The reaction mixture was quenched by dropwise addition of HCl/dioxane (6 mL) at 0°C, then concentrated under reduced pressure to give a residue that was suspended in MeOH (20 mL) and the mixture stirred at 25°C for 1 h. The mixture was filtered and the filtrate was concentrated under reduced pressure to give 4'-bromo-2'-methoxy-[1,1'-biphenyl]-4-carboximidamide (**3**) (2.00g, crude), which was used in the next step without further purification. LC-MS: 306.2 + bromo isomers (M+1).

Synthesis of 2-(4'-bromo-2'-methoxy-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



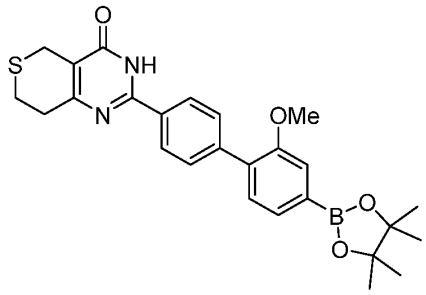
[00519] *Scheme 28C*



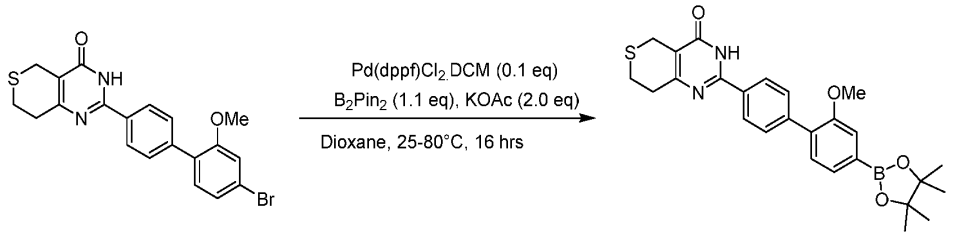
3 **4**

[00520] To a solution of 4'-bromo-2'-methoxy-[1,1'-biphenyl]-4-carboximidamide (**3**) (1.00g, 3.28 mmol, 1.00 eq) and methyl 4-oxotetrahydrothiopyran-3-carboxylate (1.14g, 6.55 mmol, 2.00 eq) in MeOH (10 mL) was added K_2CO_3 (1.81g, 13.1 mmol, 4.00 eq) at 25°C. The mixture was stirred at 50°C for 16 h. LC-MS showed that (**3**) was consumed completely. The reaction mixture was filtered and filter cake dried under reduced pressure to give a residue. H_2O (15 mL) was added to make a slurry and the mixture stirred at 20°C for 16 h. The mixture was filtered and the filter cake dried under reduced pressure to give 2-(4'-bromo-2'-methoxy-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (2.00 g, crude). The crude product was used in the next step without further purification. LC-MS: 430.3 + bromo isomers (M+1).

Synthesis of 2-(2'-methoxy-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



[00521] *Scheme 28D*

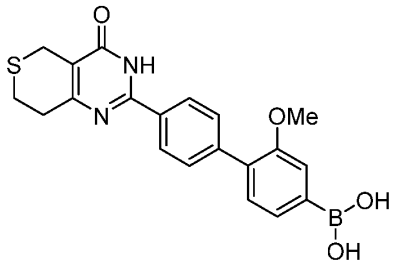


4 **5**

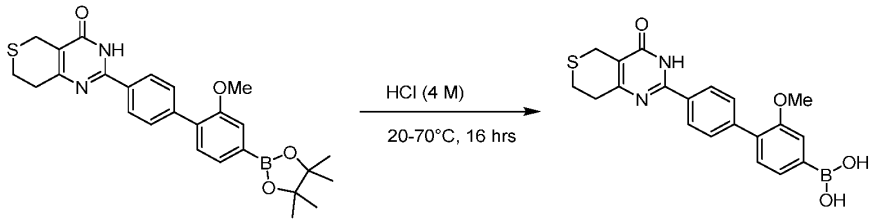
[00522] To a solution of 2-(4'-bromo-2'-methoxy-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (400mg, 932 μmol, 1 eq) and B_2Pin_2 (260mg, 1.02 mmol, 1.10 eq) in dioxane (20 mL) was added KOAc (183mg, 1.86 mmol, 2.00 eq) and $Pd(dppf)Cl_2$.DCM (76.1mg, 93.17 μmol, 0.1 eq) at 25°C. The mixture was stirred under N_2 at 80°C for 16h. LC-MS showed that (**4**) was consumed completely. The mixture was filtered and the filtrate was concentrated under reduced pressure to give 2-(2'-methoxy-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-

thiopyrano[4,3-d]pyrimidin-4-one (**5**) (700 mg, crude). The crude product was used in the next step without further purification. LC-MS: 477.2 (M+1).

Synthesis of (2-methoxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid



[00523] *Scheme 28E*



5 **6**

[00524] 2-(2'-methoxy-4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (400mg, 840 µmol, 1.0 eq) was added portion-wise to a stirred solution of HCl (4M, 20 mL, 95.3 eq) at 20°C. The mixture was stirred at 70°C for 16h. LC-MS showed that (**5**) was consumed completely. The reaction mixture was filtered, and the filtrate concentrated under reduced pressure to give (2-methoxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**6**) (490 mg, crude). The crude product was used in the next step without further purification. LC-MS: 395.1 (M+1).

[00525] *Scheme 28F*



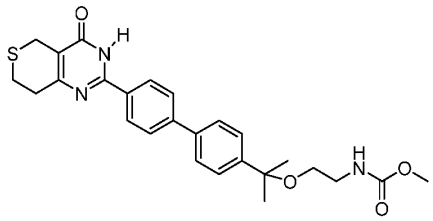
6 **7**

[00526] To a solution of (2-methoxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**6**) (200mg, 507 µmol, 1 eq) in DCM (3 mL), tribromoborane (1 M, 2.49 mL, 4.9 eq) was added dropwise at -10°C. The mixture was

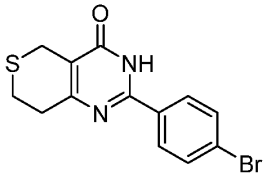
warmed to 20°C and stirred for 3 h. LC-MS showed that (**6**) was consumed completely. The reaction mixture was filtered and the filter cake dried under reduced pressure to give a residue that was purified by prep-HPLC (column: Phenomenex luna C18 100 × 40 mm × 5 μm; mobile phase: [H_2O (0.04% HCl)-ACN]; gradient: 5%-45% B over 8.0 min). (2-hydroxy-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**Compound 28**) was obtained. ^{1}H NMR (400 MHz, DMSO-d6): δ = 9.69 - 9.32 (m, 1H), 8.12 (d, J = 8.5 Hz, 2H), 7.73 (d, J = 8.4 Hz, 2H), 7.39 - 7.37 (m, 1H), 7.36 - 7.28 (m, 2H), 3.64 - 3.59 (m, 2H), 2.91 (s, 4H). LC-MS: 381.1 (M+1).

Compound 29

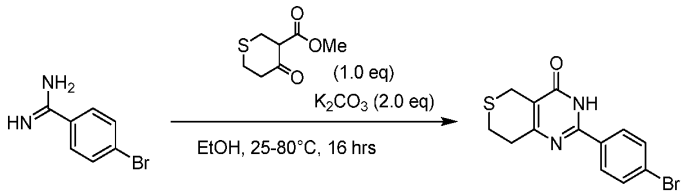
Synthesis of methyl (2-((2-(4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)propan-2-yl)oxy)ethyl)carbamate (Compound 29)



Synthesis of 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



[00527] *Scheme 29A*

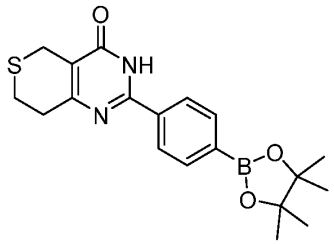


1 2

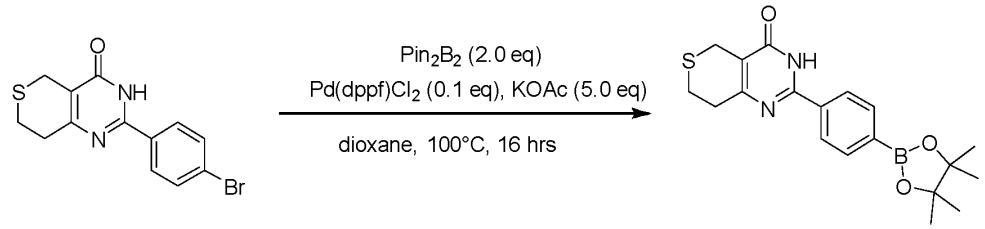
[00528] To a solution of 4-bromobenzimidamide (**1**) (5 g, 21.2 mmol, 1.00 eq) in EtOH (50 mL) was added methyl 4-oxotetrahydrothiopyran-3-carboxylate (3.70 g, 21.2 mmol, 1.00 eq) and K_2CO_3 (5.87 g, 42.5 mmol, 2.00 eq) at 25°C. The mixture was stirred at 80°C for 16 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one

(**2**) (7 g, crude) was obtained and used without further purification. ^{1}H NMR: (400 MHz, DMSO-d6): δ = 8.21 - 8.11 (m, 2H), 7.53 - 7.46 (m, 2H), 3.42 (s, 2H), 2.83 - 2.64 (m, 4H).

Synthesis of 2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



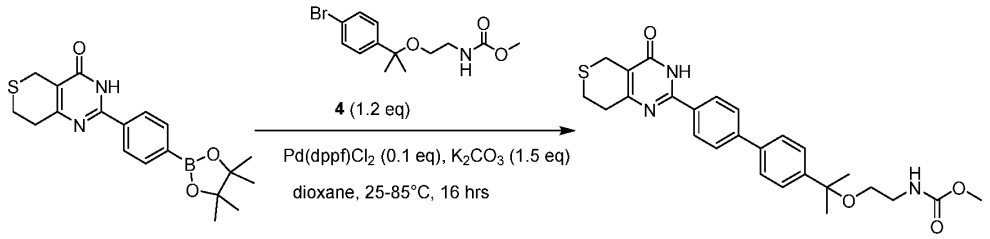
[00529] *Scheme 29B*



2 **3**

[00530] To a solution of 2-(4-bromophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**2**) (7 g, 21.7 mmol, 1.00 eq) in dioxane (70 mL) was added Pin_2B_2 (11.0 g, 43.3 mmol, 2.00 eq), KOAc (10.6 g, 108 mmol, 5.00 eq), and $Pd(dppf)Cl_2$ (1.58 g, 2.17 mmol, 0.10 eq) at 25°C under N_2. The mixture was degassed three times with N_2. The mixture was stirred at 100°C for 16 h. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was triturated with H_2O (150 mL) at 20°C for 2 h. The mixture was filtered, and the filter cake was concentrated under reduced pressure to give a residue. 2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (9 g, crude) was obtained and used immediately in the next step.

Synthesis of methyl (2-((2-(4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)propan-2-yl)oxy)ethyl)carbamate

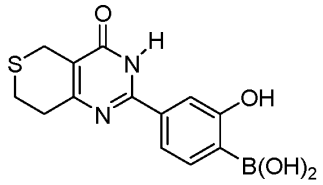


3 Compound 29

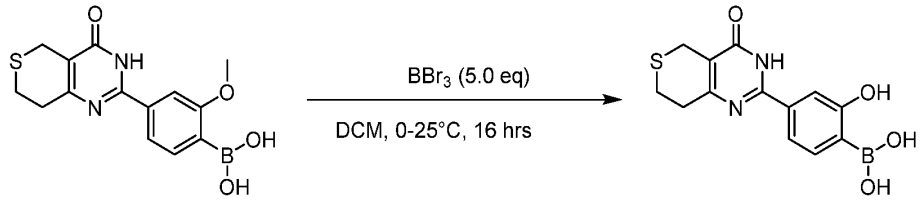
[00531] To a solution of 2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (0.8 g, 2.16 mmol, 1.00 eq) in dioxane (16 mL) was added methyl (2-((2-(4-bromophenyl)propan-2-yl)oxy)ethyl)carbamate (**4**) (820 mg, 2.59 mmol, 1.20 eq), $Pd(dppf)Cl_2$ (158 mg, 216 μmol, 0.10 eq), and K_2CO_3 (1.49 g, 10.8 mmol, 5eq) at 25°C under N_2. The mixture was degassed three times with N_2. The mixture was stirred at 85°C for 16 h. The reaction mixture was concentrated under reduced pressure and the resultant residue diluted with H_2O (20 mL) and extracted with ethyl acetate 60 mL (20 mL × 3). The combined organic layers were washed with brine (30 mL × 1), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Phenomenex Luna C18 75 * 30 mm * 3 μm; mobile phase: [$NaHCO_3$-ACN]; gradient: 40%-70% B over 16.0 min). Methyl (2-((2-(4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)propan-2-yl)oxy)ethyl)carbamate (**Compound 29**) was obtained. LCMS: 480.1 (M+1). ^{1}H NMR: (400 MHz, DMSO-d6): δ = 8.19 (d, J = 8.4 Hz, 2H), 7.82 (d, J = 8.5 Hz, 2H), 7.72 (d, J = 8.4 Hz, 2H), 7.57 - 7.47 (m, 2H), 7.17 - 7.11 (m, 1H), 3.53 (s, 2H), 3.49 (s, 3H), 3.18 - 3.08 (m, 4H), 2.90 (br s, 4H), 1.49 (s, 6H).

Compound 30

Synthesis of (2-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (Compound 30)



[00532] *Scheme 30A*



Compound 32 **Compound 30**

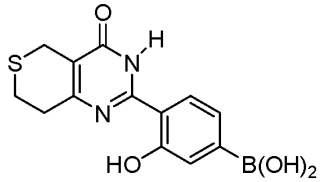
[00533] The synthesis of (2-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**Compound 32**) is shown in Schemes 32A-D.

[00534] To a solution of (2-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**Compound 32**) (0.30 g, 943 μmol, 1.00 eq) in DCM (3 mL), was added BBr_3 (1.18 g, 4.71 mmol, 454 μL, 5.00 eq) dropwise at 0°C and then stirred at 25°C for 16h. LC-MS showed that (**Compound 32**) was consumed. The reaction mixture was quenched by dropwise addition of H_2O (5 mL at 25°C) and extracted with ethyl acetate (3 mL × 3). The combined organic layers were washed and concentrated under reduced pressure to give a residue that was purified by prep-HPLC (column: Phenomenex Luna C8 250 × 50 mm × 10 μm; mobile phase: [H_2O (0.04 % HCl)-ACN]; gradient: 5%-40% B over 10.0 min). (2-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**Compound 30**) was obtained.

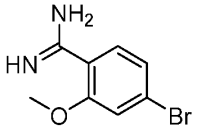
[00535] ^{1}H NMR (400 MHz, DMSO-d6): δ = 10.30 - 9.99 (m, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.55 - 7.45 (m, 2H), 3.52 (s, 2H), 2.92 - 2.88 (m, 4H).

Compound 31

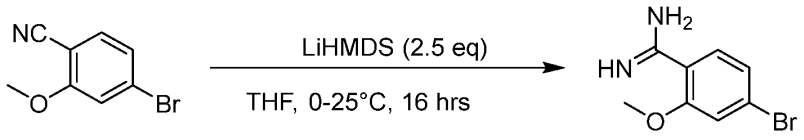
Synthesis of (3-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (Compound 31)



Synthesis of 4-bromo-2-methoxybenzimidamide



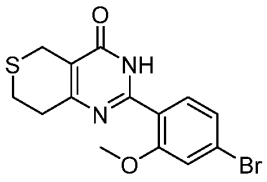
[00536] *Scheme 31A*



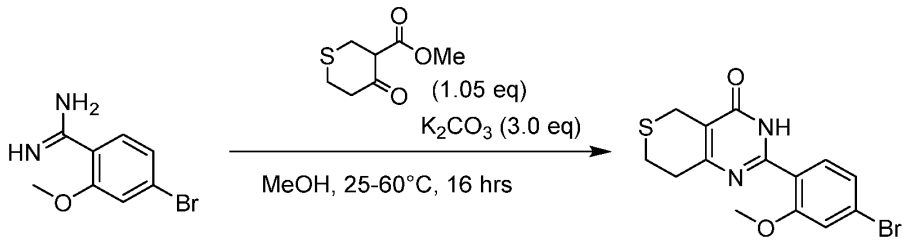
1 **2**

[00537] To a solution of 4-bromo-2-methoxybenzonitrile (**1**) (3.00 g, 14.2 mmol, 1.00 eq) in THF (30 mL), was added LiHMDS (1 M, 35.37 mL, 2.50 eq) dropwise at 0°C and then stirred at 25°C for 16 h. LC-MS showed that (**1**) was consumed. The reaction mixture was quenched by addition of 4 M HCl/dioxane (40 mL). The combined organic layers were concentrated under reduced pressure to give a residue which was suspended in MeOH (30 mL) and stirred at 15°C for 2h, then filtered. The filtrate was concentrated under reduced pressure to give 4-bromo-2-methoxybenzimidamide (**2**) (3.50 g, crude) was obtained. The crude product was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d6): δ = 7.65 - 7.55 (m, 1H), 7.52 - 7.44 (m, 1H), 7.38 - 7.32 (m, 1H), 3.56 (s, 3H).

Synthesis of 2-(4-bromo-2-methoxyphenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



[00538] *Scheme 31B*

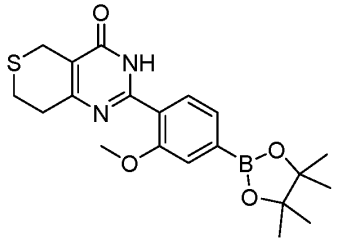


2 **3**

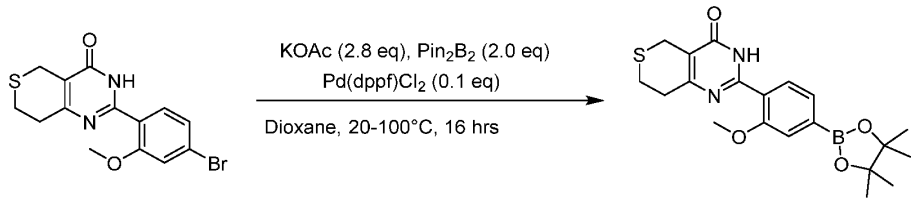
[00539] To a solution of 4-bromo-2-methoxybenzimidamide (**2**) (3.50 g, 15.3 mmol, 1.00 eq) in MeOH (35 mL) was added K_2CO_3 (6.34 g, 45.8 mmol, 3.00 eq) and methyl 4-oxotetrahydrothiopyran-3-carboxylate (2.79 g, 16.0 mmol, 1.05 eq) at 25°C and then stirred at 60°C for 16 h. LC-MS showed (**2**) was consumed. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue that was purified by

re-crystallization from MTBE (30 mL) at 25°C. 2-(4-bromo-2-methoxyphenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (3.00 g, crude) was obtained. ^{1}H NMR (400 MHz, DMSO-d6): δ = 7.24 (d, J = 8.1 Hz, 1H), 7.16 (s, 1H), 7.11 - 7.07 (m, 1H), 3.72 (s, 3H), 3.45 (s, 2H), 2.74 - 2.58 (m, 4H).

Synthesis of 2-(2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



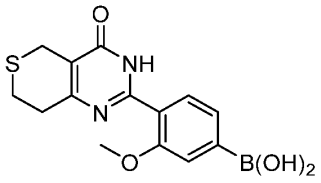
[00540] *Scheme 31C*



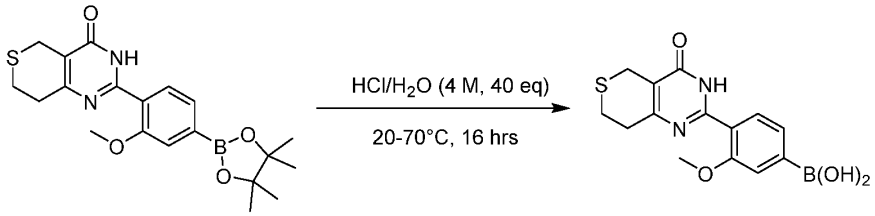
3 **4**

[00541] To a solution of 2-(4-bromo-2-methoxyphenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (3.00 g, 8.49 mmol, 1.00 eq) in dioxane (30 mL) was added Pin_2B_2 (4.31 g, 17.0 mmol, 2.00 eq), K_2CO_3 (3.29 g, 23.8 mmol, 2.80 eq), and $Pd(dppf)Cl_2$ (621 mg, 849 μmol, 0.10 eq) at 20°C under N_2. The suspension was degassed and purged three times with N_2. The mixture was stirred under N_2 at 100°C for 16 h. LC-MS showed that (**3**) was consumed. The reaction mixture was concentrated under reduced pressure to remove solvent and then diluted with H_2O (30 mL) and extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The crude product was triturated with MTBE at 25°C for 120 min. 2-(2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (2.5 g, crude) was obtained. The crude product was used in the next step without further purification. LC-MS: 401.2 (M+1).

Synthesis of (3-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid



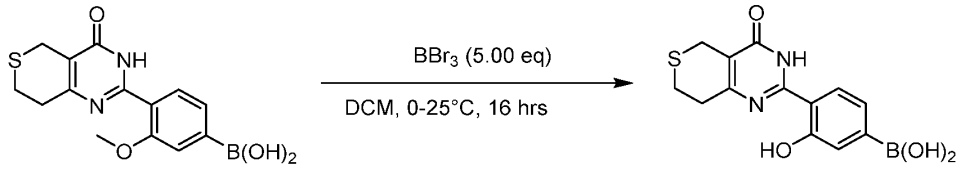
[00542] *Scheme 31D*



4 **5**

[00543] A mixture of 2-(2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (1.50 g, 3.75 mmol, 1.00 eq) in HCl/H_2O (4 M, 37.5 mL, 40.0 eq) was degassed and purged three times with N_2 at 20 °C, and then stirred at 70°C for 16 h under N_2. LC-MS showed (**4**) was consumed. The reaction mixture was concentrated under reduced pressure to give a residue that was triturated with MTBE at 25°C for 120 min. The mixture was filtered, and the solvent was concentrated under reduced pressure to give a residue that was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150 × 40 mm × 10 μm; mobile phase: [water (NH_4HCO_3)-ACN]; gradient: 10%-45% B over 8 min) to provide (3-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**5**) (1.00 g, 3.14 mmol). ^1H NMR (400 MHz, DMSO-d6): δ = 8.27 (s, 2H), 7.61 - 7.52 (m, 2H), 7.46 (d, J = 7.8 Hz, 1H), 3.86 (s, 3H), 3.51 (s, 2H), 2.91 - 2.81 (m, 4H).

[00544] *Scheme 31E*



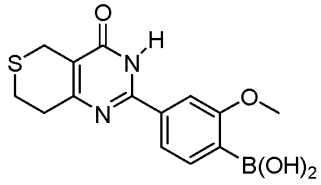
5 **Compound 31**

[00545] To a solution of (3-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**5**) (0.9 g, 2.83 mmol, 1 eq) in DCM (9 mL), was added BBr_3 (3.54 g, 14.1 mmol, 1.36 mL, 5.00 eq) dropwise at 0°C and then stirred at 25°C for 16 h.

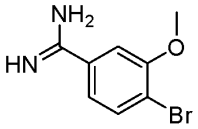
LC-MS showed that (**5**) was consumed. The reaction mixture was quenched by the addition of H_2O (5 mL at 25°C) and extracted with ethyl acetate (3 mL × 3). The combined organic layers were washed and concentrated under reduced pressure to give a residue that was purified by prep-HPLC (column: Phenomenex luna C18 100 × 40 mm × 5 µm; mobile phase: [H_2O (0.04 % HCl)-ACN]; gradient: 5%-35% B over 8.0 min) to provide (3-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**Compound 31**). LCMS: 305.1 (M=1). ^{1}H NMR (400 MHz, DMSO-d6): δ = 8.08 (br d, J = 7.9 Hz, 1H), 7.78 - 7.43 (m, 2H), 7.37 (s, 1H), 7.31 (br d, J = 7.7 Hz, 1H), 3.54 (br s, 2H), 2.98 - 2.84 (m, 4H).

Compound 32

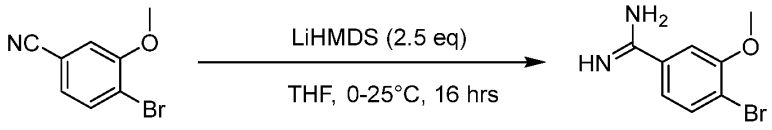
Synthesis of (2-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (Compound 32)



Synthesis of 4-bromo-3-methoxybenzimidamide



[00546] *Scheme 32A*

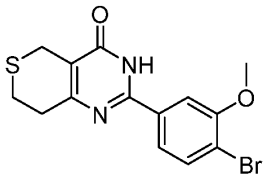


1 **2**

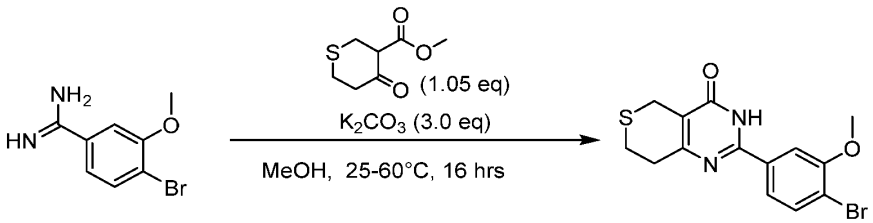
[00547] To a solution of 4-bromo-3-methoxybenzonitrile (**1**) (3.00 g, 14.2 mmol, 1.00 eq) in THF (30 mL), was added LiHMDS (1.00 M, 35.4 mL, 2.50 eq) dropwise at 0 °C. The mixture was warmed to 25°C and stirred for 16 h. LC-MS showed that (**1**) was consumed. The reaction mixture was quenched by dropwise addition of 4 M HCl/dioxane (30 mL). The combined organic layers were concentrated under reduced pressure to give a residue. The residue was suspended in MeOH (30 mL) and stirred at 15°C for 2h, filtered, and concentrated under reduced pressure to give 4-bromo-3-methoxybenzimidamide (**2**) (4.00 g, crude). The crude product was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d6):

δ = 7.81 (d, J = 8.3 Hz, 1H), 7.74 (d, J = 1.4 Hz, 1H), 7.45 (dd, J = 1.6, 8.3 Hz, 1H), 3.99 (s, 3H).

Synthesis of 2-(4-bromo-3-methoxyphenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



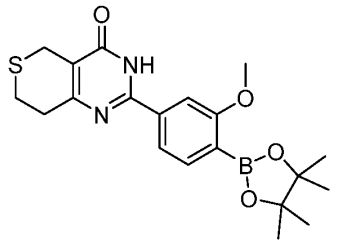
[00548] *Scheme 32B*



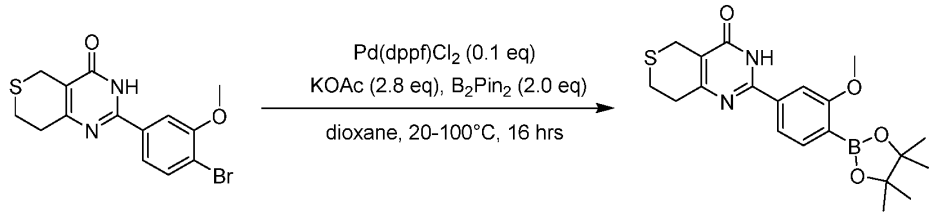
2 **3**

[00549] To a solution of 4-bromo-3-methoxybenzimidamide (**2**) (2.00 g, 8.73 mmol, 1.00 eq) in MeOH (20 mL) was added methyl 4-oxotetrahydrothiopyran-3-carboxylate (1.60 g, 9.17 mmol, 1.05 eq) and K_2CO_3 (3.62 g, 26.2 mmol, 3.00 eq) at 25°C. The mixture was stirred at 60°C for 16h. LC-MS showed that (**2**) was consumed completely. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give 2-(4-bromo-3-methoxyphenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (3.00 g, crude) was obtained. The crude product was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d6): δ = 13.16 - 12.30 (m, 1H), 7.78 (s, 1H), 7.73 - 7.68 (m, 1H), 7.66 - 7.60 (m, 1H), 3.95 (s, 3H), 3.52 (s, 2H), 2.88 (br s, 4H).

Synthesis of 2-(3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



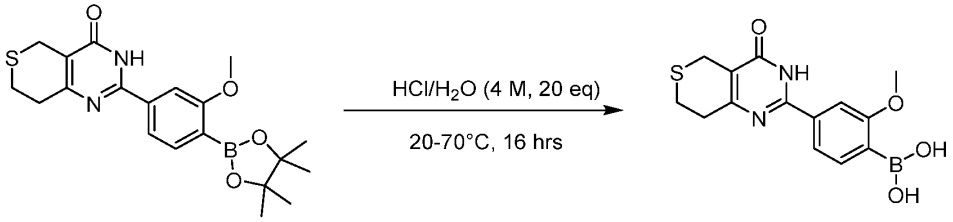
[00550] *Scheme 32C*



3 4

[00551] To a solution of 2-(4-bromo-3-methoxyphenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (2.00 g, 5.66 mmol, 1.00 eq) in dioxane (20 mL) was added B_2Pin_2 (2.88 g, 11.3 mmol, 2.00 eq), KOAc (1.56 g, 15.85 mmol, 2.80 eq), and $Pd(dppf)Cl_2$ (414 mg, 566 μmol, 0.10 eq) at 25°C. The suspension was degassed and purged three times with N_2. The mixture was stirred at 100°C for 16h. LC-MS showed that (**3**) was consumed. The reaction mixture was concentrated under reduced pressure to provide a residue that was diluted with H_2O (40 mL) and extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 2-(3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (2.50 g, crude) was obtained. The crude product was used in the next step without further purification. LC-MS: 401.3 (M+1).

[00552] *Scheme 32D*



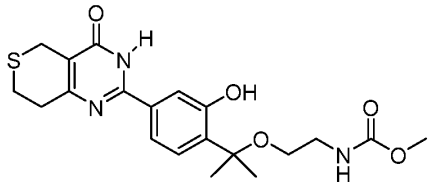
4 **Compound 32**

[00553] A mixture of 2-(3-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (1.00 g, 2.50 mmol, 1.00 eq) in HCl/H_2O (4 M, 12.5 mL, 20 eq) was degassed and purged three times with N_2, and then stirred at 70°C for 16 h under N_2. LC-MS showed that (**4**) was consumed. The reaction mixture was concentrated under reduced pressure to give a residue that was triturated with MTBE at 25°C for 120 min. The mixture was then filtered, and the filter cake dried under reduced pressure to give a residue that was purified by prep-HPLC (column: Waters Xbridge BEH C18 250 × 50 mm × 10 μm; mobile phase: [water (NH_4HCO_3)-ACN]; B%: 5%-45%,

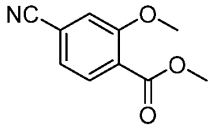
10 min). (2-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**Compound 32**) was obtained. ^{1}H NMR (400 MHz, DMSO-d6): δ = 13.14 - 12.16 (m, 1H), 7.96 - 7.81 (m, 2H), 7.74 - 7.53 (m, 3H), 3.89 (br s, 3H), 3.53 (br s, 2H), 2.89 (br s, 4H).

Compound 33

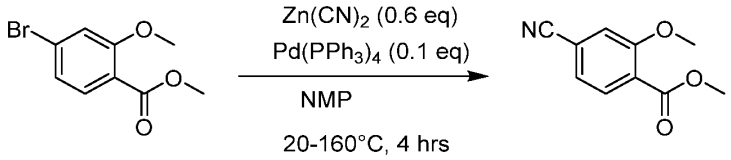
Synthesis of methyl (2-((2-(2-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (Compound 33)



Synthesis of methyl 4-cyano-2-methoxybenzoate



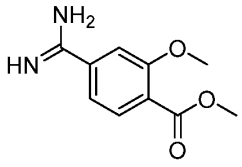
[00554] *Scheme 33A*



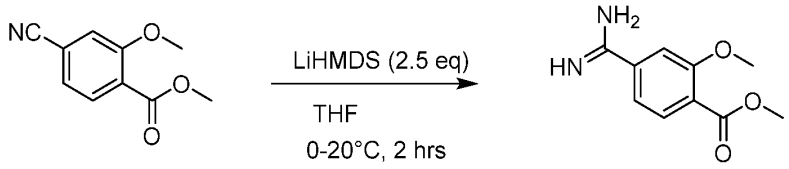
1 **2**

[00555] Methyl 4-bromo-2-methoxybenzoate (**1**) (10 g, 40.80 mmol, 1.0 eq) and $Zn(CN)_2$ (2.87 g, 24.48 mmol, 1.55 mL, 0.6 eq) were added to N-methylpyrrolidone (NMP) (100 mL) in one portion at 20°C under N_2. Then $Pd(PPh_3)_4$ (4.72 g, 4.08 mmol, 0.1 eq) was added to the mixture in one portion at 20 °C under N_2, and the mixture stirred at 160 °C for 4hr and then cooled to 20 °C and filtered. The filter cake was washed with EtOAc (100 mL x 2). The organic layer was then washed with aq. sat. Na_2CO_3 (50 mL × 2). The organic layer was concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50/1 to 0/1) to provide methyl 4-cyano-2-methoxybenzoate (**2**) (4 g, crude) was obtained and used immediately in the next step.

Synthesis of Methyl 4-carbamimidoyl-2-methoxybenzoate



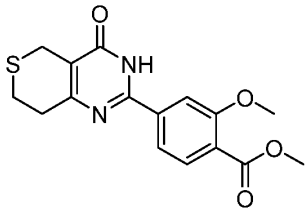
[00556] *Scheme 33B*



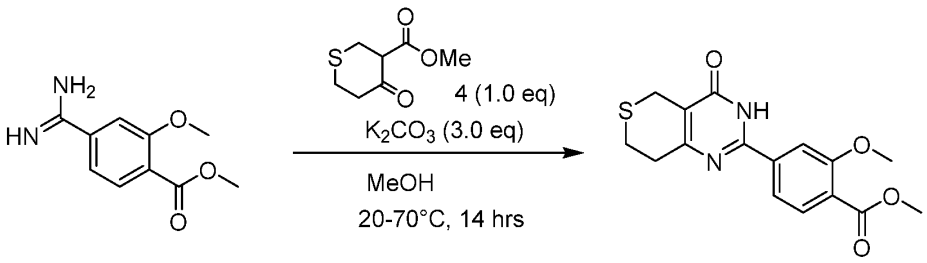
2 **3**

[00557] Methyl 4-cyano-2-methoxybenzoate (**2**) (3.0 g, 15.69 mmol, 1.0 eq) was added to THF (30 mL) at 20°C under N_2. LiHMDS (1.0 M, 39.23 mL, 2.5 eq) was added dropwise to the mixture at 0°C under N_2, and the mixture stirred at 20°C under N_2 for 2 h. LC-MS showed that (**2**) was consumed completely. The reaction mixture was quenched with HCl/dioxane (30 mL) at 0°C and then diluted with MeOH (30 mL). The mixture was filtered, and the filtrate concentrated under reduced pressure to give methyl 4-carbamimidoyl-2-methoxybenzoate (**3**) (4 g, crude, HCl) and used immediately in the next step. LC-MS: 209.2 (M+1).

Synthesis of methyl 2-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate



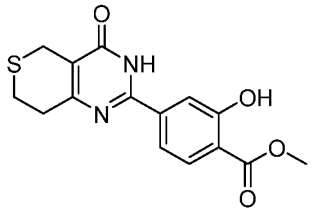
[00558] *Scheme 33C*



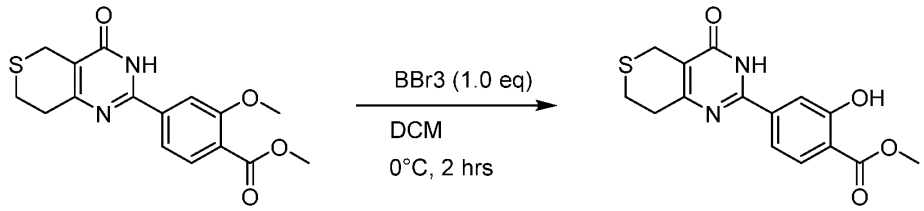
3 **5**

[00559] Methyl 4-carbamimidoyl-2-methoxybenzoate (**3**) (4.0 g, 19.21 mmol, 1.0 eq) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**4**) (3.35 g, 19.21 mmol, 1.0 eq) were added to MeOH (40 mL) at 20°C. K_2CO_3 was added (7.97 g, 57.63 mmol, 3.0 eq) to the mixture at 20°C and the mixture stirred at 70°C for 14 h under N_2. LC-MS showed that (**3**) was completely consumed. The mixture was filtered and concentrated to provide a residue that was purified by prep-HPLC (column: Agela DuraShell C18 250 × 70mm × 10um; mobile phase: [H_2O (10 mM NH_4HCO_3)-ACN]; gradient: 25%-45% B over 17.0 m) to provide Methyl 2-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**5**) (0.5 g, 1.50 mmol). LC-MS: 333.3 (M+1).

Synthesis of methyl 2-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate



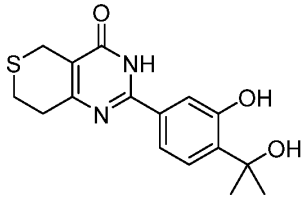
[00560] *Scheme 33D*



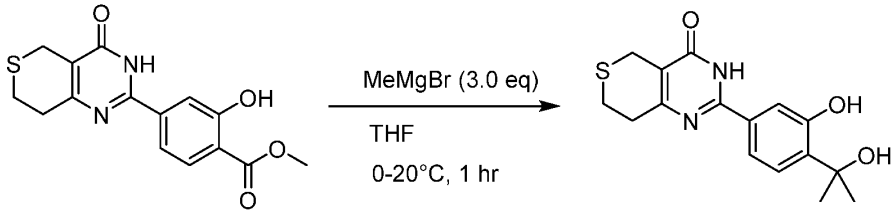
5 **6**

[00561] Methyl 2-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**5**) (250 mg, 752.17 µmol, 1.0 eq) was added to DCM (5.0 mL) at 20°C and the mixture cooled to 0°C. BBr_3 (188.43 mg, 752.17 µmol, 72.47 µL, 1.0 eq) was added dropwise to the mixture at 0°C and the reaction mixture was stirred at 0°C for 2 h under N_2. LC-MS indicated that (**6**) was detected. The reaction mixture was quenched with MeOH (2.0 mL) at 0°C under N_2. The mixture was filtered, and the filtrate concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50/1 to 0/1) to provide methyl 2-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**6**) (250 mg) that was used in the next step without further purification. LC-MS: 319.3 (M+1).

Synthesis of 2-(3-hydroxy-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



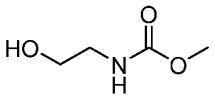
[00562] *Scheme 33E*



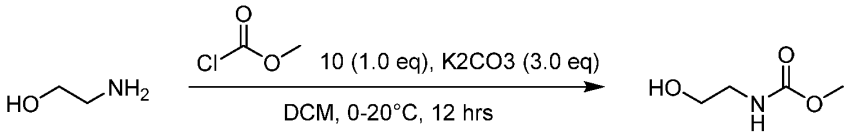
6 **7**

[00563] Methyl 2-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**6**) (80 mg, 251.30 μmol, 1.0 eq) was added to THF (0.8 mL) at 20°C and then the mixture cooled to 0°C. MeMgBr (3.0 M, 251.30 μL, 3.0 eq) was added to the mixture at 0°C and the reaction mixture stirred at 20°C under N_2 for 1 h. LC-MS showed that (**6**) was consumed. The reaction mixture was quenched with NH_4Cl (1.0 mL), and then extracted with EtOAc (1.0 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and then concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (petroleum ether/ethyl acetate = 0/1) to provide 2-(3-hydroxy-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**7**) (90 mg, crude). LC-MS: 319.3 (M+1).

Synthesis of methyl (2-hydroxyethyl)carbamate



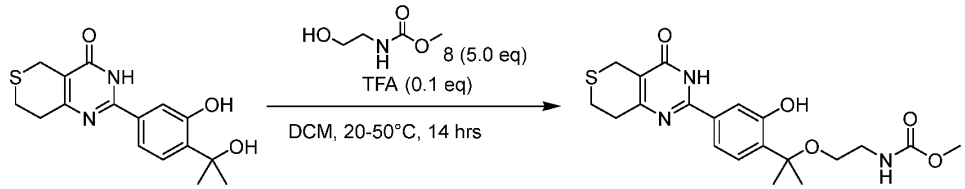
[00564] *Scheme 33F*



9 **8**

[00565] 2-aminoethan-1-ol (**9**) (6.46 g, 105.82 mmol, 6.39 mL, 1.0 eq) and K_2CO_3 (43.88 g, 317.47 mmol, 3.0 eq) were added to DCM (45 mL) at 20°C under N_2 and then cooled to 0°C. Carbonochloridate (**10**) (10 g, 105.82 mmol, 8.18 mL, 1.0 eq) was added to the mixture at 0°C under N_2 and mixture allowed to warm to 20°C under N_2 for 12 h. The reaction mixture was then quenched with $NH_3.H_2O$ (10%, 100 mL) and extracted with EtOAc (50 mL × 3). The organic layers were concentrated under reduced pressure to give crude methyl (2-hydroxyethyl)carbamate (**8**) (14.2 g, crude). ^{1}H NMR (400 MHz, $CDCl_3$-d): δ 5.36 - 5.11 (m, 1H), 3.78 - 3.61 (m, 5H), 3.35 (br s, 2H).

[00566] *Scheme 33G*

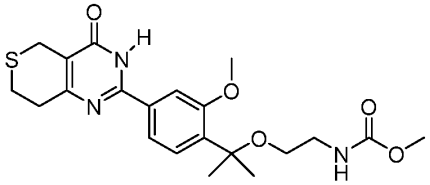


7 **Compound 33**

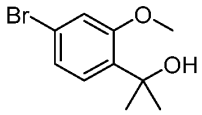
[00567] 2-(3-hydroxy-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**7**) (90 mg, 282.67 µmol, 1.0 eq) and methyl (2-hydroxyethyl)carbamate (**8**) (168.36 mg, 1.41 mmol, 5.0 eq) were added to DCM (0.9 mL) at 20°C under N_2. Then TFA (3.22 mg, 28.27 µmol, 2.10 µL, 0.1 eq) was at 20°C and the mixture stirred at 50°C under N_2 for 14 h. The reaction mixture was concentrated under reduced pressure to give a residue that was subjected to prep-HPLC (column: Agela DuraShell C18 250 × 70mm × 10um; mobile phase: [H_2O (10 mM NH_4HCO_3)-ACN]; gradient: 20%-43% B over 17.0 m) to provide methyl (2-((2-(2-hydroxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**Compound 33**). LC-MS: 420.5 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.82 - 12.46 (m, 1H), 9.49 (br d, J = 1.1 Hz, 1H), 7.58 - 7.47 (m, 2H), 7.38 (br d, J = 8.2 Hz, 1H), 7.21 (br t, J = 5.4 Hz, 1H), 3.58 - 3.47 (m, 6H), 3.18 (q, J = 5.5 Hz, 2H), 2.94 - 2.79 (m, 5H), 1.54 (s, 6H).

Compound 34

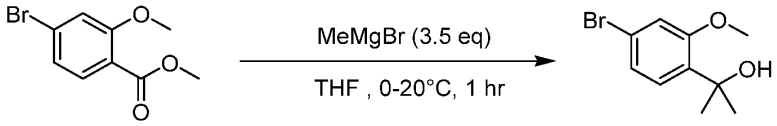
Synthesis of methyl (2-((2-(2-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (Compound 34)



Synthesis of 2-(4-bromo-2-methoxyphenyl)propan-2-ol



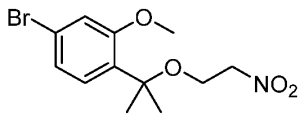
[00568] *Scheme 34A*



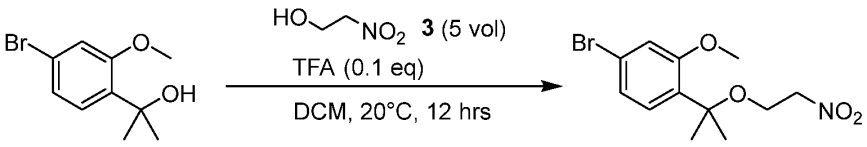
1 **2**

[00569] Methyl 4-bromo-2-methoxybenzoate (**1**) (10 g, 40.80 mmol, 1.0 eq) was added to THF (100 mL) at 20°C. MeMgBr (3 M, 47.61 mL, 3.5 eq) was then added dropwise at 0°C and stirred at 20°C under N_2 for 1h. TLC (petroleum ether/ethyl acetate = 5/1) indicated that (**1**) was consumed completely and many new spots were formed. Saturated NH_4Cl (150 mL) was added dropwise to the reaction mixture at 0°C and then the mixture extracted with EtOAc (50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50/1 to 0/1) to obtain 2-(4-bromo-2-methoxyphenyl)propan-2-ol (**2**) (8 g, 32.64 mmol). LC-MS: 229 (bromo isomers, -OH) [M+1].

Synthesis of 4-bromo-2-methoxy-1-(2-(2-nitroethoxy)propan-2-yl)benzene



[00570] *Scheme 34B*

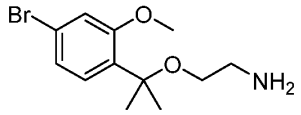


2 **4**

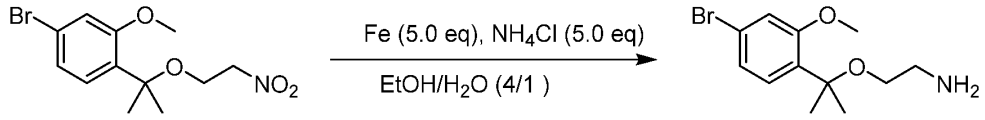
[00571] 2-(4-bromo-2-methoxyphenyl)propan-2-ol (**2**) (5.0 g, 20.40 mmol, 1.0 eq) in dichloromethane (25mL) was added (**3**) (32.30 g, 354.69 mmol, 25 mL, 17.39 eq) at 20 under N_2. TFA (232.59 mg, 2.04 mmol, 151.52 µL, 0.1 eq) was added to the mixture at 20 °C under N_2 and the mixture was stirred at 20°C under N_2 for 12h. TLC (petroleum ether/ethyl acetate = 5/1) indicated the reaction was consumed completely and a major new spot was detected. The reaction mixture was diluted with H_2O (50.0 mL) and extracted with DCM (30 mL × 3). The organic layers were dried over Na_2SO_4, filtered, and the organic layer was concentrated under

reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate=100/1 to 0/1) to provide 4-bromo-2-methoxy-1-(2-(2-nitroethoxy)propan-2-yl)benzene (**4**) (4.0 g, 12.57 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.21 (br d, J = 8.2 Hz, 1H), 7.07 (br d, J = 8.3 Hz, 1H), 7.02 (s, 1H), 4.51 (br t, J = 5.0 Hz, 2H), 3.86 - 3.76 (m, 5H), 1.57 (s, 6H).

Synthesis of 2-((2-(4-bromo-2-methoxyphenyl)propan-2-yl)oxy)ethan-1-amine



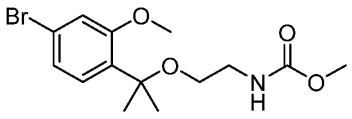
[00572] *Scheme 34C*



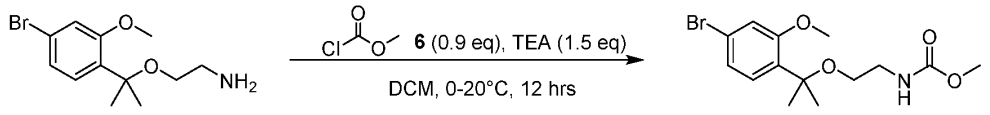
4 **5**

[00573] 4-bromo-2-methoxy-1-(2-(2-nitroethoxy)propan-2-yl)benzene (**4**) (4.0 g, 12.57 mmol, 1.0 eq) in EtOH (32 mL) and H_2O (8 mL) was added Fe (3.51 g, 62.86 mmol, 5.0 eq) and NH_4Cl (3.36 g, 62.86 mmol, 5.0 eq) at 20°C and stirred at 80°C under N_2 for 12h. TLC (petroleum ether/ethyl acetate = 2/1) indicated the reaction was consumed completely and a major new spot was detected. The reaction mixture was filtered and the filtrate concentrated. Then the resulting oil was diluted with H_2O (50 mL) and the mixture extracted with EtOAc (20 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 2-((2-(4-bromo-2-methoxyphenyl)propan-2-yl)oxy)ethan-1-amine (**5**) (3.0 g, crude). The crude product was used in the next step without purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.80 (br s, 2H), 7.37 (d, J = 8.3 Hz, 1H), 7.19 (d, J = 1.7 Hz, 1H), 7.11 (dd, J = 1.7, 8.3 Hz, 1H), 3.83 (s, 3H), 3.42 (t, J = 5.4 Hz, 2H), 2.97 (t, J = 5.4 Hz, 2H), 1.52 (s, 6H).

Synthesis of methyl (2-((2-(4-bromo-2-methoxyphenyl)propan-2-yl)oxy)ethyl)carbamate



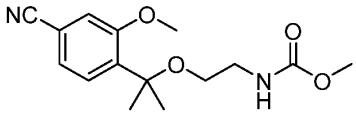
[00574] *Scheme 34D*



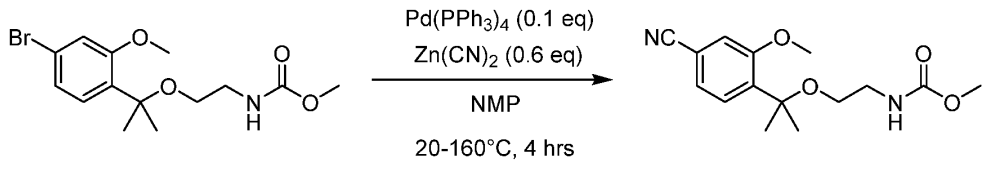
5 **7**

[00575] 2-((2-(4-bromo-2-methoxyphenyl)propan-2-yl)oxy)ethan-1-amine (**5**) (3.0 g, 10.41 mmol, 1.0 eq) in DCM (30mL) was added TEA (1.58 g, 15.62 mmol, 2.17 mL, 1.5 eq) at 20°C. Methyl carbonochloridate (**6**) (885.35 mg, 9.37 mmol, 723.92 µL, 0.9 eq) was added at 0°C under N_2 and the mixture was stirred at 20°C under N_2 for 12h. LC-MS showed that (**5**) was consumed. The reaction mixture was quenched with $NH_3.H_2O$ (10%, 20 mL) and extracted with DCM (20 mL × 3). The organic layers were concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1) to provide methyl (2-((2-(4-bromo-2-methoxyphenyl)propan-2-yl)oxy)ethyl)carbamate (**7**) (1.0 g, 2.89 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.11 (d, J = 8.2 Hz, 1H), 7.01 - 6.95 (m, 2H), 5.40 - 5.22 (m, 1H), 3.80 (s, 3H), 3.59 (s, 3H), 3.28 - 3.18 (m, 4H), 1.50 (s, 6H).

Synthesis of methyl (2-((2-(4-cyano-2-methoxyphenyl)propan-2-yl)oxy)ethyl)carbamate



[00576] *Scheme 34E*

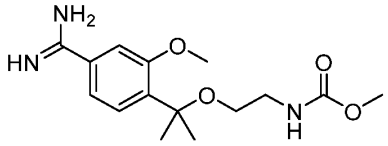


7 **8**

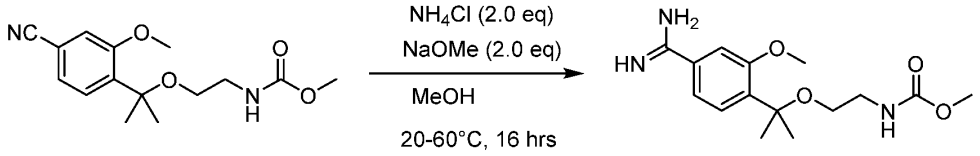
[00577] Methyl (2-((2-(4-bromo-2-methoxyphenyl)propan-2-yl)oxy)ethyl)carbamate (**7**) (1.0 g, 2.89 mmol, 0.9 eq) was added into NMP (10 mL) at 20°C under N_2. $Zn(CN)_2$ (226.10 mg, 1.93 mmol, 122.22 µL, 0.6 eq) and $Pd(PPh_3)_4$ (370.85 mg, 320.93 µmol, 0.1 eq) were added to the mixture at 20°C under N_2. The reaction mixture was stirred at 160°C and microwaved under N_2 for 4h. TLC (petroleum ether/ethyl acetate = 2/1) indicated some (**7**) remained, and some major new spots were detected. The reaction mixture was cooled to 20°C and saturated Na_2CO_3 (50 mL) was added to the mixture at 0°C. The mixture was extracted with EtOAc (20 mL × 3) and the combined organic layers were concentrated under reduced

pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50/1 to 0/1) to provide methyl (2-((2-(4-cyano-2-methoxyphenyl)propan-2-yl)oxy)ethyl)carbamate (**8**) (0.34 g, 1.16 mmol). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.48 (d, J = 7.9 Hz, 1H), 7.25 (d, J = 1.5 Hz, 1H), 7.14 (d, J = 1.0 Hz, 1H), 5.28 (br s, 1H), 3.91 (s, 3H), 3.68 (s, 3H), 3.35 (br s, 4H), 1.59 (s, 6H).

Synthesis of methyl (2-((2-(4-carbamimidoyl-2-methoxyphenyl)propan-2-yl)oxy)ethyl)carbamate



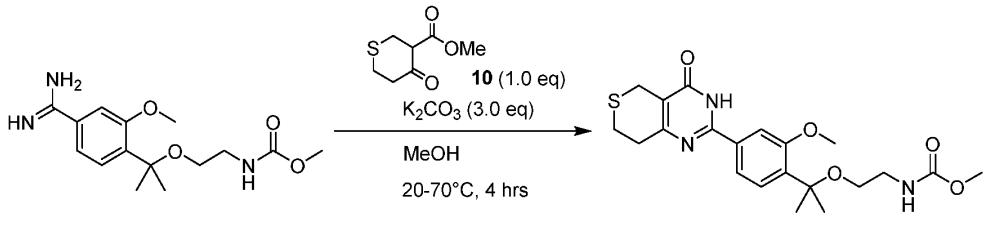
[00578] *Scheme 34F*



8 **9**

[00579] Methyl (2-((2-(4-cyano-2-methoxyphenyl)propan-2-yl)oxy)ethyl)carbamate (**8**) (0.34 g, 1.16 mmol, 1.0 eq) was added to MeOH (3.4 mL) at 20°C under N_2. NaOMe (125.66 mg, 2.33 mmol, 2.0 eq) was then added to the mixture at 20°C under N_2 and stirred at 40°C under N_2 for 12 h. NH_4Cl (124.43 mg, 2.33 mmol, 2.0 eq) was added to the mixture at 40°C and the mixture was stirred at 60°C under N_2 for 4h. LC-MS showed the desired compound was detected. The mixture was then filtered and the filtrate was concentrated under reduced pressure to give methyl (2-((2-(4-carbamimidoyl-2-methoxyphenyl)propan-2-yl)oxy)ethyl)carbamate (**9**) (1.0 g, crude). LC-MS: 310.3 (M+1).

[00580] *Scheme 34G*



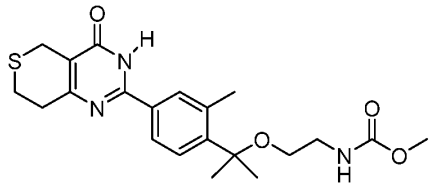
9 **Compound 36**

[00581] Methyl (2-((2-(4-carbamimidoyl-2-methoxyphenyl)propan-2-yl)oxy)ethyl)carbamate (**9**) (1.0 g, 3.23 mmol, 1.0 eq) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**10**) (563.15 mg, 3.23 mmol, 1.0 eq) were added to MeOH (10 mL)

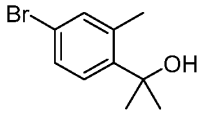
at 20°C under N_2. K_2CO_3 (1.34 g, 9.70 mmol, 3.0 eq) was added to the mixture at 20°C and the mixture stirred at 70°C and microwaved under N_2 for 4h. LC-MS showed that (**9**) was consumed. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give a residue that was purified by prep-HPLC (column: Agela DuraShell C18 250 × 70mm × 10um; mobile phase: [H_2O (10 mM NH_4HCO_3)-ACN]; gradient: 30%-55% B over 20.0 min) to provide methyl (2-((2-(2-methoxy-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**Compound 34**). LC-MS: 435.1 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.79 (s, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.39 (br d, J = 8.1 Hz, 1H), 7.16 - 7.07 (m, 1H), 3.86 (s, 3H), 3.52 (s, 3H), 3.45 (s, 2H), 3.30 - 3.24 (m, 2H), 3.16 (q, J = 5.9 Hz, 2H), 2.79 (br dd, J = 5.3, 18.5 Hz, 4H), 1.52 (s, 6H).

Compound 35

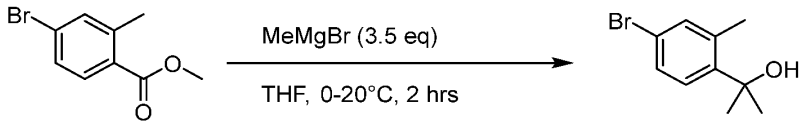
Synthesis of methyl (2-((2-(2-methyl-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (Compound 35)



Synthesis of 2-(4-bromo-2-methylphenyl)propan-2-ol



[00582] *Scheme 35A*

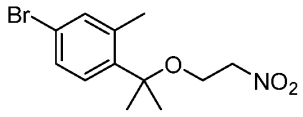


1 **2**

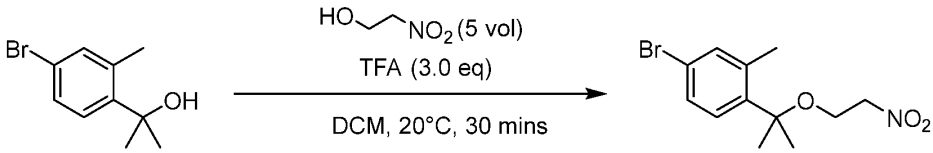
[00583] To a solution of methyl 4-bromo-2-methylbenzoate (**1**) (10 g, 43.65 mmol, 10.00 mL, 1 eq) in THF (100 mL), was added MeMgBr (3 M, 50.93 mL, 3.5 eq) dropwise at 0°C. The mixture was stirred at 20°C for 2h. TLC (petroleum ether/ethyl acetate = 8/1, Rf(Cpd **1**) = 0.60) indicated that (**1**) was consumed, and three new spots were detected. The reaction mixture was poured into sat. NH_4Cl (150 mL) at 0°C and extracted with EtOAc (50 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum

ether/ethyl acetate=50/1 to 0/1) to provide 2-(4-bromo-2-methylphenyl)propan-2-ol (**2**) (6 g, 26.19 mmol). LCMS: 214, 215 (-OH, bromo isomers) [M+1].

Synthesis of 4-bromo-2-methyl-1-(2-(2-nitroethoxy)propan-2-yl)benzene



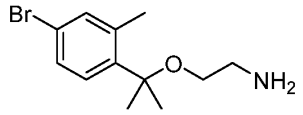
[00584] *Scheme 35B*



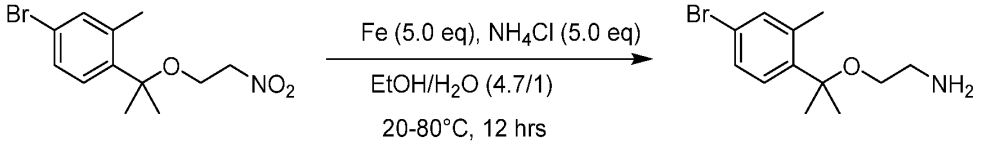
2 **3**

[00585] To a solution of 2-(4-bromo-2-methylphenyl)propan-2-ol (**2**) (4.59 g, 20.03 mmol, 1 eq) in DCM (50 mL) was added trifluoroacetic acid (TFA) (6.85 g, 60.10 mmol, 4.46 mL, 3 eq) and 2-nitroethanol (32.30 g, 354.69 mmol, 25 mL, 17.70 eq) at 20°C and stirred for 30 min. TLC (petroleum ether/ethyl acetate = 5/1) indicated that (**2**) was consumed completely and one major new spot with lower polarity was detected. The reaction was quenched with water (60 mL) and extracted with EtOAc (20 × 10 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50/1 to 0/1) to provide 4-bromo-2-methyl-1-(2-(2-nitroethoxy)propan-2-yl)benzene (**3**) (2.68 g, 8.87 mmol). LCMS: 303.5 + bromo isomers (M+1).

Synthesis of 2-((2-(4-bromo-2-methylphenyl)propan-2-yl)oxy)ethan-1-amine



[00586] *Scheme 35C*

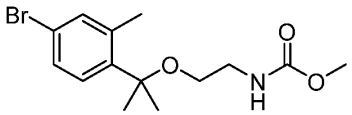


3 **4**

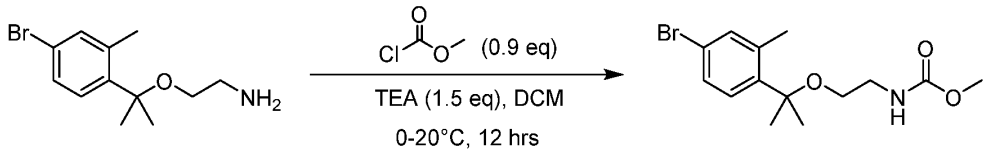
[00587] To a solution of 4-bromo-2-methyl-1-(2-(2-nitroethoxy)propan-2-yl)benzene (**3**) (1.7 g, 5.63 mmol, 1 eq) in EtOH (14 mL) and H_2O (3 mL), was added Fe (1.57 g, 28.13 mmol, 5 eq) and NH_4Cl (1.50 g, 28.13 mmol, 5 eq) at 20°C. The mixture was stirred at 80°C for 12 h.

The reaction mixture was diluted with H_2O (30 mL), extracted with EtOAc (15 mL × 2) and the organic layers combined and concentrated under reduced pressure to give 2-((2-(4-bromo-2-methylphenyl)propan-2-yl)oxy)ethan-1-amine (**4**) (1.4 g, 5.14 mmol). The crude product was used in the next step without purification. ^{1}H NMR (400 MHz, CDCl3): δ = 7.30 (d, J = 1.8 Hz, 1H), 7.25 (d, J = 1.9 Hz, 1H), 7.12 (d, J = 8.3 Hz, 1H), 3.72 (br s, 2H), 3.22 (t, J = 5.3 Hz, 2H), 2.94 (t, J = 5.3 Hz, 2H), 2.53 (s, 3H), 1.62 (s, 6H).

Synthesis of methyl (2-((2-(4-bromo-2-methylphenyl)propan-2-yl)oxy)ethyl)carbamate



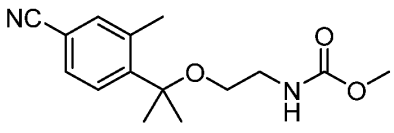
[00588] *Scheme 35D*



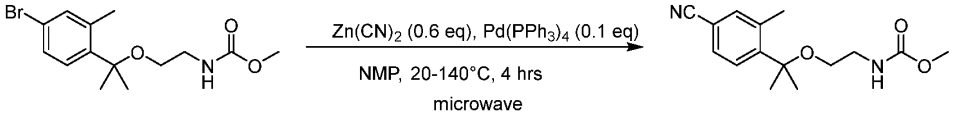
4 **5**

[00589] To a solution of 2-((2-(4-bromo-2-methylphenyl)propan-2-yl)oxy)ethan-1-amine (**4**) (1.4 g, 5.14 mmol, 1 eq) in dichloromethane (DCM) (14 mL), was added triethylamine (TEA) (780.73 mg, 7.72 mmol, 1.07 mL, 1.5 eq) at 20°C. The mixture was cooled to 0°C and methyl carbonochloridate (437.47 mg, 4.63 mmol, 357.70 µL, 0.9 eq) was added dropwise at 0°C. The mixture was stirred at 20°C for 12h. The reaction was quenched with $NH_3.H_2O$ (10%, 20 mL) and extracted with DCM (10 mL × 3). The combined organic layers were concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1) to provide methyl (2-((2-(4-bromo-2-methylphenyl)propan-2-yl)oxy)ethyl)carbamate (**5**) (1.3 g, 3.94 mmol). ^{1}H NMR (400 MHz, CHLOROFORM-d): δ = 7.30 (d, J = 2.0 Hz, 1H), 7.27 - 7.22 (m, 1H), 7.11 (d, J = 8.5 Hz, 1H), 5.01 (br s, 1H), 3.67 (s, 3H), 3.36 - 3.24 (m, 2H), 3.16 (br t, J = 5.1 Hz, 2H), 2.53 (s, 3H), 1.58 (s, 6H).

Synthesis of methyl (2-((2-(4-cyano-2-methylphenyl)propan-2-yl)oxy)ethyl)carbamate



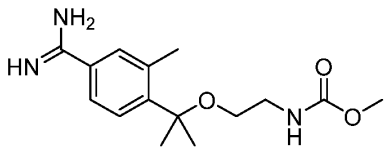
[00590] *Scheme 35E*



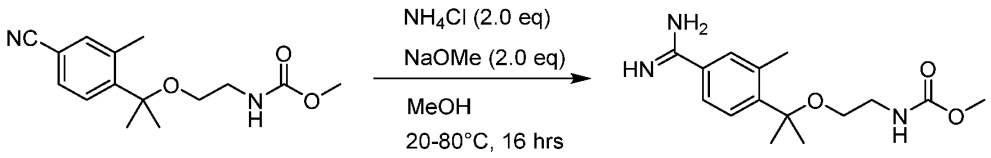
5 6

[00591] To a solution of methyl (2-((2-(4-bromo-2-methylphenyl)propan-2-yl)oxy)ethyl)carbamate (**5**) (0.6 g, 1.82 mmol, 1 eq) in NMP (6 mL) was added $Zn(CN)_2$ (128.01 mg, 1.09 mmol, 69.19 µL, 0.6 eq) and $Pd(PPh_3)_4$ (209.96 mg, 181.70 µmol, 0.1 eq) at 20°C under N_2. The mixture was stirred at 140°C for 4h and microwaved under an N_2 atmosphere. The resulting mixture was cooled to 20°C and diluted with saturated Na_2CO_3 (30 mL). The mixture was extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50/1 to 0/1) to provide methyl (2-((2-(4-cyano-2-methylphenyl)propan-2-yl)oxy)ethyl)carbamate (**6**) (0.3 g, 1.09 mmol). ^{1}H NMR (400 MHz, CDCl3): δ = 7.46 - 7.41 (m, 2H), 7.38 - 7.34 (m, 1H), 5.12 - 4.91 (m, 1H), 3.67 (s, 3H), 3.37 - 3.28 (m, 2H), 3.17 (t, *J* = 5.1 Hz, 2H), 2.59 (s, 3H), 1.61 (s, 6H).

Synthesis of methyl (2-((2-(4-carbamimidoyl-2-methylphenyl)propan-2-yl)oxy)ethyl)carbamate



[00592] *Scheme 35F*

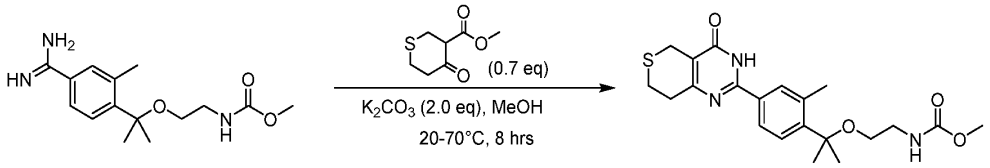


6 7

[00593] To a solution of methyl (2-((2-(4-cyano-2-methylphenyl)propan-2-yl)oxy)ethyl)carbamate (**6**) (0.3 g, 1.09 mmol, 1 eq) in MeOH (3 mL) was added NaOMe (117.30 mg, 2.17 mmol, 2 eq) at 20°C and then stirred at 70°C for 4h. NH_4Cl (116.15 mg, 2.17 mmol, 2 eq) was added the mixture stirred at 80°C for 12h. The reaction mixture was filtered, and the filtrate concentrated under reduced pressure to give methyl (2-((2-(4-

carbamimidoyl-2-methylphenyl)propan-2-yl)oxy)ethyl)carbamate (**7**) (0.32 g, crude). The crude product was used in the next step without purification. LCMS: 294.7 (M+1).

[00594] *Scheme 35G*

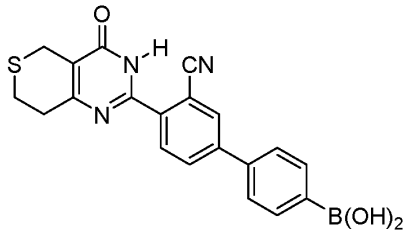


7 **Compound 35**

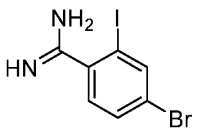
[00595] To a solution of methyl (2-((2-(4-carbamimidoyl-2-methylphenyl)propan-2-yl)oxy)ethyl)carbamate (**7**) (0.32 g, 1.09 mmol, 1 eq) in MeOH (3.2 mL) was added K_2CO_3 (301.52 mg, 2.18 mmol, 2 eq) and methyl 4-oxotetrahydrothiopyran-3-carboxylate (133.03 mg, 763.57 μmol, 0.7 eq) at 20°C under N_2. The mixture was stirred at 70°C for 8h. LC-MS showed that (**7**) was consumed. The reaction mixture was filtered and concentrated under reduced pressure to give a residue that was purified by prep-HPLC (neutral condition: column: Waters Xbridge BEH C18 250 × 50 mm × 10 μm; mobile phase: [H_2O (10mM NH_4HCO_3)-ACN]; gradient: 20%-50% B over 10.0 min) to provide methyl (2-((2-(2-methyl-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**Compound 35**). LC-MS: 418.1 (M+1). ^{1}H NMR (400 MHz, CD3OD): δ = 7.82 (s, 1H), 7.77 (br d, J = 7.9 Hz, 1H), 7.50 (d, J = 8.3 Hz, 1H), 3.63 (s, 3H), 3.62 (s, 2H), 3.28 - 3.22 (m, 2H), 3.21 - 3.16 (m, 2H), 2.98 (br dd, J = 5.2, 14.1 Hz, 4H), 2.68 (s, 3H), 1.66 (s, 6H).

Compound 36

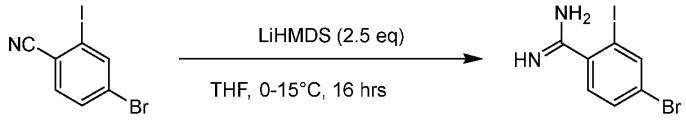
Synthesis of (3'-cyano-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (Compound 36)



Synthesis of 4-bromo-2-iodobenzimidamide



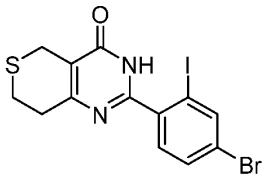
[00596] *Scheme 36A*



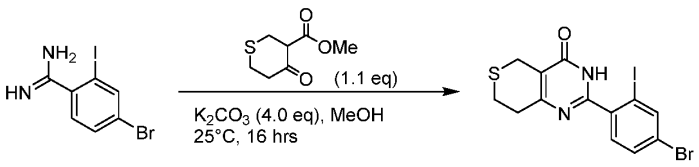
1 2

[00597] To a solution of 4-bromo-2-iodobenzonitrile (**1**) (5.00 g, 16.24 mmol, 1.0 eq) in THF (50 mL), LiHMDS (1 M, 40.60 mL, 2.5 eq) was added dropwise at 0 °C. The mixture was stirred at 15°C for 16 h. TLC (petroleum ether/ethyl acetate = 3/1, R_f (Cpd.**1**) = 0.72) indicated that (**1**) was consumed completely, and one new spot formed. The reaction mixture was diluted with 4 N HCl/dioxane (10 mL). The combined organic layers were concentrated under reduced pressure to give a residue. The residue was triturated with MeOH (10 mL), then filtered. The filter cake was dried *in vacuo* to give a residue. 4-bromo-2-iodobenzimidamide (**2**) (6 g, crude, HCl) was obtained and used immediately in the next step.

Synthesis of 2-(4-bromo-2-iodophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



[00598] *Scheme 36B*

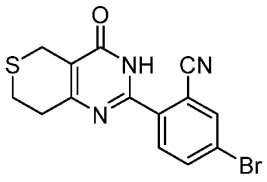


2 3

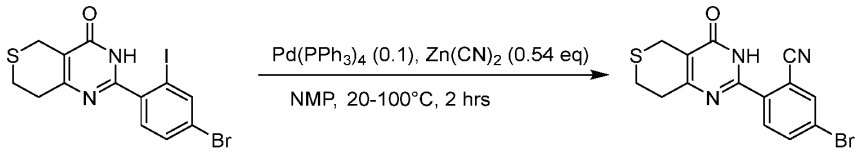
[00599] To a solution of 4-bromo-2-iodobenzimidamide (**2**) (5.87 g, 16.24 mmol, 1.0 eq, HCl) and methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (3.11 g, 17.8 mmol, 1.1 eq) in MeOH (60 mL) was added K_2CO_3 (8.98 g, 64.97 mmol, 4.0 eq) at 25°C. The mixture was stirred at 25°C for 16 h under N_2 atmosphere. LC-MS indicated most of (**2**) was consumed, and one main peak with the desired MS was detected. The resulting mixture was filtered. The filter cake was triturated with H_2O (50 mL) at 25°C for 4 h. Then the mixture was filtered, and the filter cake was dried under reduced pressure to give 2-(4-bromo-2-iodophenyl)-3,5,7,8-

tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (3.78 g, 8.42 mmol, crude), which was used directly in the next step without further purification.

Synthesis of 5-bromo-2-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzonitrile



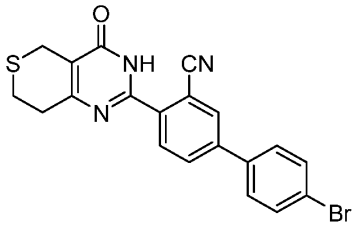
[00600] *Scheme 36C*



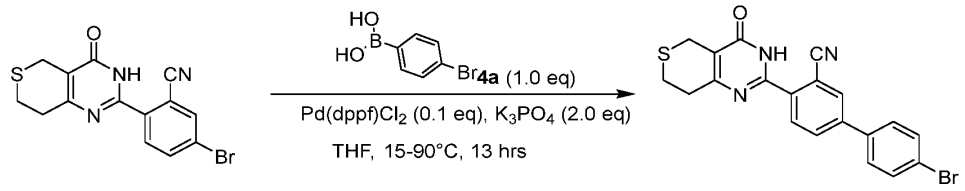
3 **4**

[00601] To a solution of 2-(4-bromo-2-iodophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (3.28 g, 7.30 mmol, 1.0 eq) in NMP (33 mL) was added $Zn(CN)_2$ (463 mg, 3.94 mmol, 250.33 μL, 0.54 eq) in one portion and $Pd(PPh_3)_4$ (844 mg, 730.34 μmol, 0.1 eq) at 20°C under N_2. The mixture was stirred at 100°C for 2 h under N_2. LC-MS showed **3** was consumed completely. The resulting mixture was cooled to 20°C, and diluted with saturated Na_2CO_3 (60 mL) at 0°C; then the mixture was extracted with 60 mL of EtOAc (20 mL × 3). The organic layers were combined, washed with brine (120 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 50/1 to 0/1) to provide 5-bromo-2-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzonitrile (**4**) (1.56 g, 4.48 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.59 (s, 1H), 8.33 (br d, J = 8.3 Hz, 1H), 7.99 - 7.82 (m, 1H), 3.84 - 3.60 (m, 2H), 2.88 (br d, J = 3.5 Hz, 4H).

Synthesis of 4'-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-3-carbonitrile

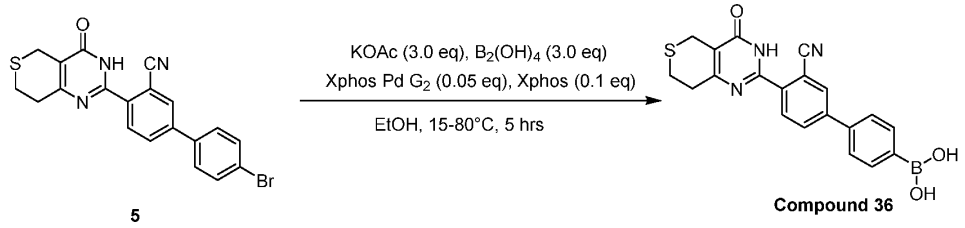


[00602] *Scheme 36D*



[00603] To a solution of 5-bromo-2-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzonitrile (**4**) (300 mg, 861.53 μmol, 1.0 eq) in THF (10 mL) was added (4-bromophenyl)boronic acid (**4a**) (173.02 mg, 861.53 μmol, 1.0 eq), K_3PO_4 (1 M, 1.72 mL, 2.0 eq), and $Pd(dppf)Cl_2$ (63.04 mg, 86.15 μmol, 28.72 μL, 0.1 eq) at 15°C. The mixture was stirred at 90°C for 13 h under N_2. TLC (petroleum ether/ethyl acetate = 2/1, R_f (Cpd.**4**) = 0.50) indicated that (**4**) was consumed completely, and one major new spot formed. After cooling to 15°C, the mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO_2, petroleum ether/ethyl acetate = 2/1) to provide 4'-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-3-carbonitrile (**5**) (400 mg, crude). The compound was used crude in next step.

[00604] *Scheme 36E*

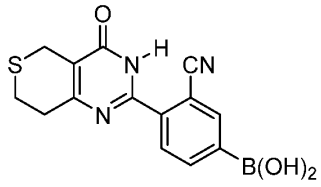


[00605] To a solution of $B_2(OH)_4$ (41.6 mg, 463.81 μmol, 3.0 eq) in EtOH (1 mL) was added4'-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-3-carbonitrile (**5**) (65.6 mg, 154.60 μmol, 1.0 eq), KOAc (45.5 mg, 463.81 μmol, 3.0 eq), XPHOS-pD-G2 (6.08 mg, 7.73 μmol, 0.05 eq), and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (XPhos) (7.37 mg, 15.46 μmol, 0.1 eq) at 15°C. The mixture was stirred at 80°C for 5 h under N_2. LC-MS showed that (**5**) was consumed completely, and one main peak with the desired mass was detected. After cooling to 15°C, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (neutral condition; column: Waters Xbridge Prep OBD C18 150 × 40mm × 10um; mobile phase: [H_2O (10 mM NH_4HCO_3)-ACN]; gradient: 20% - 50% B over 8.0 min) to provide (3'-cyano-4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-

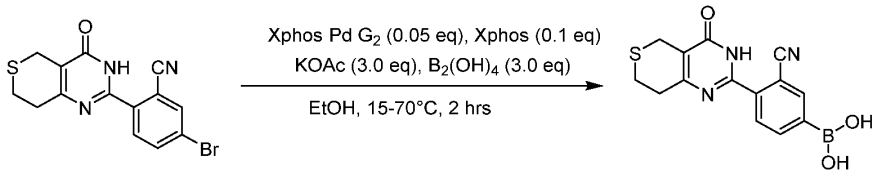
yl)-[1,1'-biphenyl]-4-yl)boronic acid **(Compound 36)**. LC-MS: 369.1 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.70 (d, J = 1.5 Hz, 1H), 8.52 (br d, J = 8.0 Hz, 1H), 8.30 (s, 2H), 8.02 (d, J = 8.0 Hz, 2H), 7.87 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 8.1 Hz, 2H), 3.62 (s, 2H), 2.99 (s, 4H).

Compound 37

Synthesis of (3-cyano-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (Compound 37)



[00606] *Scheme 37A*



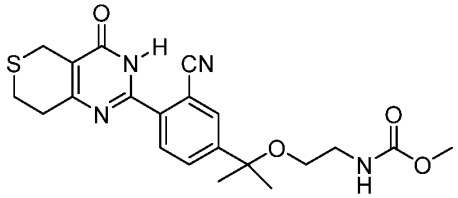
4 **Compound 37**

[00607] The synthesis of 5-bromo-2-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzonitrile (**4**) is shown in Schemes 36A-C.

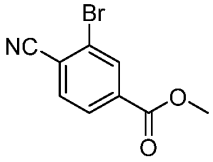
[00608] To a solution of $B_2(OH)_4$ (232 mg, 2.58 mmol, 3.0 eq) in EtOH (12 mL) was added 5-bromo-2-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzonitrile (**4**) (300 mg, 861.53 μmol, 1.0 eq), KOAc (254 mg, 2.58 mmol, 3.0 eq), XPHOS-Pd-G2 (33.9 mg, 43.08 μmol, 0.05 eq), and XPhos (41.1 mg, 86.15 μmol, 0.1 eq) at 15°C under N_2. The mixture was stirred at 70°C under N_2 for 2 h. LC-MS showed that (**4**) was consumed completely, and one main peak with the desired mass was detected. After cooling to 15°C, the mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (neutral condition; column: Waters Xbridge BEH C18 100 × 30 mm × 10 μm; mobile phase: [H_2O (10 mM NH_4HCO_3)-ACN]; gradient: 1% - 30% B over 8.0 mins) to provide (3-cyano-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**Compound 37**). LC-MS: 314.1 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.87 - 12.47 (m, 1H), 8.53 - 8.39 (m, 1H), 8.21 - 7.81 (m, 2H), 7.75 - 7.62 (m, 1H), 7.57 - 7.50 (m, 1H), 3.55 (br s, 2H), 2.91 (br s, 4H).

Compound 38

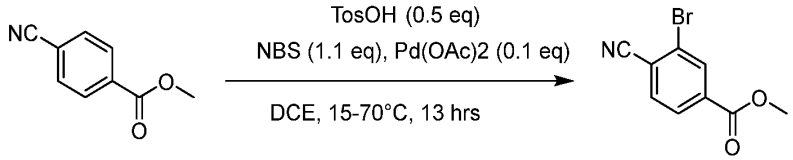
Synthesis of methyl (2-((2-(3-cyano-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (Compound 38)



Synthesis of methyl 3-bromo-4-cyanobenzoate



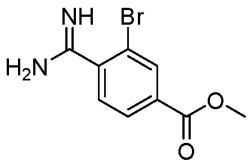
[00609] *Scheme 38A*



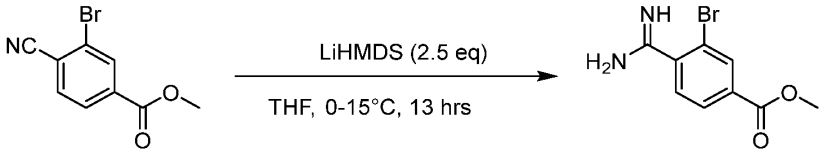
1 **2**

[00610] To a solution of methyl 4-cyanobenzoate (**1**) (10.0 g, 62.1mmol, 1.00 eq) in DCE (100 mL) was added N-bromosuccinimide (NBS) (12.2 g, 68.3 mmol, 1.10 eq), tosOH (5.34 g, 31.0 mmol, 0.5 eq) and $Pd(OAc)_2$ (1.39 g, 6.21 mmol, 0.1 eq) at 15°C. The mixture was stirred at 70°C for 13 h under N_2. TLC (Petroleum ether/Ethyl acetate = 8/1, R_f (**1**) = 0.60) showed some of (**1**) remained. After cooling to 15°C, the mixture was filtered through celatom and the filtrate concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 100/1 to 0/1) to provide methyl 3-bromo-4-cyanobenzoate (**2**) (4.50 g, 18.8 mmol). ^{1}H NMR (400 MHz, $CDCl_3$-*d*):δ 8.27 (d, *J* = 1.3 Hz, 1H), 8.00 (dd, *J* = 1.3, 8.1 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 1H), 3.90 (s, 3H).

Synthesis of methyl 3-bromo-4-carbamimidoylbenzoate



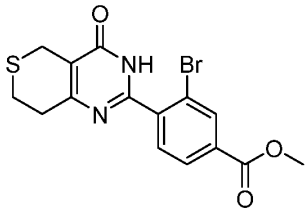
[00611] *Scheme 38B*



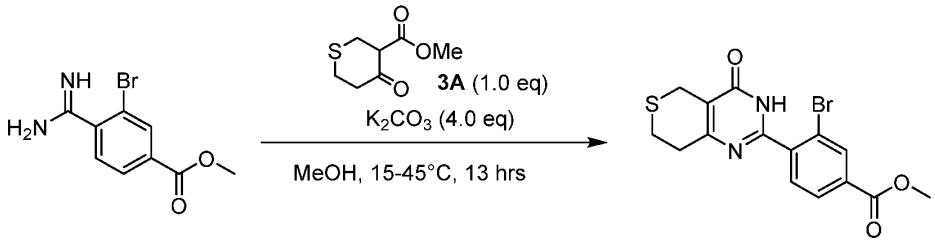
2 **3**

[00612] To a solution of methyl 3-bromo-4-cyanobenzoate (**2**) (4.00 g, 16.7 mmol, 1 eq) in THF (40 mL) was added dropwise LiHMDS (1.00 M, 41.7 mL, 2.50 eq) at 0°C. The mixture was warmed to 15°C and stirred for 13 hrs. LC-MS showed that (**2**) was consumed completely and one main peak with desired mass was detected. The reaction mixture was quenched by addition of HCl/dioxane (4M, 20 mL) at 0°C and then concentrated under reduced pressure to give a residue that was suspended in MeOH (80.0 ml), and stirred at 20°C for 1 hr. The mixture was filtered and the filtrate concentrated under reduced pressure to give methyl 3-bromo-4-carbamimidoylbenzoate (**3**) (4.90 g, crude, HCl), which was used in the next step without further purification. LC-MS (product: R_t = 0.219 min).

Synthesis of methyl 3-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate



[00613] *Scheme 38C*

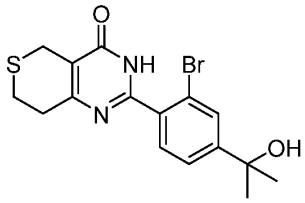


3 **4**

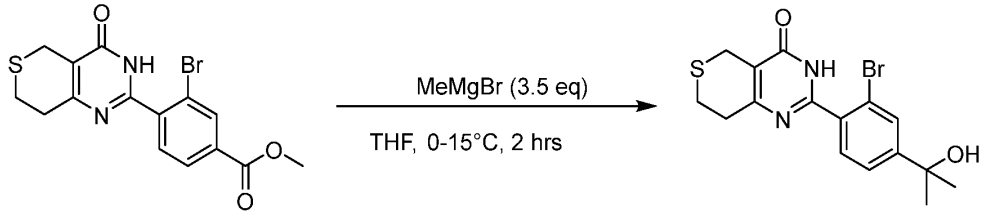
[00614] To a solution of methyl 3-bromo-4-carbamimidoylbenzoate (**3**) (4.90 g, 16.7 mmol, 1 eq, HCl) in MeOH (50 mL) was added methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**3A**) (2.91 g, 16.7 mmol, 1 eq) and K_2CO_3 (9.23 g, 66.8 mmol, 4 eq) at 15°C. The mixture heated to 45°C and stirred for 13 hrs. LC-MS showed that (**3**) was consumed completely and one main peak with desired mass was detected. After cooling to 15°C, the reaction mixture was

filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was suspended in water (50.0 mL), and stirred at 15°C for 4 hrs. The mixture was filtered, and the filter cake dried under vacuum to give methyl 3-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**4**) (3.80 g, crude). ^{1}H NMR: (400 MHz, DMSO-d_6) δ 13.04 - 12.82 (m, 1H), 8.21 (d, J = 1.2 Hz, 1H), 8.05 (dd, J = 1.3, 7.9 Hz, 1H), 7.72 (d, J = 8.1 Hz, 1H), 3.91 (s, 3H), 3.54 (s, 2H), 2.92 - 2.84 (m, 4H). LC-MS (product: R_t = 0.395 min).

Synthesis of 2-(2-bromo-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



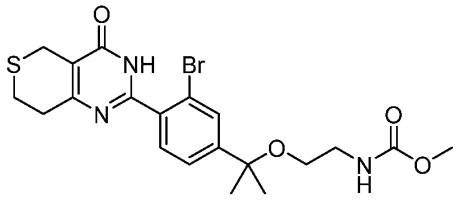
[00615] *Scheme 38D*



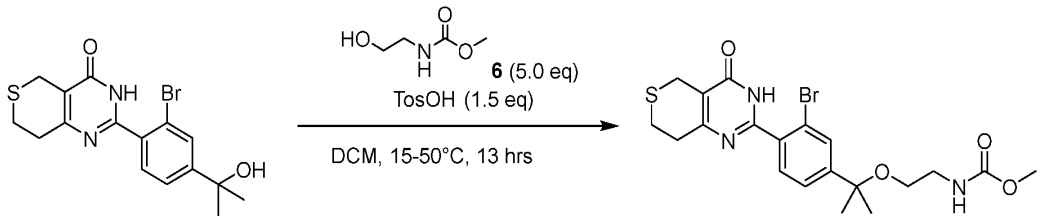
4 **5**

[00616] To a solution of methyl 3-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**4**) (3.80 g, 9.97 mmol, 1 eq) in THF (27.0 mL) was added dropwise, MeMgBr (3M, 11.6 mL, 3.50 eq) at 0°C. The mixture was warmed to 15°C and stirred for 2 hrs. TLC (Petroleum ether/Ethyl acetate = 1/1, R_f (**4**) = 0.46) indicated that (**4**) was consumed completely and many new spots formed. The reaction mixture was poured into saturated NH_4Cl (60 mL) at 0°C and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue that was purified by prep-HPLC (neutral condition; column: Waters Xbridge BEH C18 250 * 50 mm * 10 μm; mobile phase: [H_2O (10 mM NH_4HCO_3) - ACN]; gradient: 20% - 50% B over 10.0 mins). 2-(2-bromo-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (2.00 g, 5.19 mmol) was obtained. LC-MS (product: R_t = 0.358 mins).

Synthesis of methyl (2-((2-(3-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate

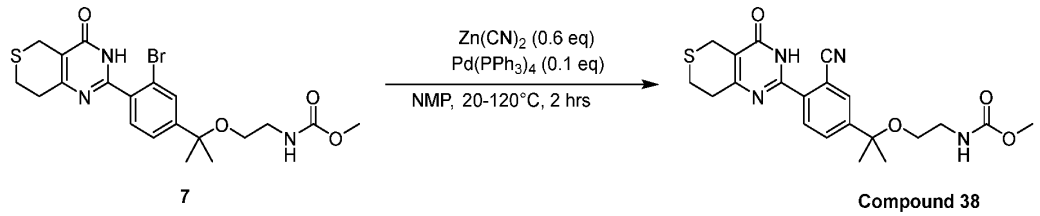


[00617] *Scheme 38E*



[00618] To a solution of 2-(2-bromo-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**5**) (600 mg, 1.57 mmol, 1 eq) and methyl (2-hydroxyethyl)carbamate (**6**) (937 mg, 7.87 mmol, 5 eq) in DCM (5 mL) was added TosOH (406 mg, 2.36 mmol, 1.50 eq) at 15°C. The mixture was heated to 50°C and stirred for 13 hrs. LC-MS showed some of (**5**) remained. After cooling to 15°C, the reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (neutral condition; column: Waters Xbridge Prep OBD C18 150 * 40 mm * 10 μm; mobile phase: [H_2O (10 mM NH_4HCO_3) - ACN]; gradient: 20% - 50% B over 8.0 mins) to provide methyl (2-((2-(3-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**7**) (100 mg, crude). ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ 7.62 (d, J = 1.3 Hz, 1H), 7.50 (s, 1H), 7.46 (d, J = 1.5 Hz, 1H), 5.07 - 4.93 (m, 1H), 3.61 (s, 3H), 3.55 (s, 2H), 3.29 - 3.19 (m, 4H), 2.96 (s, 2H), 2.86 (s, 2H), 1.46 (s, 6H). LC-MS (product: R_t = 1.291 min).

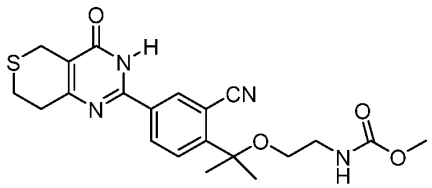
[00619] *Scheme 38F*



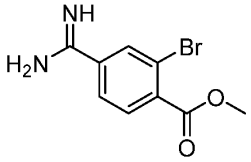
[00620] To a solution of methyl (2-((2-(3-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**7**) (75 mg, 155.48 μmol, 1.0 eq) in NMP (1.5 mL) was added in portions, both $Zn(CN)_2$ (10.95 mg, 93.29 μmol, 5.92 μL, 0.6 eq) and $Pd(PPh_3)_4$ (17.97 mg, 15.55 μmol, 0.1 eq) at 20°C. The mixture was heated to 120°C and stirred for 2 hrs. LC-MS showed that (**7**) was consumed completely. The resulting mixture was cooled to 20°C, diluted with saturated Na_2CO_3 (3.00 mL) at 0°C, and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue that was purified by prep-HPLC (neutral condition; column: Waters xbridge 150 * 25 mm 10 μm; mobile phase: [water (NH_4HCO_3) - ACN]; gradient: 25% - 55% B over 10 mins) to provide methyl (2-((2-(3-cyano-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**Compound 38**). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.03 - 7.93 (m, 1H), 7.91 - 7.82 (m, 1H), 7.27 - 7.15 (m, 1H), 3.65 - 3.53 (m, 2H), 3.53 - 3.49 (m, 3H), 3.21 - 3.12 (m, 4H), 2.98 - 2.83 (m, 4H), 1.56 - 1.45 (m, 6H). LC-MS (EC16472-22-P1J2, product: R_t = 1.600/2.078 min).

Compound 39

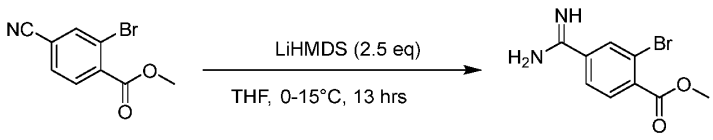
Synthesis of methyl (2-((2-(2-cyano-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (Compound 39)



Synthesis of methyl 2-bromo-4-carbamimidoylbenzoate



[00621] *Scheme 39A*

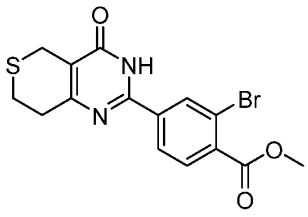


1 2

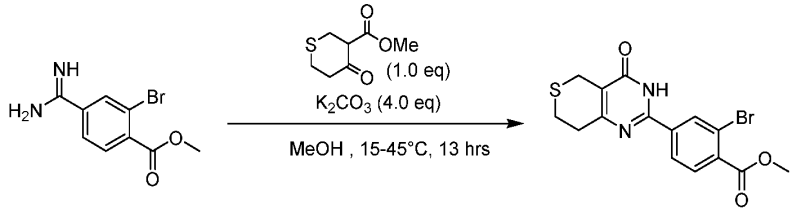
[00622] To a solution of Methyl 2-bromo-4-cyanobenzoate (**1**) (2.5 g, 10.41 mmol, 1 eq) in THF (25 mL) was added drop-wise LiHMDS (1 M, 26.04 mL, 2.5 eq) at 0°C. The mixture was

stirred at 15°C for 13 hrs. LC-MS showed that (**1**) was consumed completely and one main peak with desired mass was detected. The reaction mixture was quenched by addition of HCl/dioxane (4M,13 mL) at 0°C and concentrated under reduced pressure to give a residue that was suspended in MeOH (50 ml) and stirred at 15°C for 1 hr. The mixture was filtered and the filtrate was concentrated under reduced pressure to give a methyl 2-bromo-4-carbamimidoylbenzoate (**2**) (3.1 g, crude), which was used into next step without further purification. LC-MS (product: R_t = 0.231 mins).

Synthesis of methyl 2-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate



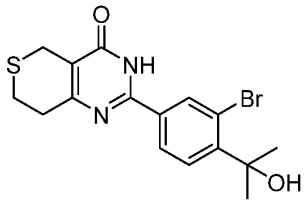
[00623] *Scheme 39B*



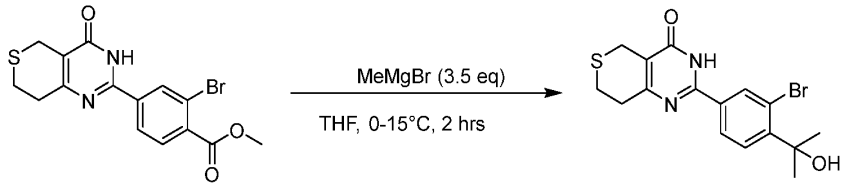
2 **3**

[00624] To a solution of methyl 2-bromo-4-carbamimidoylbenzoate (**2**) (3.1 g, 10.56 mmol, 1 eq, HCl) in MeOH (30 mL) was added methyl 4-oxotetrahydrothiopyran-3-carboxylate (1.84 g, 10.56 mmol, 1 eq) and K_2CO_3 (5.84 g, 42.24 mmol, 4 eq) at 15°C. The mixture was stirred at 45°C for 13 hrs. LC-MS showed that (**2**) was consumed completely and one main peak with desired mass was detected. After cooling to 15°C, the reaction mixture was filtered and the filtrate concentrated under reduced pressure to give a residue that was suspended in water (40 mL) and stirred at 15°C for 4 hrs. The mixture was then filtered and the filter cake dried under reduced pressure to give methyl 2-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**3**) (2.89 g, crude). ^{1}H NMR**:**(400 MHz, DMSO-d_6) δ 8.45 (s, 1H), 8.19 (br d, J = 7.9 Hz, 1H), 7.88 (d, J = 8.2 Hz, 1H), 3.92 - 3.86 (m, 3H), 3.58 - 3.51 (m, 2H), 2.90 (s, 4H). LC-MS (product: R_t = 1.559 mins).

Synthesis of 2-(3-bromo-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



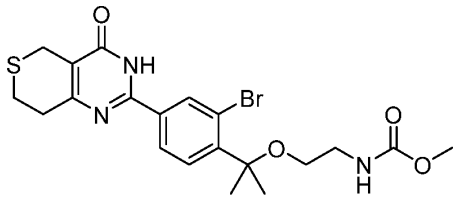
[00625] *Scheme 39C*



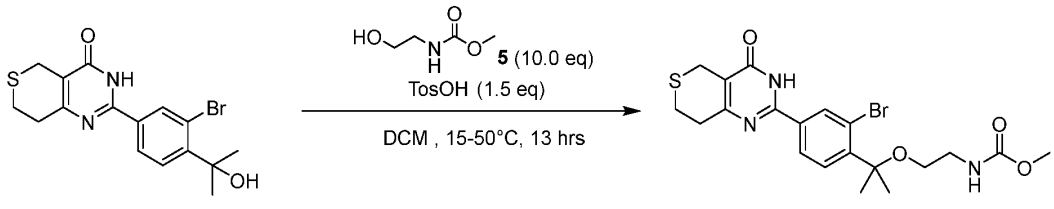
3 **4**

[00626] To a solution of methyl 2-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**3**) (890 mg, 2.33 mmol, 1.0 eq) in THF (8.9 mL) was added dropwise MeMgBr (3 M, 2.72 mL, 3.5 eq) at 0°C. The mixture was stirred at 15°C for 2 hrs. LC-MS showed that (**3**) was consumed completely and desired mass was detected. The mixture was poured into saturated NH_4Cl (20 ml) at 0°C and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give 2-(3-bromo-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (800 mg, crude). LC-MS (product: R_t = 0.418 mins).

Synthesis of methyl (2-((2-(2-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate



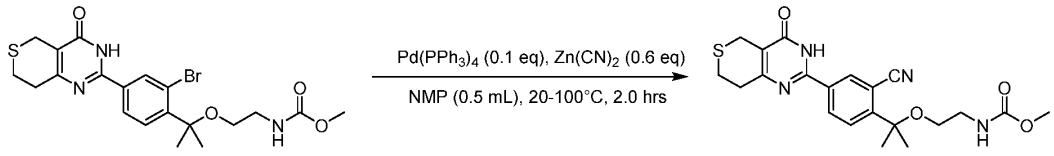
[00627] *Scheme 39D*



4 **6**

[00628] To a solution of compound2-(3-bromo-4-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (500 mg, 1.31 mmol, 1.0 eq) and methyl (2-hydroxyethyl)carbamate (**5**) (1.56 g, 13.1 mmol, 10.0 eq) in dichloromethane (DCM) (5.0 mL) was added TosOH (338.72 mg, 1.97 mmol, 1.5 eq) at 15°C. The mixture was stirred at 50°C for 13 hrs under N_2. LC-MS showed some of (**4**) remained. After cooling to 15°C, the mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (neutral condition; column: Waters X bridge BEH C18100 * 30 mm * 10 μm; mobile phase: [H_2O (10 mM NH_4HCO_3) - ACN]; gradient: 20%-50% B over 8.0 min). Compound methyl (2-((2-(2-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**6**) (60 mg, 124.38 μmol) was obtained. LC-MS (product: R_t = 1.478 mins).

[00629] *Scheme 39E*

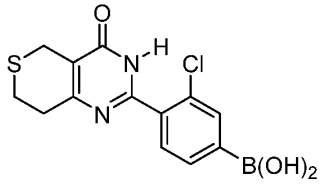


6 Compound 39

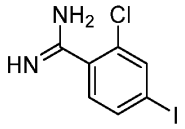
[00630] To a solution of methyl (2-((2-(2-bromo-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**6**) (30 mg, 62.19 μmol, 1 eq) in NMP (0.5 mL) was added $Zn(CN)_2$ (4.38 mg, 37.31 μmol, 2.37 μL, 0.6 eq) and $Pd(PPh_3)_4$ (7.19 mg, 6.22 μmol, 0.1 eq) at 20°C under N_2. The mixture was stirred at 100°C for 2 hrs. The resulting mixture was cooled to 0°C and diluted with saturated Na_2CO_3 (5 mL) at 0°C. The mixture was extracted with EtOAc and washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue that was purified by prep-HPLC (neutral condition; column: Waters X bridge BEH C18 100 * 30 mm * 10 μm; mobile phase: [H_2O (10 mM NH_4HCO_3) - ACN]; gradient: 15%-50% B over 8.0 min) to provide Methyl (2-((2-(2-cyano-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**Compound 39**). ^1H NMR (400 MHz, DMSO-d_6): δ 13.12 - 12.68 (m, 1H), 8.53 (s, 1H), 8.33 (br d, J = 8.1 Hz, 1H), 7.73 (d, J = 8.3 Hz, 1H), 7.08 - 7.00 (m, 1H), 3.55 (br s, 2H), 3.50 (s, 3H), 3.25 - 3.17 (m, 4H), 2.97 - 2.86 (m, 4H), 1.62 (s, 5H). LC-MS (product: R_t = 2.412 mins).

Compound 40

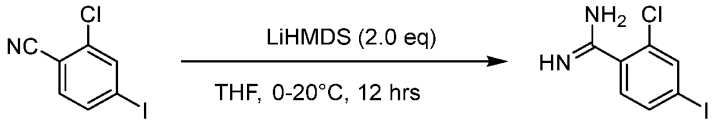
Synthesis of (3-chloro-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (Compound 40)



Synthesis of 2-chloro-4-iodobenzimidamide



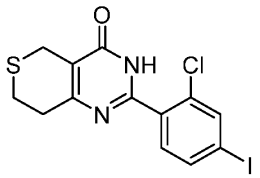
[00631] *Scheme 40A*



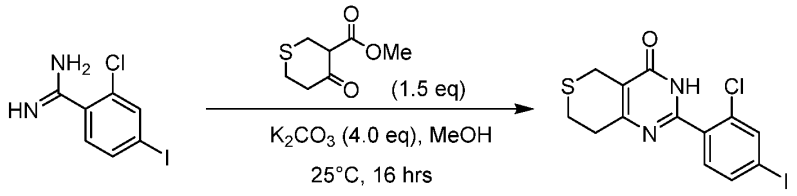
1 **2**

[00632] To a solution of 2-chloro-4-iodobenzonitrile (**1**) (500 mg, 1.90 mmol, 1 eq) in THF (10 mL) was added LiHMDS (1 M, 3.80 mL, 2 eq) dropwise at 0°C. The mixture was stirred at 20°C for 12h. LCMS showed that (**1**) was consumed. The reaction mixture was quenched by addition HCl/ dioxane (4 mL) at 0°C, then concentrated under reduced pressure to give a residue that was dissolved in MeOH (5 mL) and stirred at 20°C for 0.5 hrs. The mixture was filtered and the filtrate concentrated under reduced pressure to give 2-chloro-4-iodobenzimidamide (**2**) (3.2 g, crude). LCMS: 280.9 (M+1).

Synthesis of 2-(2-chloro-4-iodophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



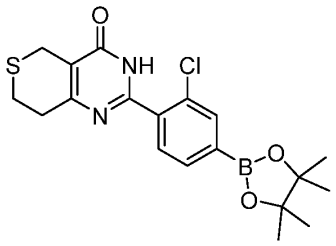
[00633] *Scheme 40B*



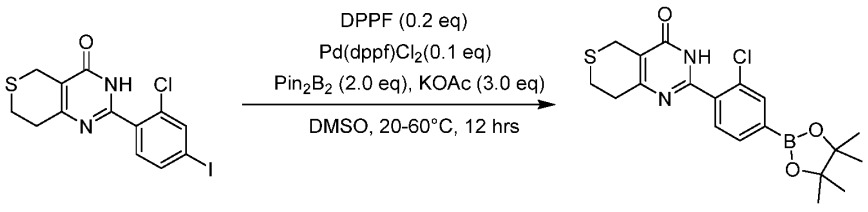
2 3

[00634] To a solution of 2-chloro-4-iodobenzimidamide (**2**) (0.5 g, 1.78 mmol, 1.0 eq) and methyl 4-oxotetrahydrothiopyran-3-carboxylate (465.83 mg, 2.67 mmol, 1.5 eq) in MeOH (10 mL) was added K_2CO_3 (985.48 mg, 7.13 mmol, 4 eq) at 25°C and stirred for 16 h. LCMS showed that (**2**) was consumed. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to give a residue that was suspended in water (5 mL) and stirred at 25°C for 1 h and then filtered. The filtrate was dried in vacuo to give 2-(2-chloro-4-iodophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (1.5 g, crude). The crude product was used in the next step without purification. LCMS: 404.9 (M+1).

Synthesis of 2-(2-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



[00635] *Scheme 40C*

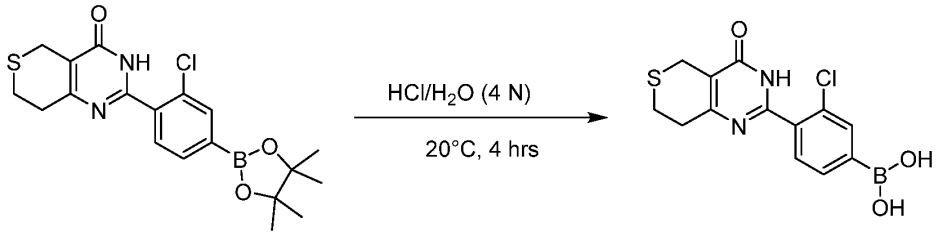


3 4

[00636] To a solution of 2-(2-chloro-4-iodophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**3**) (500 mg, 1.24 mmol, 1 eq) in DMSO (5.0 mL) was added Pin_2B_2 (470.66 mg, 1.85 mmol, 1.5 eq) and KOAc (485.06 mg, 4.94 mmol, 4 eq) at 20°C. The mixture was degassed and purged with N_2 3 times. DPPF (137.00 mg, 247.13 μmol, 0.2 eq) and $Pd(dppf)Cl_2$ (90.41 mg, 123.56 μmol, 0.1 eq) were added in one portion at 20°C and stirred at

60°C for 12 h under N_2. The mixture was cooled to 20°C and diluted with EtOAc (10 mL). The mixture was filtered and the filtrate was washed brine. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give 2-(2-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (900 mg, crude) which was used in next step without further purification. LCMS: 405.6 (M+1).

[00637] *Scheme 40D*

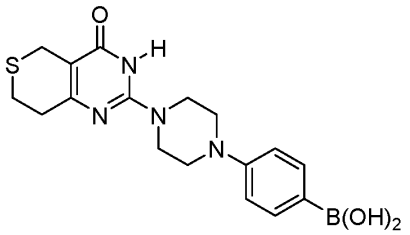


4 **Compound 40**

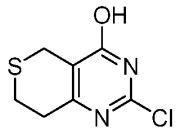
[00638] 2-(2-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (300 mg, 741.26 µmol, 1 eq) in HCl/H_2O (4 M, 1.5 mL) was stirred at 20°C for 4h. The reaction mixture was filtered and the filter cake was dried in vacuo to give a residue. The residue was purified by prep-HPLC (column: Phenomenex luna C18 100 * 40 mm * 3 µm; mobile phase: [H_2O (0.04% HCl)-ACN]; gradient: 10%-35% B over 18.0 min) to provide (3-chloro-4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid (**compound 40**). LCMS: 323.0 (M+1). ^{1}H NMR: (400 MHz, DMSO-d_6) δ= 7.91 (s, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.54 (d, J = 7.5 Hz, 1H), 3.53 (s, 2H), 2.94 - 2.81 (m, 4H).

Compound 41

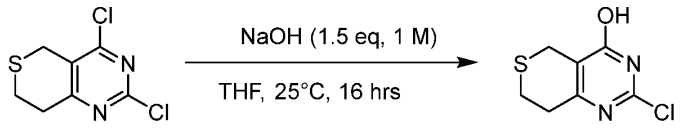
Synthesis of (4-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)piperazin-1-yl)phenyl)boronic acid (Compound 41)



Synthesis of 2-chloro-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol



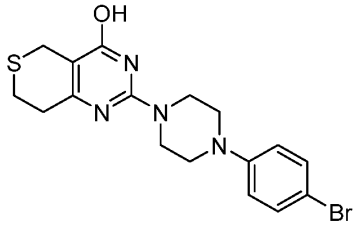
[00639] *Scheme 41A*



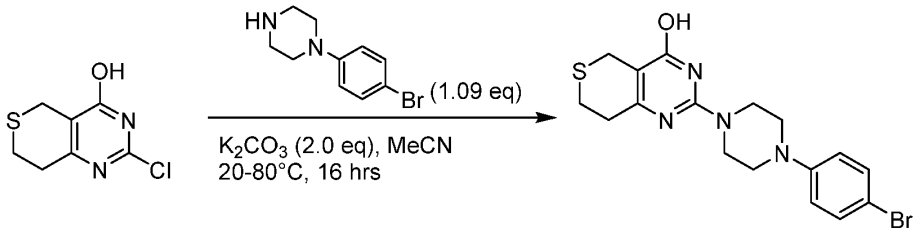
1 **2**

[00640] To a solution of 2,4-dichloro-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidine (**1**) (0.5 g, 2.26 mmol, 1 eq) in THF (1 mL) was added NaOH (1 M, 3.39 mL, 1.5 eq) at 25°C. The mixture was stirred at 25°C for 16 h and followed by TLC. The mixture was quenched with H_2O (10 mL) and extracted with EtOAc (10 mL). The aqueous phase was acidified with 0.1 N HCl to pH = 3-4 and stirred at 25°C for 12 h. Then the mixture was extracted with 60 mL of EtOAc (20 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 2-chloro-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol (**2**) (0.28 g, crude), which was used as a crude in next step. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.49 (br s, 2H), 2.85 (br dd, J = 4.5, 14.6 Hz, 4H).

Synthesis of 2-(4-(4-bromophenyl)piperazin-1-yl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol



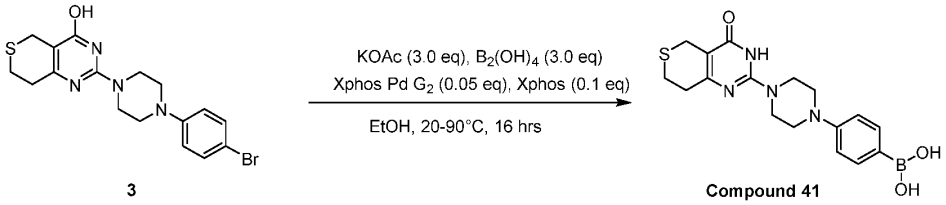
[00641] *Scheme 41B*



2 **3**

[00642] To a solution of 2-chloro-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol (**2**) (0.2 g, 986.87 μmol, 1 eq) in MeCN (2 mL) was added 1-(4-bromophenyl)piperazine (0.26 g, 1.08 mmol, 1.09 eq) and K_2CO_3 (272.78 mg, 1.97 mmol, 2 eq) at 20°C. The mixture was stirred at 80°C for 16 h. LC-MS showed that (**2**) was consumed completely, and the desired mass was detected. The mixture was diluted with water (5 mL) and stirred at 20°C for 4 h. The mixture was filtered, and the filter cake was dried under reduced pressure to give 2-(4-(4-bromophenyl)piperazin-1-yl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol (**3**) (0.2 g, crude) and used immediately in the next step.

[00643] *Scheme 41C*

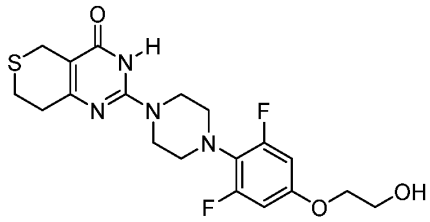


3 Compound 41

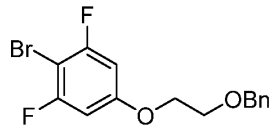
[00644] To a solution of 2-(4-(4-bromophenyl)piperazin-1-yl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol (**3**) (380 mg, 932.91 μmol, 1 eq) and $B_2(OH)_4$ (250.91 mg, 2.80 mmol, 3 eq) in EtOH (19 mL) was added KOAc (274.67 mg, 2.80 mmol, 3.0 eq), XPHOS-PD-G2 (36.70 mg, 46.65 μmol, 0.05 eq), and XPhos (44.47 mg, 93.29 μmol, 0.1 eq) at 20°C. The mixture was stirred at 90 °C for 16 h. LC-MS showed that (**3**) was consumed partly, and one main peak with the desired mass was detected. The reaction mixture was filtered, then the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150 × 40 mm × 10 μm; mobile phase: [H_2O (10 mm NH_4HCO_3)-ACN]; gradient: 15%-55% B over 8.0 min) to provide (4-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)piperazin-1-yl)phenyl)boronic acid (**Compound 41**). LC-MS: 373.1 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.29 (br s, 1H), 7.72 (s, 2H), 7.70 - 7.64 (m, J = 8.5 Hz, 2H), 6.98 - 6.85 (m, J = 8.6 Hz, 2H), 3.77 - 3.62 (m, 4H), 3.42 - 3.35 (m, 2H), 3.29 - 3.19 (m, 4H), 2.82 (t, J = 5.9 Hz, 2H), 2.66 (br t, J = 5.4 Hz, 2H).

Compound 42

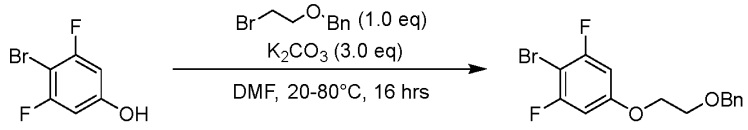
Synthesis of 2-(4-(2,6-difluoro-4-(2-hydroxyethoxy)phenyl)piperazin-1-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 42)



Synthesis of 5-(2-(benzyloxy)ethoxy)-2-bromo-1,3-difluorobenzene



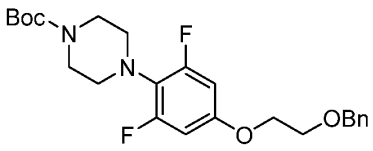
[00645] *Scheme 42A*



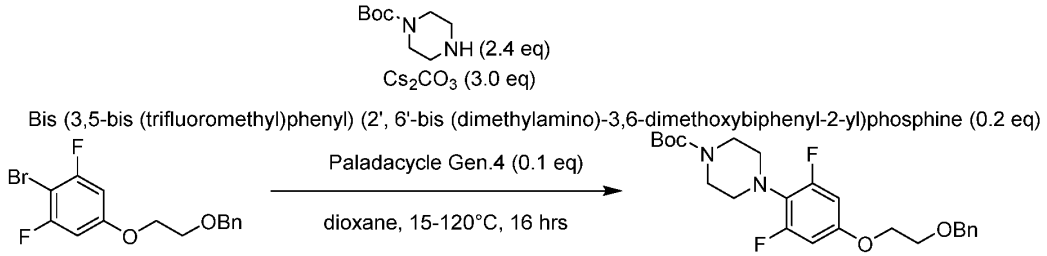
1 **2**

[00646] To a solution of 4-bromo-3,5-difluorophenol (**1**) (2.00 g, 9.57 mmol, 1.00 eq) in DMF (12.0 mL) was added 2-bromoethoxymethylbenzene (2.06 g, 9.57 mmol, 1.51 mL, 1.00 eq) and K_2CO_3 (3.97 g, 28.7 mmol, 3.00 eq) at 20°C. The mixture was heated to 80°C and stirred for 16 h. The mixture was cooled to 20°C, then diluted with EtOAc (10.0 mL) and washed with NaCl (10.0 mL × 3). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 5-(2-(benzyloxy)ethoxy)-2-bromo-1,3-difluorobenzene (**2**) (3.00 g, crude). The compound was used without further purification in the next step. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.34 (m, 5H), 6.60 (m, 2H), 4. 64 (m, 2H), 4.13 (m, 2H), 3.83 (m, 2H). ^{19}F NMR (400 MHz, $CDCl_3$-*d*) δ -104.84 (s).

Synthesis of tert-butyl 4-(4-(2-(benzyloxy)ethoxy)-2,6-difluorophenyl)piperazine-1-carboxylate



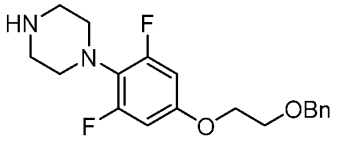
[00647] *Scheme 42B*



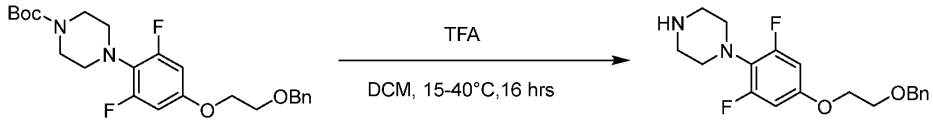
2 **3**

[00648] To a solution of 5-(2-(benzyloxy)ethoxy)-2-bromo-1,3-difluorobenzene (**2**) (1.00 g, 2.91 mmol, 1.00 eq) in dioxane (20.0 mL) was added tert-butyl piperazine-1-carboxylate (1.30 g, 6.99 mmol, 2.40 eq), Cs_2CO_3 (2.85 g, 8.74 mmol, 3.00 eq), Bis (3,5-bis(trifluoromethyl)phenyl) (2', 6'-bis(dimethylamino)-3,6-dimethoxybiphenyl-2-yl)phosphine (440.93 mg, 582.81 μmol, 0.20 eq), and Paladacycle Gen.4 (332.5 mg, 291.41 μmol, 0.10 eq) at 15°C under N_2. The mixture was heated to 120°C and stirred for 16 h under N_2. LC-MS showed that (**2**) was consumed. After cooling to 15°C, the mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 100/1 to 0/1) to provide tert-butyl 4-(4-(2-(benzyloxy)ethoxy)-2,6-difluorophenyl)piperazine-1-carboxylate (**3**) (350 mg, 780.38 μmol). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) *δ:*7.29-7.21 (m, 5H), 6.38 (m, 2H), 4.55 (m, 2H), 3.94 (m, 2H), 3.71 (m, 2H), 3.46 (m, 4H), 2.96 (m, 4H), 1.41 (M, 9H). ^{19}F NMR (400 MHz, $CDCl_3$-*d*) *δ:* -118.34 (s).

Synthesis of 1-(4-(2-(benzyloxy)ethoxy)-2,6-difluorophenyl)piperazine



[00649] *Scheme 42C*

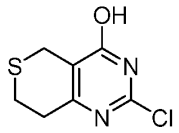


3 **4**

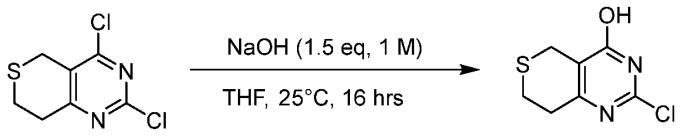
[00650] To a solution of tert-butyl 4-(4-(2-(benzyloxy)ethoxy)-2,6-difluorophenyl)piperazine-1-carboxylate (**3**) (350 mg, 780.38 μmol, 1.00 eq) in DCM (3.50 mL) was added TFA (2.69 g, 23.56 mmol, 1.75 mL) at 15°C. The mixture was heated to

40°C and stirred for 16 h. After cooling to 15°C, the reaction mixture was concentrated under reduced pressure to give 1-(4-(2-(benzyloxy)ethoxy)-2,6-difluorophenyl)piperazine (**4**) (350 mg, crude, TFA). LC-MS: 349.2 (M+1).

Synthesis of 2-chloro-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol



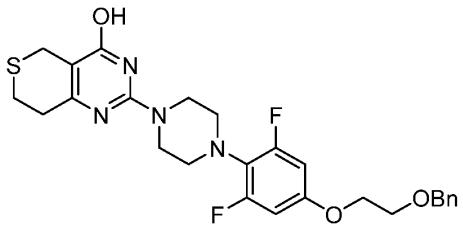
[00651] *Scheme 42D*



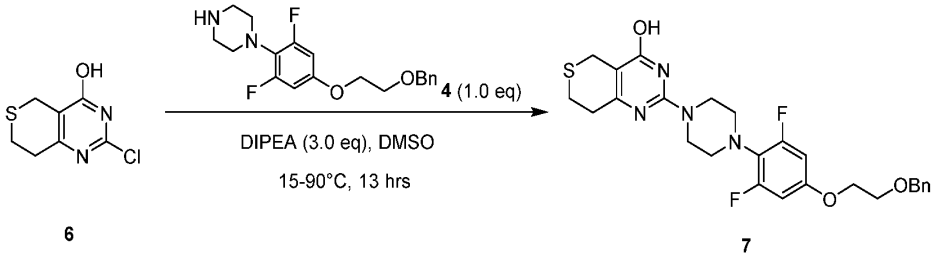
5 **6**

[00652] To a solution of 2,4-dichloro-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidine (**5**) (500 mg, 2.26 mmol, 1.00 eq) in THF (1.00 mL) was added NaOH (1 M, 3.39 mL, 1.50 eq) at 25°C and stirred for 16 h. The mixture was quenched with H_2O (10.0 mL) and extracted with EtOAc (10.0 mL). The aqueous phase was acidified with 1N HCl (20.0 mL) to adjust the pH to 3-4, and then stirred at 25°C for 12 h. The mixture was extracted with EtOAc (10.0 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 2-chloro-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol (**6**) (0.8 g, 2.84 mmol). ^{1}H NMR (400 MHz, $CDCl_3$-*d*) *δ:* 3.54 (s, 2H), 2.87 (m, 2H), 2.82 (m, 2H). LC-MS: 203.2 (M+1).

Synthesis of 2-(4-(4-(2-(benzyloxy)ethoxy)-2,6-difluorophenyl)piperazin-1-yl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol



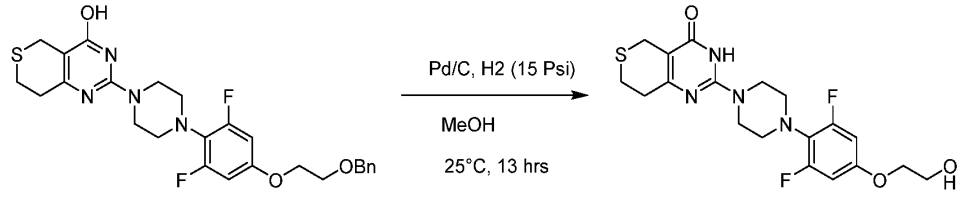
[00653] *Scheme 42E*



6 7

[00654] To a solution of 1-(4-(2-(benzyloxy)ethoxy)-2,6-difluorophenyl)piperazine (**4**) (300 mg, 779.53 μmol, 1.00 eq, TFA) and 2-chloro-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol (**6**) (157.98 mg, 779.53 μmol, 1.00 eq) in DMSO (3.00 mL) was added N,N-diisopropylethylamine (DIPEA also called Hünig's base) (302.25 mg, 2.34 mmol, 407.34 μL, 3.00 eq) at 15°C. The mixture was stirred at 90°C for 13 hrs and then cooled to 15°C. The mixture was diluted with water (10.0 mL) and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue that was purified by prep-HPLC (neutral condition; Waters Xbridge Prep OBD C18 150 × 40 mm × 10 μm; mobile phase: [H_2O (0.05% $NH_3H_2O^+$; 10 mM NH_4HCO_3) - ACN]; gradient: 40%-70% B over 8.0 m) to provide 2-(4-(4-(2-(benzyloxy)ethoxy)-2,6-difluorophenyl)piperazin-1-yl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol (**7**) (200 mg, 376.15 μmol). LC-MS: 516.2 (M+1).

[00655] *Scheme 42F*



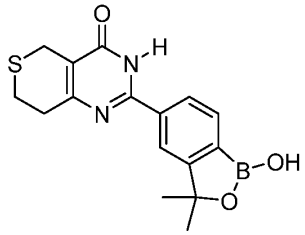
7 Compound 42

[00656] To a solution of 2-(4-(4-(2-(benzyloxy)ethoxy)-2,6-difluorophenyl)piperazin-1-yl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-ol (**7**) (100 mg, 194.33 μmol, 1.00 eq) in MeOH (5 mL) was added Pd/C (100 mg, 194.33 μmol, 1.00 eq) at 25°C under N_2 atmosphere. The suspension was degassed and purged three times with H_2. The mixture was stirred under H_2 (15 psi) at 25°C for 13 h. After cooling to 15°C, the reaction mixture was filtered through diatomite and the filtrate was concentrated under reduced pressure to give a residue that was purified by prep-HPLC (neutral conditions; column: Waters X bridge Prep OBD C18 150 × 40 mm × 10 μm; mobile phase: [H_2O (10 mM NH_4HCO_3) - ACN]; gradient: 20% - 50% B over

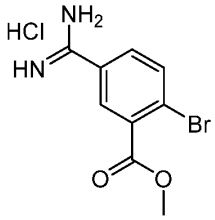
8.0 m) to provide 2-(4-(2,6-difluoro-4-(2-hydroxyethoxy)phenyl)piperazin-1-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 42**). LC-MS: 425.0 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.74 - 6.69 (m, 2H), 4.88 (t, J = 5.5 Hz, 1H), 3.97 (t, J = 4.9 Hz, 2H), 3.71 - 3.65 (m, 6H), 3.41 - 3.36 (m, 2H), 3.03 (br s, 4H), 2.84 - 2.78 (m, 2H), 2.70 - 2.61 (m, 2H). ^{19}F NMR (400 MHz, DMSO-d_6): δ -118.26.

Compound 44

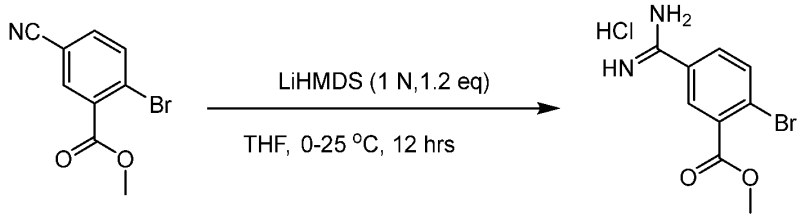
Synthesis of 2-(1-hydroxy-3,3-dimethyl-1,3-dihydrobenzo[c][1,2]oxaborol-5-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (Compound 44)



Synthesis of methyl 2-bromo-5-carbamimidoylbenzoate hydrochloride



[00657] *Scheme 44A*

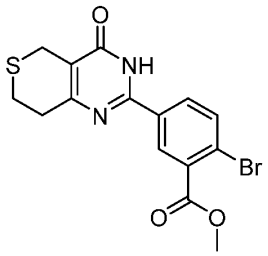


1 **2**

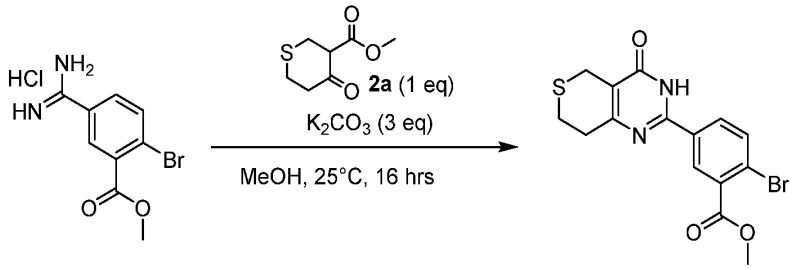
[00658] To a solution of methyl 2-bromo-5-cyanobenzoate (**1**) (3 g, 12.50 mmol, 1 eq) in THF (30 mL) was added LiHMDS (1 M, 15 mL, 1.2 eq) dropwise at 0°C. The mixture was warmed to 25°C and stirred for 12 h. LCMS indicated that (**1**) was consumed completely and one main peak formed. The mixture was quenched by addition of HCl/dioxane (4M, 15 mL) at 0°C and warmed to 15°C while stirring for 4 h. The mixture was concentrated under reduced pressure to give methyl 2-bromo-5-carbamimidoylbenzoate hydrochloride (**2**) (5.5 g, crude,

HCl). The crude product was used into the next step without further purification. LCMS: (EC16609-21-P1A1, product: R_t = 0.211 mins).

Synthesis of methyl 2-bromo-5-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate



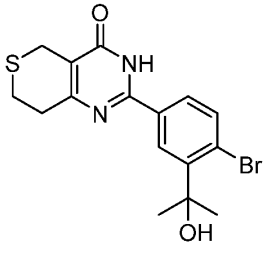
[00659] *Scheme 44B*



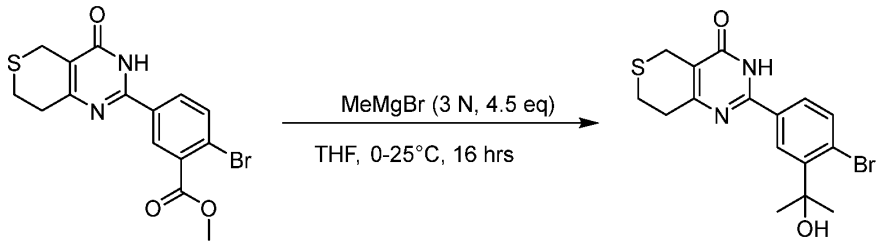
2 **3**

[00660] To a solution of methyl 2-bromo-5-carbamimidoylbenzoate hydrochloride (**2**) (5.6 g, 19.08 mmol, 1 eq, HCl) in MeOH (56 mL) was added methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**2a**) (3.32 g, 19.08 mmol, 1 eq) and K_2CO_3 (7.91 g, 57.23 mmol, 3 eq) at 25°C and then stirred for 16 h. LCMS indicated that (**2**) was consumed completely and one main peak formed. The reaction mixture was filtered and the filtrate was concentrated in vacuum to give a residue that was added to H_2O (50mL) at 25°C and stirred for 2 h. The mixture was filtered and methyl 2-bromo-5-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**3**) (3.8 g, 9.97 mmol, crude) was obtained, which was used in the next step without further purification. LCMS: (EC16609-21-P1A1, product: R_t = 0.430 mins).

Synthesis of 2-(4-bromo-3-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



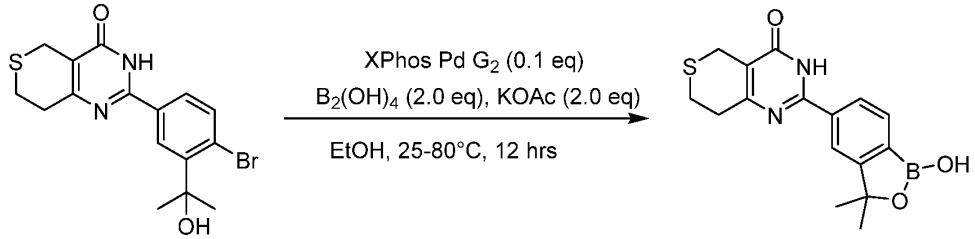
[00661] *Scheme 44C*



3 **4**

[00662] To a solution of methyl 2-bromo-5-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)benzoate (**3**) (1.5 g, 3.93 mmol, 1 eq) in THF (15 mL) was added MeMgBr (3 M, 5.90 mL, 4.5 eq) dropwise at 0°C. The mixture was heated to 25°C and stirred for 16 h. TLC (Petroleum ether/ Ethyl acetate = 2:1) indicated some of (**3**) remained. The reaction mixture was quenched by addition saturated NH_4Cl at 0°C, and then extracted with ethyl acetate. The organic layers were combined and concentrated under reduced pressure to give 2-(4-bromo-3-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (1.2 g, 3.15 mmol, crude), which was used in the next step without further purification. LCMS: (EC16609-14-P1A, product: R_t = 0.415 mins).

[00663] *Scheme 44D*



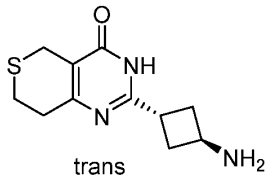
4 **Compound 44**

[00664] To a solution of 2-(4-bromo-3-(2-hydroxypropan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**4**) (1.2 g, 3.15 mmol, 1 eq), $B_2(OH)_4$ (564.31 mg, 6.29 mmol, 2 eq) and KOAc (617.75 mg, 6.29 mmol, 2 eq) in EtOH (24 mL) was added XPhos Pd G_2 (247.63 mg, 314.72 μmol, 0.1 eq) at 25°C. The mixture was heated to 80°C and stirred for 12 hr. LCMS indicated that (**4**) was consumed completely. The mixture was filtered and the filtrate concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Waters Xbridge BEH C18 250 * 50 mm * 10 μm; mobile phase: [water (NH_3H_2O)-ACN]; gradient: 1%-30% B over 10 min) to provide 2-(1-hydroxy-3,3-dimethyl-1,3-dihydrobenzo[c][1,2]oxaborol-5-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 44**). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 9.16 (br s, 1 H) 8.10 (s, 1 H)

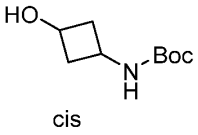
8.05 (br d, J = 7.38 Hz, 1 H) 7.74 (d, J = 7.50 Hz, 1 H) 3.52 (s, 2 H) 2.87 (br s, 4 H) 1.49 (s, 6 H). LCMS: (EC16609-26-P1A3, product: R_t = 0.372 mins).

Compound A1

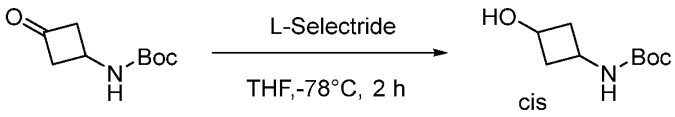
Synthesis of 2-((1r,3r)-3-aminocyclobutyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (A1)



Synthesis of (Z)-tert-butyl-3-hydroxycyclobutyl)carbamate



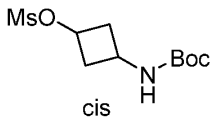
[00665] *Scheme A1-A*



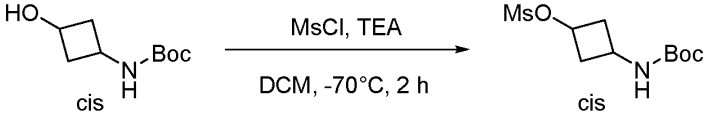
1 **2**

[00666] A solution of *tert*-butyl (3-oxocyclobutyl)carbamate (**1**) (2.5 g, 13.5 mmol, 1 eq) in 80 mL THF was cooled to -78°C and treated with a 1 N solution of L-Selectride (16.2 mL, 16.2 mmol, 1.2 eq) in THF. After stirring for 1 h, the reaction was quenched with 5 mL water and warmed to room temperature (rt). The reaction mixture was concentrated and purified by silica gel chromatography (EA:PE = 0~50 %) to provide (Z)-*tert*-butyl-3-hydroxycyclobutyl)carbamate (**2**). ^{1}H NMR (400 MHz, $CDC1_3$): δ= 4.68 (brs, 1H), 4.05-3.98 (m, 1H), 3.67-3.65 (m, 1H), 2.78-2.75 (m, 2H), 2.08 (brs, 1H), 1.81-1.78 (m, 2H), 1.44 (s, 9H).

Synthesis of (Z)-3-((tert-butoxycarbonyl)amino)cyclobutyl methanesulfonate



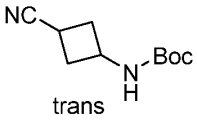
[00667] *Scheme A1-B*



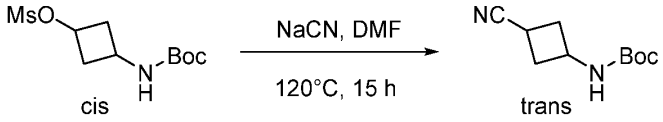
2 **3**

[00668] Methanesulfonyl chloride (1.3 g, 11.6 mmol, 1.2 eq) was added dropwise to a solution consisting of (Z)-*tert*-butyl-3-hydroxycyclobutyl)carbamate (**2**) (1.8 g, 9.6 mmol, 1 eq), TEA (1.5 g, 14.8 mmol, 1.5 eq) and dichloromethane (60 mL) at -70°C. The resulting solution was stirred for 2 h at -70°C, and then diluted with water. The resulting solution was extracted with dichloromethane and the organic layers combined, washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give (Z)-3-((*tert*-butoxycarbonyl)amino)cyclobutyl methanesulfonate (**3**). ^{1}H NMR (400 MHz, $CDC1_3$): δ (ppm): 4.75-4.67 (m, 2H), 3.85-3.82 (m, 1H), 2.98 (s, 3H), 2.93-2.88 (m, 2H), 2.22-2.14 (m, 2H), 1.44 (s, 9H).

Synthesis of (E)-tert-butyl (3-cyanocyclobutyl)carbamate



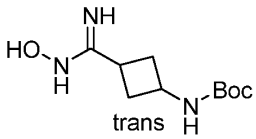
[00669] *Scheme A1-C*



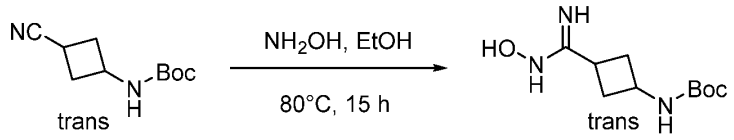
3 **4**

[00670] (Z)-3-((*tert*-butoxycarbonyl)amino)cyclobutyl methanesulfonate (**3**) (1.2 g, 4.5 mmol, 1 eq) in DMF (30 mL) was treated with NaCN (665.7 mg, 13.6 mmol, 3 eq) and the reaction was heated to 120°C for 15 h. The mixture was then cooled to 0°C and diluted with water. The resulting solution was extracted with ethyl acetate and the organic layers were combined. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give (E)-*tert*-butyl (3-cyanocyclobutyl)carbamate (**4**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.77 (br s, 1H), 4.41-4.39 (m, 1H), 3.06-3.01 (m, 1H), 2.74-2.41 (m, 2H), 2.27-2.24 (m, 2H), 1.45 (s, 9H).

Synthesis of (E)-tert-butyl (3-(N-hydroxycarbamimidoyl)cyclobutyl)carbamate



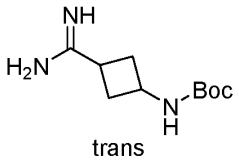
[00671] *Scheme A1-D*



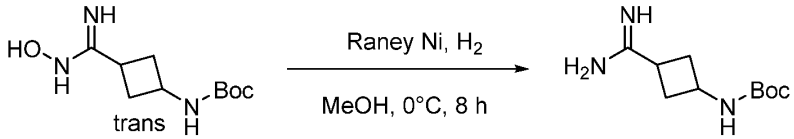
4 **5**

[00672] To a solution of (E)-*tert*-butyl (3-cyanocyclobutyl)carbamate (**4**) (730.0 mg, 3.7 mmol, 1 eq) in EtOH (25 mL) was added hydroxylamine in ethanol (2.2 g/2.0 mL of ethanol, 33.3 mmol, 9 eq). The mixture was heated to 80°C and stirred for 15 h. The reaction mixture was then cooled to RT and concentrated under reduced pressure followed by prep-HPLC purification to give (E)-*tert*-butyl (3-(N-hydroxycarbamimidoyl)cyclobutyl)carbamate (**5**). LCMS (ESI): m/z 230.2 $[M+H]^+$.

Synthesis of (E)-tert-butyl (3-carbamimidoylcyclobutyl)carbamate



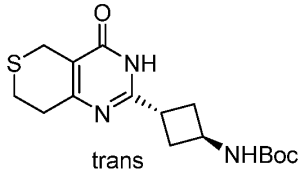
[00673] *Scheme A1-E*



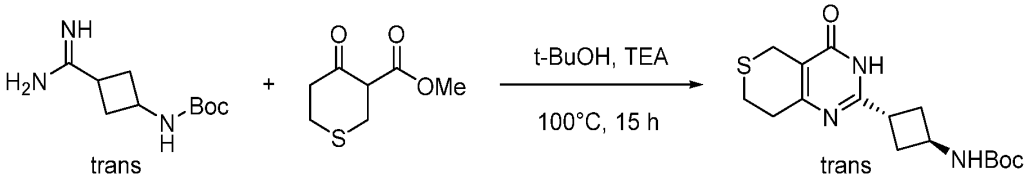
5 **6**

[00674] To a solution of (E)-*tert*-butyl (3-(N-hydroxycarbamimidoyl)cyclobutyl)carbamate (**5**) (520.0 mg, 2.3 mmol, 1 eq) in MeOH (100 mL) was added Raney nickel (Raney Ni) (200 mg, 2.3 mmol, 1 eq). The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under an H_2 atmosphere at 0°C for 8 h. The reaction mixture was then filtered, and the filtrate was concentrated under reduced pressure to give (E)-*tert*-butyl (3-carbamimidoylcyclobutyl)carbamate (**6**) (480.0 mg). LCMS (ESI): m/z 214.2 $[M+H]^+$.

Synthesis of (E)-tert-butyl ((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)carbamate

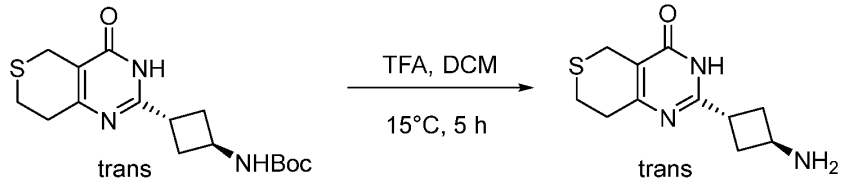


[00675] *Scheme A1-F*



[00676] To a solution of E)-*tert*-butyl (3-carbamimidoylcyclobutyl)carbamate (**6**) (480.0 mg, 2.3 mmol, 1 eq) and (**7**) (431.3 mg, 2.5 mmol, 1.1 eq) in *t*-BuOH (30 mL) was added TEA (1.3 g, 12.8 mmol, 5.6 eq) in one portion at 15°C. The mixture was heated to 100°C and stirred for 15 h. The reaction was concentrated in vacuo. The resultant residue was purified by silica gel column chromatography (DCM/MeOH = 100% to 95% DCM/MeOH) to give (E)-*tert*-butyl ((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)carbamate (**8**) (630.1 mg). LCMS (ESI): m/z 338.1 $[M+H]^+$.

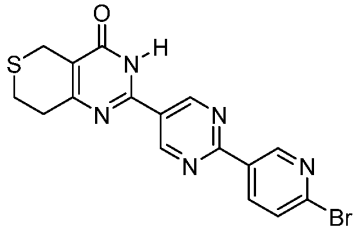
[00677] *Scheme A1-G*



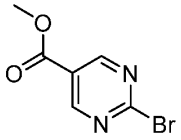
[00678] To a stirred solution of (E)-*tert*-butyl ((1r,3r)-3-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)cyclobutyl)carbamate (**8**) (630 mg, 1.9 mmol, 1 eq) in DCM (30 mL) was added TFA (3 mL, 39.5 mmol, 20.8 eq) at 25°C. The reaction mixture was stirred for 5 h. The reaction mixture was concentrated in vacuum to give (E)-2-((1r,3r)-3-aminocyclobutyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**A1**). LCMS (ESI): m/z 238.0 $[M+H]^+$. ^{1}H-NMR (400MHz, DMSO-d_6): δ = 8.41 (brs, 3H), 3.75-3.71 (m, 1H), 3.47 (s, 2H), 3.43-3.80 (m, 1H), 2.90-2.87 (m, 4H), 2.66-2.53 (m, 4H).

Compound A4

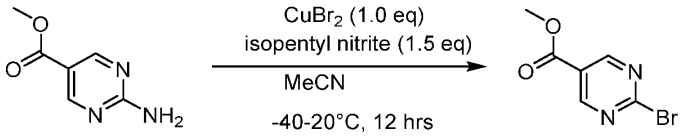
Synthesis of 2-(2-(6-bromopyridin-3-yl)pyrimidin-5-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (A4)



Synthesis of methyl 2-bromopyrimidine-5-carboxylate



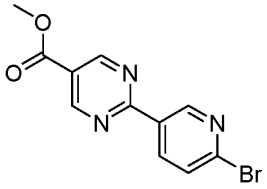
[00679] *Scheme A4-A*



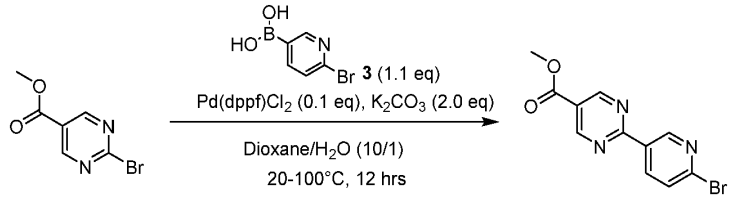
1 **2**

[00680] To a solution of methyl 2-aminopyrimidine-5-carboxylate (**1**) (5.00 g, 32.6 mmol, 1 eq) in MeCN (50 mL) was added $CuBr_2$ (7.29 g, 32.6 mmol, 1.53 mL, 1 eq) at 20°C under N_2. The mixture was cooled to -40°C and then isopentyl nitrite (5.74 g, 48.9 mmol, 6.59 mL, 1.50 eq) added dropwise. The mixture was warmed to 20°C and stirred for 12 hrs. The mixture was diluted with EtOAc, filtered, and concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 100/1 to 5/1) to provide methyl 2-(6-bromopyridin-3-yl)pyrimidine-5-carboxylate (**2**) (1.60 g, 7.37 mmol).

Synthesis of methyl 2-(6-bromopyridin-3-yl)pyrimidine-5-carboxylate

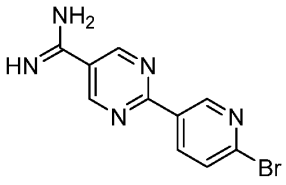


[00681] *Scheme A4-B*

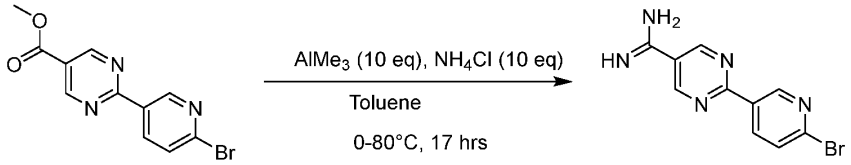


[00682] To a solution of methyl 2-bromopyrimidine-5-carboxylate (**2**) (1.60 g, 7.36 mmol, 1 eq) in dioxane (16 mL) and H_2O (1.60 mL) was added K_2CO_3 (2.03 g, 14.7 mmol, 2 eq) and (6-bromopyridin-3-yl)boronic acid (**3**) (1.63 g, 8.09 mmol, 1.10 eq) at 20°C followed by Pd(dppf)Cl_2 (538 mg, 735 µmol, 0.10 eq). The mixture was heated to 100°C and stirred for 12 hrs. The reaction mixture was filtered, and the filtrate concentrated under reduced pressure to give a residue that was purified by prep-HPLC (column: Phenomenex luna C18 (250 * 70 mm, 15 µm); mobile phase: [water (HCl) -ACN]; B%: 35% - 65%, 18 mins) to provide methyl 2-(6-bromopyridin-3-yl)pyrimidine-5-carboxylate (**4**) (600 mg, 2.04 mmol). ^{1}H NMR (400 MHz, chloroform-d) δ = 9.49 (d, *J* = 2.4 Hz, 1H), 9.36 (s, 2H), 8.65 (dd, *J* = 2.4, 8.4 Hz, 1H), 7.67 (d, *J* = 8.3 Hz, 1H), 4.04 (s, 3H).

Synthesis of 2-(6-bromopyridin-3-yl)pyrimidine-5-carboximidamide



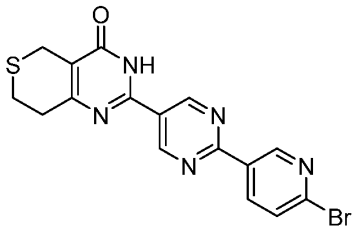
[00683] *Scheme A4-C*



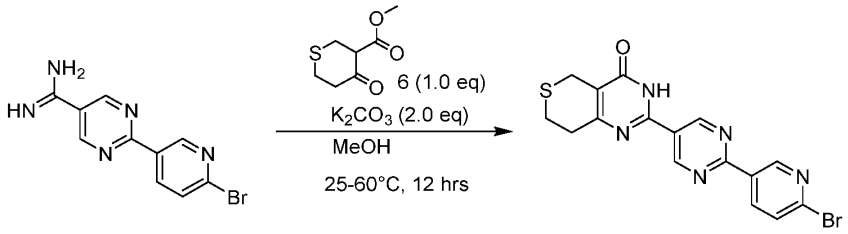
[00684] NH_4Cl (672 mg, 12.5 mmol, 10 eq) was added to toluene (5.55 mL) at 25°C. $AlMe_3$ (1.00 M, 6.29 mL, 10 eq) was added dropwise at 0°C. The mixture was stirred at 0°C for 1 hr. Then, methyl 2-(6-bromopyridin-3-yl)pyrimidine-5-carboxylate (**4**) (370 mg, 1.26 mmol, 1 eq) in toluene (1.85 mL) was added dropwise at 0°C. The mixture was warmed to 80°C and stirred for 16 hrs under N_2. The reaction mixture was cooled to 0°C and MeOH added dropwise at 0°C. The mixture was then filtered, and the filtrate concentrated under reduced pressure to give

a residue that was purified by prep-HPLC (0.1% NH_4HCO_3) to provide 2-(6-bromopyridin-3-yl)pyrimidine-5-carboximidamide (**5**) (50.0 mg, 179 µmol).

Synthesis of 2-(2-(6-bromopyridin-3-yl)pyrimidin-5-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one



[00685] *Scheme A4-D*

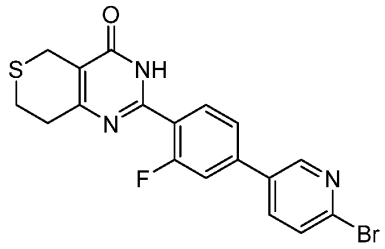


5 **A4**

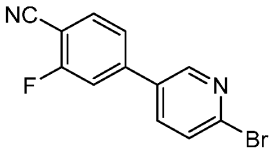
[00686] To a mixture of 2-(6-bromopyridin-3-yl)pyrimidine-5-carboximidamide (**5**) (50.0 mg, 179 µmol, 1 eq) in MeOH (1 mL) was added methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**6**) (31.3 mg, 179 µmol, 1 eq) and K_2CO_3 (49.7 mg, 359 µmol, 2 eq) at 25°C. The mixture was warmed to 60°C and stirred for 12 hrs. The reaction mixture was filtered, and the filtrate concentrated under reduced pressure to give a residue that was purified by prep-HPLC (column: Phenomenex luna C18 80 * 40mm * 3 µm; mobile phase: [water (HCl) - ACN]; B%: 20% - 50%, 8 mins) to provide 2-(2-(6-bromopyridin-3-yl)pyrimidin-5-yl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**A4**). ^{1}H NMR (400 MHz, DMSO-d6): δ = 13.51 - 12.57 (m, 1H), 9.50 (s, 2H), 9.35 (d, *J* = 1.9 Hz, 1H), 8.66 (dd, *J* = 2.5, 8.4 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 3.58 (s, 2H), 2.93 (s, 4H).

Compound A5

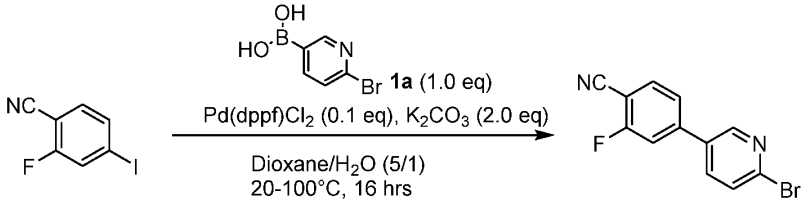
Synthesis of 2-(4-(6-bromopyridin-3-yl)-2-fluorophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (A5)



Synthesis of 4-(6-bromopyridin-3-yl)-2-fluorobenzonitrile



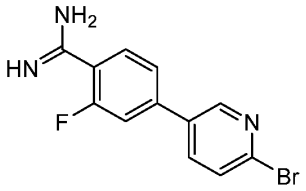
[00687] *Scheme A5-A*



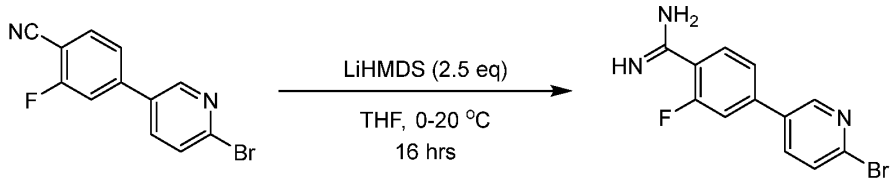
1 **2**

[00688] To a solution of 2-fluoro-4-iodobenzonitrile (**1**) (0.5 g, 2.02 mmol, 1.0 eq) in dioxane (5 mL)/H_2O (1 mL) was added (6-bromopyridin-3-yl)boronic acid (**1a**) (408.52 mg, 2.02 mmol, 1.0 eq), K_2CO_3 (559.52 mg, 4.05 mmol, 2.0 eq), and $Pd(dppf)Cl_2$ (148.11 mg, 202.42 μmol, 0.1 eq) at 20°C under N_2. The mixture was stirred at 100°C for 16 h under N_2. TLC (petroleum ether/ethyl acetate = 5/1, R_f (cpd. 1) = 0.77) indicated that (**1**) was consumed, and one new spot with larger polarity was detected. After cooling to 20°C, the mixture was filtered, and then the filtrate was concentrated under reduced pressure to give a residue. 4-(6-bromopyridin-3-yl)-2-fluorobenzonitrile (**2**) (650 mg, crude) was obtained. ^1H NMR: (400 MHz, $CDCl_3$): δ = 8.53 (d, J = 2.4 Hz, 1H), 7.71 - 7.65 (m, 2H), 7.56 (d, J = 8.3 Hz, 1H), 7.41 - 7.33 (m, 2H).

Synthesis of 4-(6-bromopyridin-3-yl)-2-fluorobenzimidamide



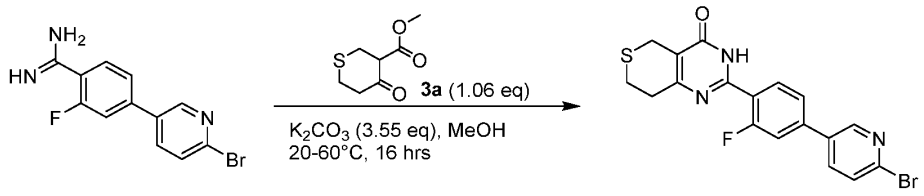
[00689] *Scheme A5-B*



2 **3**

[00690] To a solution of 4-(6-bromopyridin-3-yl)-2-fluorobenzonitrile (**2**) (500 mg, 1.80 mmol, 1.0 eq) in THF (5 mL) was added LHMDS (1 M, 4.51 mL, 2.5 eq) at 0°C under N_2. The mixture was warmed to 20°C and stirred at 20°C for 16 h. The reaction mixture was quenched by addition of HCl/dioxane (4 mol/L, 2.5 mL) at 0°C, and then concentrated under reduced pressure to give a residue. The residue was suspended in MeOH (15 ml), and the mixture was stirred at 20°C for 1 hr. The mixture was then filtered, and the filtrate was concentrated under reduced pressure to give a residue. 4-(6-bromopyridin-3-yl)-2-fluorobenzimidamide (**3**) (540 mg, crude, HCl) was obtained and used immediately in the next step.

[00691] *Scheme A5-C*



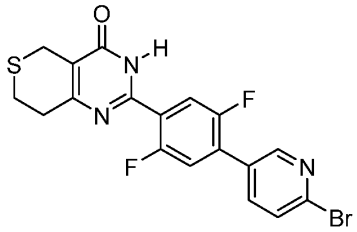
3 **A5**

[00692] To a solution of 4-(6-bromopyridin-3-yl)-2-fluorobenzimidamide (**3**) (220 mg, 747.99 μmol, 1.0 eq) in MeOH (2.2 mL) was added methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**3a**) (137.50 mg, 789.25 μmol, 1.06 eq) and K_2CO_3 (366.68 mg, 2.65 mmol, 3.55 eq) at 20°C; the mixture was stirred at 60°C for 16 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (FA condition; column: Phenomenex luna C18 80 * 40 mm * 3 μm; mobile phase:

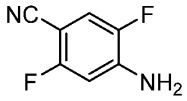
[water (FA)-ACN]; B%: 30%-70%, 8 mins). 2-(4-(6-bromopyridin-3-yl)-2-fluorophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**A5**) (30 mg) was obtained. ^{1}H NMR: (400 MHz, DMSO-d_6): δ = 8.85 (d, J = 2.4 Hz, 1H), 8.19 (dd, J = 2.5, 8.4 Hz, 1H), 7.88 - 7.84 (m, 1H), 7.83 - 7.79 (m, 2H), 7.77 - 7.73 (m, 1H), 3.54 (s, 2H), 2.89 (br dd, J = 4.5, 11.8 Hz, 4H). LC-MS: 420 + bromo isomers (M+1).

Compound A6

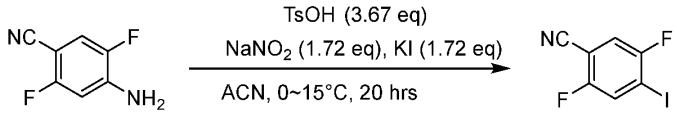
Synthesis of 2-(4-(6-bromopyridin-3-yl)-2,5-difluorophenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (A6)



Synthesis of 4-amino-2,5-difluorobenzonitrile



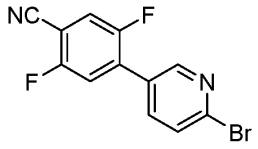
[00693] *Scheme A6-A*



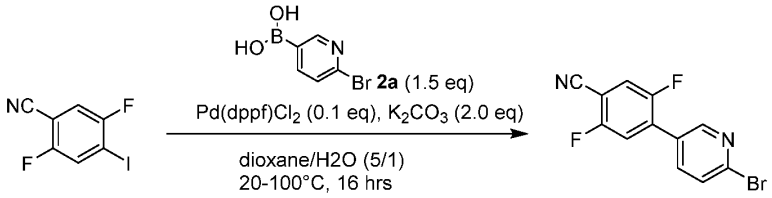
1 **2**

[00694] A mixture of 4-amino-2,5-difluorobenzonitrile (**1**) (3.00 g, 19.5 mmol, 1.00 eq), TsOH (12.3 g, 71.4 mmol, 3.67 eq) in acetonitrile (ACN) (60.0 mL) was stirred for 4 hrs at 15°C. Then $NaNO_2$ (2.31 g, 33.5 mmol, 1.72 eq) and KI (5.56 g, 33.5 mmol, 1.72 eq) were added into the reaction mixture at 0°C. Then the mixture was stirred at 15°C for 16 hrs. LCMS showed that (**1**) was consumed completely. The reaction mixture was diluted with Na_2SO_3 (20 mL) and extracted with EtOAc 120 mL (40.0 mL * 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 10/1 to 0/1) and obtained 2.8 g crude. 1.3 g of crude product was purified by prep-HPLC (column: Phenomenex luna C18 80 * 40 mm * 3 µm; mobile phase: [water (HCl)-ACN]; B%: 35%-65%, 7 min) to give 0.5 g desired product. 2,5-Difluoro-4-iodobenzonitrile (**2**) (1.5 g, crude) was obtained, verified by NMR, and used into the next step.

Synthesis of 4-(6-bromopyridin-3-yl)-2,5-difluorobenzonitrile



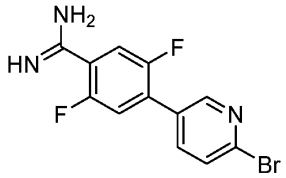
[00695] *Scheme A6-B*



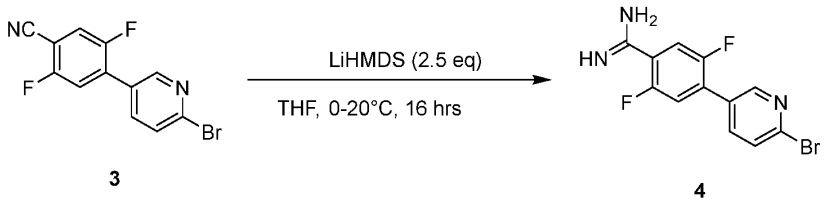
2 3

[00696] To a solution of 2,5-difluoro-4-iodobenzonitrile (**2**) (400 mg, 1.51 mmol, 1.00 eq) and (6-bromopyridin-3-yl)boronic acid (**2a**) (457 mg, 2.26 mmol, 1.50 eq) in dioxane (4.00 mL) and H_2O (0.80 mL) was added K_2CO_3 (417 mg, 3.02 mmol, 2.00 eq) at 20°C. The suspension was degassed and purged with N_2 for 3 times. To the mixture was added $Pd(dppf)Cl_2$ (110 mg, 151 μmol, 0.10 eq) at 20°C under N_2. The suspension was degassed and purged with N_2 for 3 times. The mixture was stirred at 100°C for 16 hrs under N_2. LCMS showed that (**2**) was consumed completely and one main peak with desired mass was detected. The reaction mixture was diluted with H_2O 5.00 mL and extracted with EtOAc 30.0 mL (15.0 mL * 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO_2, Petroleum ether/ Ethyl acetate = 2/ 1). 4-(6-bromopyridin-3-yl)-2,5-difluorobenzonitrile (**3**) (80.0 mg, 244.00 μmol) was obtained.

Synthesis of 4-(6-bromopyridin-3-yl)-2,5-difluorobenzimidamide



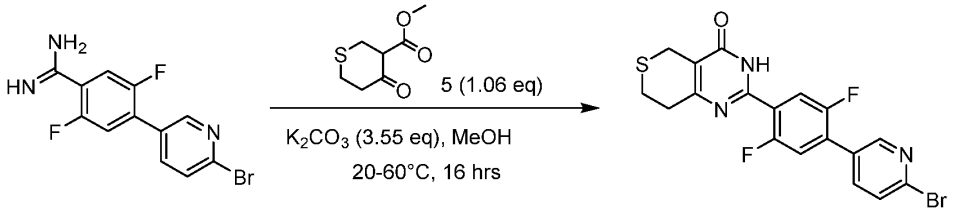
[00697] *Scheme A6-C*



3 4

[00698] To a solution of 4-(6-bromopyridin-3-yl)-2,5-difluorobenzonitrile (**3**) (360 mg, 1.22 mmol, 1.00 eq) in THF (3.60 mL) was added LiHMDS (1 M, 3.05 mL, 2.50 eq) at 0°C. The mixture was stirred at 20°C for 16 hrs. LCMS indicated that (**3**) was consumed completely. The reaction mixture was quenched by addition of HCl/Dioxane (4 M, 1.50 mL) at 0°C and then concentrated under reduced pressure to give a residue. The residue was suspended in MeOH (5.00 ml) and the mixture was stirred at 25°C for 1 hr. Then the mixture was filtered and the filtrate was concentrated under reduced pressure to give a residue. 4-(6-bromopyridin-3-yl)-2,5-difluorobenzimidamide (**4**) (390 mg, crude) was obtained.

[00699] *Scheme A6-D*



4 **A6**

[00700] To a solution of 4-(6-bromopyridin-3-yl)-2,5-difluorobenzimidamide (**4**) (160 mg, 513 µmol, 1.00 eq) in MeOH (1.60 mL) was added methyl 4-oxotetrahydro-2H-thiopyran-3-carboxylate (**5**) (94.7 mg, 544 µmol, 1.06 eq) and K_2CO_3 (252 mg, 1.82 mmol, 3.55 eq) at 25°C. The mixture was stirred at 25°C for 12 hrs. LCMS indicated that (**4**) was consumed completely. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (FA condition; column: Phenomenex Luna C18 200 * 40 mm * 10 µm; mobile phase: [water (FA)-ACN]; B%: 40%-70%, 8 min). (**A6**) (28.0 mg, 64.2 µmol) was obtained. Confirmed by HNMR and FNMR. ^{1}H NMR: (400 MHz, DMSO-d6). δ = 8.68 (s, 1H), 8.39 (s, 1H), 8.07 - 8.03 (m, 1H), 7.83 (d, J = 8.3 Hz, 1H), 7.76 - 7.70 (m, 2H), 3.53 (s, 2H), 2.86 (br dd, J = 4.9, 15.0 Hz, 4H). LC-MS (product: R_t = 2.500 mins).

Examples

[00701] The procedure of preparing the composition is disclosed in United States Patent Nos. 11,291,730 B2 and 11,369,685 B2. In brief, graphene oxide (1.25 g, 250 mL of 5 mg/mL GO dispersion in de-ionized water; Goographene Inc.) was added to 250 mL of ultra-pure de-ionized water and stirred for 5 minutes. Sodium hydroxide pellets (3 g, 0.075 moles; Sigma-Aldrich) were added in small solid portions to the mixture over 30 minutes. Once addition was complete, it was stirred for 1 hour at room temperature. Next the solution was ultrasonicated

for 30 minutes, and then chloroacetic acid (3.54 g, 0.0375 moles; Alfa Aesar) added in small, solid portions over 20 minutes. The reaction mixture was then stirred for 18 hours at room temperature. The reaction mixture was acidified with hydrochloric acid (7 mL, 12N). The solution was then transferred to centrifuge tubes and centrifuged for 15 minutes at 5,000 rpm. The water layer was decanted and more ultra-pure deionized water (~30 mL) added to the tubes before re-centrifugation. This process was repeated 3 times. Methanol (~30 mL) was added to the precipitated, modified graphene oxide remaining in the centrifuged tubes and centrifuged at 5,000 rpm for 15 minutes. This process was repeated 3 times. Once the methanol was decanted, the tubes were put under vacuum for 48 hours at room temperature for drying to obtain modified GO.

[00702] Hyaluronic acid (100mg, MW=10,000 (n=13.5 in the scheme), 0.00001 moles; supplier: Creativepegworks) in ultra-pure deionized water (20mL) was stirred for 5 minutes at room temperature. 1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC) (52mg, 0.00027 moles, supplier: Alfa Aesar) was added to the mixture and stirred for 3 hours at room temperature. During this time, the pH of the solution was maintained at approximately 5 to 6 by small additions of 0.1N hydrochloric acid. This mixture was added dropwise to a separate mixture containing adipic dihydrazide (ADH) (17mg, 0.00027 moles; supplier: Alfa Aesar) in 5mL of ultra-pure deionized water at room temperature. Once the addition was complete, it was stirred at room temperature for 18 hours. The solution was then subjected to dialysis (MWC=3500) for 24 hours. The mixture was then lyophilized to obtain derivatized HA as a powder.

[00703] The modified GO was added to ultra-pure deionized water (100mL) and stirred for 5 minutes, followed by ultra-sonication for 15 minutes. 2-Succinimido-1,1,1,3-tetramethyluronium tetrafluoroborate (TSTU) (0.32g, 0.001 moles; supplier: Alfa Aesar), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, Hexafluorophosphate Benzotriazole Tetramethyl Uronium (HBTU) (0.41g, 0.001 moles; supplier: Aldrich) and diisopropylethyl amine (DIEA) (0.15mL, 0.001 moles; supplier: Sigma-Aldrich) was added to the mixture at room temperature and stirred for 5 minutes. Then a solution of hyaluronic acid–adipic hydrazide (derivatized HA) (0.90g in 150mL of ultra-pure deionized water) was added dropwise to the activated graphene oxide solution at room temperature. Once the addition was complete, it was stirred at room temperature for 18 hours. The solution was then subjected to dialysis (MWCO=3500) for 24 hours and lyophilized, resulting in the GO-HA referenced further in the following Examples.

[00704] Pharmaceutical compositions were prepared as shown in Examples 1 to 6 (Formulations 1 to 27).

[00705] Example 1: Formulation 1

Table 2. Formulation 1, component mass and weight %

	Formulation 1	
	mass (g)	wt%
WNT inhibitor [a]	0.011	0.093%
GO-HA	0.011	0.093%
Glycol [b]	0.565	4.793%
Solubilizer	-	-
Thickener [e]	0.200	1.697%
Phospholipid	-	-
Carrier	-	-
Stabilizer	-	-
Buffer	-	-
Preservative	-	-
Water/saline [k]	11.0	93.323%
Total	11.787	100%

[a] **Compound A** (XAV939)
[b] PEG-400 (d = 1.13 g/mL)
[e] hydroxypropyl cellulose
[k] water

[00706] *Preparation of Formulation 1 composition*

[00707] Formulation 1 (Table 2) was prepared by dissolving 11 mg of GO-HA into 11 mL of ultra-pure water, to create an effective concentration of 1 mg/mL GO-HA solution. The solution was ultrasonicated for 10 minutes. XAV939 (11 mg; supplier: APEBIO) was added to PEG-400 (0.5 mL) and subjected to ultrasonication for 30 minutes, to form an XAV939 solution. The XAV939 solution was added dropwise to the GO-HA solution and vigorously stirred for 5 minutes. The combined solution was then subjected to ultrasonication for 1 hour and then stirred at room temperature for 18 hours. Hydroxypropyl cellulose (0.2 g; supplier: Sigma-Aldrich) was added in small portions at room temperature with vigorous stirring. Once addition was complete, the solution was stirred at room temperature for 24 hours to form a viscous solution of the GO-HA / XAV939 complex. Formulation 1 was used with a concentration of 1.0 mg/mL XAV939 in 1.0 mg/mL GO-HA, and 0.182 g/mL hydroxypropyl cellulose.

[00708] Example 2: Formulation 2

Table 3. Formulation 2, component mass and weight %

	Formulation 2	
	mass (g)	wt%
WNT inhibitor [a]	0.040	0.09%
GO-HA	0.040	0.09%
Glycol [c]	2.04	4.60%
Solubilizer [d]	2.04	4.60%
Thickener [e]	0.333	0.75%
Phospholipid [f]	0.532	1.20%
Carrier [h]	2.04	4.60%
Stabilizer	-	-
Buffer	-	-
Preservative	-	-
Water/saline [k]	37.283	84.07%
Total	44.348	100%

[a] **Compound A** (XAV939)
[c] butylene glycol (d = 1.01 g/mL)
[d] polysorbate 20 (d = 1.1 g/mL)
[e] hydroxypropyl cellulose
[f] phosphatidylcholine
[h] ethanol (d = 0.79 g/mL)
[k] water

[00709] *Preparation of Formulation 2 composition*

[00710] Formulation 2 (Table 3) was prepared by suspending XAV939 0.09 wt% in about 2 mL (4.6 wt%) of butylene glycol and about 2 mL (4.6 wt% of polysorbate 20 and heated to 60°C for 10 min while stirring. The mixture was then placed in a sonication bath and sonicated for 30 minutes. Phosphatidylcholine (1.2 wt%) was slowly dissolved in ethanol (4.6 wt%) to form a phosphatidylcholine solution. Once dissolved, the phosphatidylcholine solution was added the XAV939/butylene glycol/polysorbate 20 mixture and stirred at room temperature for 5 min and then subjected to sonication for 30 minutes. After 30 minutes, the solution was added to a previously made solution of GO-HA (0.09 wt%) in water (84 wt%) and this new solution was sonicated for 30 min. After sonication, hydroxypropylcellulose (0.75 wt%) was added to the mixture and the mixture stirred at room temperature for 24 hours before use, providing Formulation 2 (a water-based liposomal composition).

[00711] Example 3: Formulations 3 to 5

Table 4. Formulations 3 to 5, component mass and weight %

	Formulations 3 to 5	
	mass (g)	wt%
WNT inhibitor [a1, a7, or a8]	0.011	0.093%
GO-HA	0.011	0.093%
Glycol [b]	0.565	4.793%
Solubilizer	-	-
Thickener [e]	0.200	1.697%
Phospholipid	-	-
Carrier	-	-
Stabilizer	-	-
Buffer	-	-
Preservative	-	-
Water/saline [k]	11.0	93.323%
Total	11.787	100%

[a1] **Compound 1** (Formulation 3)
[a7] **Compound 7** (Formulation 4)
[a8] **Compound 8** (Formulation 5)
[b] PEG-400 (d = 1.13 g/mL)
[e] hydroxypropyl cellulose
[k] water

[00712] *Preparation of Formulations 3 to 5 compositions*

[00713] Formulations 3 to 5 (Table 4) GO-HA (11 mg) was added to ultra-pure water (11 mL), to create an effective concentration of 1 mg/mL. The solution was ultrasonicated for 10 minutes. The respective Wnt inhibitor (11 mg), a Wnt inhibitor of Formula (I), was added in its respective Formulation to PEG-400 (0.5 mL) and subjected to ultrasonication for 30 minutes. The Wnt inhibitor solution was added dropwise to the GO-HA and vigorously stirred for 5 minutes. The combined solution was then subjected to ultrasonication for 1 hour and then stirred at room temperature for 18 hours. Hydroxypropyl cellulose (0.2 g; supplier: Sigma-Aldrich) was added in small portions at room temperature with vigorous stirring. Once addition was complete, the solution was stirred at room temperature for 24 hours to form a viscous solution of the GO-HA + Wnt inhibitor.

[00714] The respective Wnt inhibitors of Formulations 3 to 5 are as follows. In Formulation 3, **Compound 1** is the Wnt inhibitor. In Formulation 4, **Compound 7** is the Wnt inhibitor. In Formulation 5, **Compound 8** is the Wnt inhibitor.

[00715] Formulations 3 to 5 were used with a final concentration of 1.0 mg/mL Wnt inhibitor in 1.0 mg/mL GO-HA, and 0.182 g/mL hydroxypropyl cellulose.

[00716] Example 4: Formulations 6 to 13

Table 5. Formulations 6 to 10 and Control Formulation 11, component mass and weight %

	Formulations 6 to 10 and Control Formulation 11	
	mass (g)	wt%
WNT inhibitor [a1, a7, a8, a10, a18, or a]	0.030	0.098%
GO-HA	-	-
Glycol [c]	1.616	5.300%
Solubilizer [d]	1.760	5.772%
Thickener [e]	0.260	0.853%
Phospholipid [f]	0.660	2.165%
Carrier [h]	1.264	4.146%
Stabilizer	-	-
Buffer	-	-
Preservative	-	-
Water/saline [k]	24.900	81.666%
Total	30.490	100%

[a1] **Compound 1** (Formulation 6)
[a7] **Compound 7** (Formulation 7)
[a8] **Compound 8** (Formulation 8)
[a10] **Compound 10** (Formulation 9)
[a18] **Compound 18** (Formulation 10)
[a] **Compound A** (XAV939) (Control Formulation 11)
[c] butylene glycol (d = 1.01 g/mL)
[d] polysorbate 20 (d = 1.1 g/mL)
[e] hydroxypropyl cellulose
[f] phosphatidylcholine
[h] ethanol (d = 0.79 g/mL)
[k] water

[00717] *Preparation of Formulations 6 to 11 compositions*

[00718] Formulations 6 to 11 (Table 5) were prepared as follows. The Wnt Inhibitor (30 mg) was suspended in butylene glycol (1.6 mL) and polysorbate 20 (1.6 mL) and heated to 60°C for 10 min while stirring. The mixture was then placed in a sonication bath and sonicated for 30 minutes. Phosphatidylcholine (0.66 g) was slowly dissolved in ethanol (1.6 mL) to form a phosphatidylcholine solution. Once dissolved, the phosphatidylcholine solution was added to the Wnt inhibitor/butylene glycol/polysorbate 20 mixture and stirred at room temperature for 5 min and then subjected to sonication for 30 minutes. After 30 minutes, the solution was added to ultra-pure water (24.9 mL) and this solution was sonicated for 30 minutes. After sonication, hydroxypropyl cellulose (0.26 g) was added to the mixture and the mixture stirred at room temperature for 24 hours before use, providing Formulations 6 to 11.

[00719] The respective Wnt inhibitors of Formulations 6 to 11 are as follows. In Formulation 6, **Compound 1** is the Wnt inhibitor. In Formulation 7, **Compound 7** is the Wnt inhibitor. In Formulation 8, **Compound 8** is the Wnt inhibitor. In Formulation 9, **Compound 10** is the Wnt inhibitor. In Formulation 10, **Compound 18** is the Wnt inhibitor. In Control Formulation 11, XAV939 is the Wnt inhibitor.

Table 6. Formulations 12 and 13, component mass and weight %

	Formulations 12 and 13	
	mass (g)	wt%
WNT inhibitor [a18 or a]	0.045	0.096%
GO-HA	0.045	0.096%
Glycol [c]	2.020	4.317%
Solubilizer [d]	2.200	4.702%
Thickener [e]	0.400	0.855%
Phospholipid [f]	0.500	1.069%
Carrier [h]	1.58	3.377%
Stabilizer	-	-
Buffer	-	-
Preservative	-	-
Water/saline [k]	40.0	85.488%
Total	46.790	100

[a18] **Compound 18** (Formulation 12)
[a] **Compound A** (XAV939) (Formulation 13)
[c] butylene glycol (d = 1.01 g/mL)
[d] polysorbate 20 (d = 1.1 g/mL)
[e] hydroxypropyl cellulose
[f] phosphatidylcholine
[h] ethanol (d = 0.79 g/mL)
[k] water

[00720] *Preparation of Formulations 12 and 13 compositions*

[00721] Formulations 12 and 13 (Table 6) were prepared as follows. The Wnt inhibitor (0.096 wt%) was suspended in 2 mL (4.317 wt%) of butylene glycol and 2 mL (4.702 wt%) of polysorbate 20 and heated to 60°C for 10 min while stirring. The mixture was then placed in a sonication bath and sonicated for 30 minutes. Phosphatidylcholine (1.069 wt%) was slowly dissolved in 2 mL (3.377 wt%) of ethanol to form a phosphatidylcholine solution. Once dissolved, the phosphatidylcholine solution was added to the Wnt inhibitor/butylene glycol/polysorbate 20 mixture and stirred at room temperature for 5 min and then subjected to sonication for 30 minutes. After 30 minutes, the solution was added to a previously made solution of GO-HA (0.096 wt%) in water (85.5 wt%) and this new solution was sonicated for 30 min. After sonication, hydroxypropylcellulose (0.855 wt%) was added to the mixture and

the mixture stirred at room temperature for 24 hours before use, providing Formulations 12 and 13 (water-based liposomal compositions).

[00722] The respective Wnt inhibitors of Formulations 12 and 13 are as follows. In Formulation 12, **Compound 18** is the Wnt inhibitor. In Formulation 13, XAV939 is the Wnt inhibitor.

[00723] Example 5: Formulations 14 and 15

Table 7. Formulations 14 and 15, component mass and weight %

	Formulations 14 and 15	
	mass (g)	wt%
WNT inhibitor [a8 or a18]	0.045	0.097%
GO-HA	-	-
Glycol [c]	2.020	4.359%
Solubilizer [d]	2.200	4.747%
Thickener	-	-
Phospholipid [f]	0.500	1.079%
Carrier [h]	1.580	3.409%
Stabilizer	-	-
Buffer	-	-
Preservative	-	-
Water/saline [k]	40.000	86.309%
Total	46.345	100%

[a8] **Compound 8** (Formulation 14)
[a18] **Compound 18** (Formulation 15)
[c] butylene glycol (d = 1.01 g/mL)
[d] polysorbate 20 (d = 1.1 g/mL)
[f] phosphatidylcholine
[h] ethanol (d = 0.79 g/mL)
[k] water

[00724] *Preparation of Formulations 14 and 15 compositions*

[00725] Formulations 14 and 15 (Table 7) were prepared as follows. The Wnt Inhibitor (45 mg) was suspended in butylene glycol (2 mL) and polysorbate 20 (2 mL) and heated to 60°C for 10 min while stirring. The mixture was then placed in a sonication bath and sonicated for 30 minutes. Phosphatidylcholine (0.5 g) was slowly dissolved in ethanol (2 mL) to form a phosphatidylcholine solution. Once dissolved, the phosphatidylcholine solution was added to the Wnt inhibitor/butylene glycol/polysorbate 20 mixture and stirred at room temperature for 5 min and then subjected to sonication for 30 minutes. After 30 minutes, the solution was added to ultra-pure water (40 mL) and this solution was sonicated for 30 minutes. After sonication, the mixture stirred at room temperature for 24 hours before use, providing Formulations 14 and 15.

[00726] The respective Wnt inhibitors of Formulations 14 and 15 are as follows. In Formulation 14, **Compound 8** is the Wnt inhibitor. In Formulation 15, **Compound 18** is the Wnt inhibitor.

[00727] **Example 6: Formulations 16 to 17**

Table 8. Formulations 16 and 17, component mass and weight %

	Formulations 16 and 17	
	mass (g)	wt%
WNT inhibitor [a7 or a8]	0.010	0.028%
GO-HA	-	-
Glycol [c]	1.818	5.056%
Solubilizer [d]	1.980	5.507%
Thickener [e]	0.285	0.793%
Phospholipid [f]	0.440	1.224%
Carrier [h]	1.422	3.955%
Stabilizer	-	-
Buffer	-	-
Preservative	-	-
Water/saline [k]	30.000	83.438%
Total	35.955	100%

[a7] **Compound 7** (Formulation 16)
[a8] **Compound 8** (Formulation 17)
[c] butylene glycol (d = 1.01 g/mL)
[d] polysorbate 20 (d = 1.1 g/mL)
[e] hydroxypropyl cellulose
[f] phosphatidylcholine
[h] ethanol (d = 0.79 g/mL)
[k] water

[00728] *Preparation of Formulations 16 and 17 compositions*

[00729] Formulations 16 and 17 (Table 8) were prepared as follows. The Wnt Inhibitor (10 mg) was suspended in butylene glycol (1.80 mL) and polysorbate 20 (1.80 mL) and heated to 60°C for 10 min while stirring. The mixture was then placed in a sonication bath and sonicated for 30 minutes. Phosphatidylcholine (0.44 g) was slowly dissolved in ethanol (1.8 mL) to form a phosphatidylcholine solution. Once dissolved, the phosphatidylcholine solution was added to the Wnt inhibitor/butylene glycol/polysorbate 20 mixture and stirred at room temperature for 5 min and then subjected to sonication for 30 minutes. After 30 minutes, the solution was added to ultra-pure water (30 mL) and this solution was sonicated for 30 minutes. After sonication, hydroxypropylcellulose (0.285 g) was added to the mixture and the mixture stirred at room temperature for 24 hours before use, providing Formulations 16 and 17.

[00730] The respective Wnt inhibitors of Formulations 16 and 17 are as follows. In Formulation 16, **Compound 7** is the Wnt inhibitor. In Formulation 17, **Compound 8** is the Wnt inhibitor.

Table 9. Formulations 18 and 19, component mass and weight %

	Formulations 18 and 19	
	mass (g)	wt%
WNT inhibitor [a7 or a8]	0.035	0.097%
GO-HA	0.035	0.097%
Glycol [c]	1.818	5.048%
Solubilizer [d]	1.980	5.498%
Thickener [e]	0.285	0.791%
Phospholipid [f]	0.440	1.222%
Carrier [h]	1.422	3.948%
Stabilizer	-	-
Buffer	-	-
Preservative	-	-
Water/saline [k]	30.000	83.299%
Total	36.015	100%

[a7] **Compound 7** (Formulation 18)
[a8] **Compound 8** (Formulation 19)
[c] butylene glycol (d = 1.01 g/mL)
[d] polysorbate 20 (d = 1.1 g/mL)
[e] hydroxypropyl cellulose
[f] phosphatidylcholine
[h] ethanol (d = 0.79 g/mL)
[k] water

[00731] *Preparation of Formulations 18 and 19 compositions*

[00732] Formulations 18 and 19 (Table 9) were prepared as follows. The Wnt inhibitor (35 mg) was suspended in butylene glycol (1.8 mL) and polysorbate 20 (1.8 mL) and heated to 60°C for 10 min while stirring. The mixture was then placed in a sonication bath and sonicated for 30 minutes. Phosphatidylcholine (0.44 g) was slowly dissolved in ethanol (1.8 mL) to form a phosphatidylcholine solution. Once dissolved, the phosphatidylcholine solution was added to the Wnt inhibitor/butylene glycol/polysorbate 20 mixture and stirred at room temperature for 5 min and then subjected to sonication for 30 minutes. After 30 minutes, the solution was added to a previously made solution of GO-HA (35 mg) in ultra-pure water (30 mL) and this new solution was sonicated for 30 min. After sonication, hydroxypropylcellulose (0.285 g) was added to the mixture and the mixture stirred at room temperature for 24 hours before use, providing Formulations 18 and 19 (water-based liposomal compositions).

[00733] The respective Wnt inhibitors of Formulations 18 and 19 are as follows. In Formulation 18, **Compound 7** is the Wnt inhibitor. In Formulation 19, **Compound 8** is the Wnt inhibitor.

Table 10. Formulations 20 to 23, component mass and weight %

	Formulations 20 to 23	
	mass (g)	wt%
WNT inhibitor [a1, a7, a8, or a10]	0.004	0.021%
GO-HA	-	-
Glycol [c]	0.808	4.171%
Solubilizer [d]	0.440	2.271%
Thickener [e]	0.120	0.619%
Phospholipid	-	-
Carrier	-	-
Stabilizer	-	-
Buffer	-	-
Preservative	-	-
Water/saline [k]	18.000	92.918%
Total	19.372	100%

[a1] **Compound 1** (Formulation 20)
[a7] **Compound 7** (Formulation 21)
[a8] **Compound 8** (Formulation 22)
[a10] **Compound 10** (Formulation 23)
[c] butylene glycol (d = 1.01 g/mL)
[d] polysorbate 20 (d = 1.1 g/mL)
[e] hydrofxypropyl cellulose
[k] water

[00734] *Preparation of Formulations 20 to 23 compositions*

[00735] Formulations 20 to 23 (Table 10) were prepared as follows. The Wnt Inhibitor (4 mg) was suspended in butylene glycol (0.80 mL) and polysorbate 20 (0.40 mL) and heated to 60°C for 10 min while stirring. The mixture was then placed in a sonication bath and sonicated for 30 minutes. After 30 minutes, the solution was added to ultra-pure water (18 mL) and this solution was sonicated for 30 minutes. After sonication, hydroxypropylcellulose (0.120 g) was added to the mixture and the mixture stirred at room temperature for 24 hours before use, providing Formulations 20 to 23.

[00736] The respective Wnt inhibitors of Formulations 20 to 23 are as follows. In Formulation 20, **Compound 1** is the Wnt inhibitor. In Formulation 21, **Compound 7** is the Wnt inhibitor. In Formulation 22, **Compound 8** is the Wnt inhibitor. In Formulation 23, **Compound 10** is the Wnt inhibitor.

Table 11. Formulations 24 to 27, component mass and weight %

	Formulations 24 to 27	
	mass (g)	wt%
WNT inhibitor [a1, a7, a8, or a10]	0.018	0.094%
GO-HA	0.009	0.047%
Glycol [b]	0.904	4.745%
Solubilizer [e]	0.120	0.630%
Thickener	-	-
Phospholipid	-	-
Carrier	-	-
Stabilizer	-	-
Buffer	-	-
Preservative	-	-
Water/saline [k]	18.000	94.483%
Total	19.051	100%

[a1] **Compound 1** (Formulation 24)
[a7] **Compound 7** (Formulation 25)
[a8] **Compound 8** (Formulation 26)
[a10] **Compound 10** (Formulation 27)
[b] PEG-400 (d = 1.13 g/mL)
[e] hydroxypropyl cellulose
[k] water

[00737] *Preparation of Formulations 24 to 27 compositions*

[00738] Formulations 24 to 27 (Table 11) were prepared as follows. 9 mg of GO-HA was dissolved in 18 mL of ultra-pure water. The solution was ultrasonicated for 10 minutes. The Wnt inhibitor (18 mg) was added to PEG-400 (0.8 mL) and subjected to ultrasonication for 30 minutes, to form a Wnt inhibitor solution. The Wnt inhibitor solution was added dropwise to the GO-HA solution and vigorously stirred for 5 minutes. The combined solution was then subjected to ultrasonication for 1 hour and then stirred at room temperature for 18 hours. Hydroxypropyl cellulose (0.12 g; supplier: Sigma-Aldrich) was added in small portions at room temperature with vigorous stirring. Once addition was complete, the solution was stirred at room temperature for 24 hours to form viscous solutions of Formulations 24 to 27.

[00739] The respective Wnt inhibitors of Formulations 24 to 27 are as follows. In Formulation 24, **Compound 1** is the Wnt inhibitor. In Formulation 25, **Compound 7** is the Wnt inhibitor. In Formulation 26, **Compound 8** is the Wnt inhibitor. In Formulation 27, **Compound 10** is the Wnt inhibitor.

Biological Examples: WNT inhibitors

[00740] Biological Example A (WNT inhibitor)

[00741] *HEK CELL Wnt Assay*

[00742] In order to determine the effect of the compounds of Formula (I) on Wnt inhibition, an *in vitro* assay using HEK293 SuperTOPFlash reporter cells, with the Wnt promoter driving a luciferase reporter gene, were utilized. 40,000 HEK293 SuperTOPFlash reporter cells were seeded, per well, in a 48 well plate for 24 hours, prior to administration of serial dilutions of the compound of Formula (I) to individual wells. Serial dilutions ranging from 10 μm to 1 nM of the compound of Formula (I) were added with or without 50 ng/mL $Wnt_{3}\alpha$ (Time Biosciences) and allowed to incubate with the reporter cells for 18 hours. Reporter cells with no treatment were used as negative control. Following the 18-hour incubation, luciferase activity was measured using Steady-Glo® Luciferase Assay System (Promega) and a Luminometer; with luciferase activity correlated to Wnt activation, and 50% inhibition of the Wnt promoter (IC_{50}) calculated. Results are shown in Table A.

[00743] Biological Example B (WNT inhibitor)

[00744] *TNK2 Binding Assay*

[00745] Tankyrase 2 binding assay (n=3) was performed with the following steps: step 1, coating a 96-well plate with 50 μL/well of histone solution; step 2, conducting ribosylation reaction; and step 3, detection. Results are shown in Table A.

[00746] Step 1: Coating a 96-well plate with 50 μL/well of histone solution.

[00747] A 5x histone mixture was diluted 1:5 with PBS to make a 1x histone in PBS mixture. 50 μL of the histone mixture was added to each well and incubated at 4 °C overnight. The plate was washed three times using 200 μL PBST buffer (1x PBS containing 0.05% Tween®20 (polysorbate 20) per well. The plate was tapped onto a clean paper towel to remove the liquid. The wells were blocked by adding 200 μL of blocking buffer 3 to each well. The plate was incubated at room temperature for at least 90 minutes. The plate was washed three times with 200 μL PBST buffer. The plate was tapped onto a clean paper towel to remove the liquid.

[00748] Step 2: Ribosylation reaction.

[00749] A fresh solution of 10 mM DTT in water was prepared.

[00750] A master mix was prepared and delivered to each well (25 μL/well). Master mix delivered to each well: (2.5 μL of 10x PARP assay buffer (purchased from BPS Bioscience) + 2.5 μL of PARP substrate mixture 2 + 17.5 μL of water + 2.5 μL of 10 mM fresh DTT). The concentration of DTT in the master mix was 1 mM. 25 μL of master mix was added to each well.

[00751] 1x PARP buffer with DTT was prepared. 10x PARP assay buffer was diluted to 1x PARP assay buffer containing DTT by adding 1 volume of 10x PARP assay buffer + 1 volume of 10 mM DTT + 8 volumes of water. The concentration of DTT in the 1x PARP assay buffer was 1 mM.

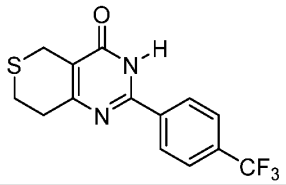
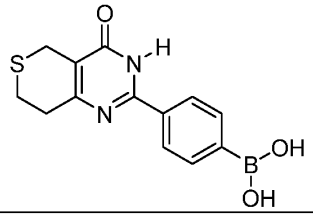
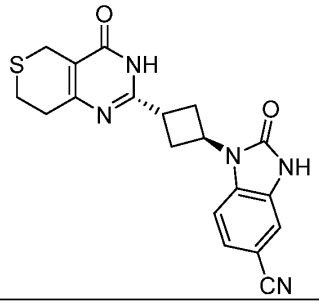
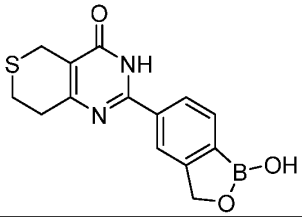
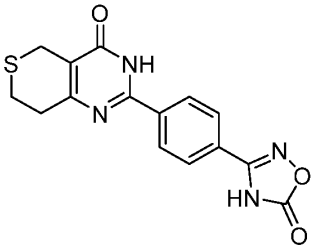
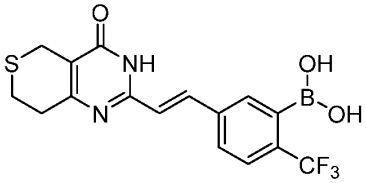
[00752] Serial dilutions of each test inhibitor were made (in 1x PARP assay buffer with DTT), starting from 10 μM to 0 μM (10 μM, 1 μM, 0.1 μM, 0.05 μM, 0.025 μM, 0.01 μM, 0.001 μM, 0 μM). 5 μL of a test inhibitor was added to each well labeled as "Test Inhibitor." For the "Positive Control," the PARP inhibitor, XAV939, was used. For the negative control, no inhibitor was added to the assay mix.

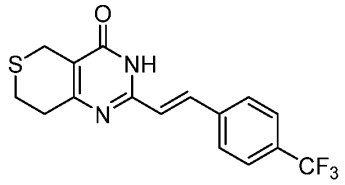
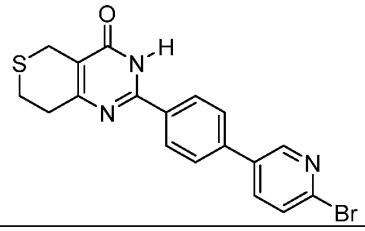
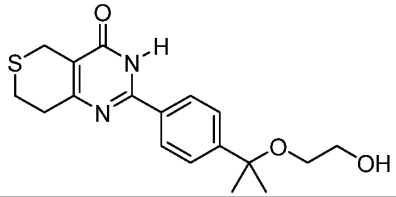
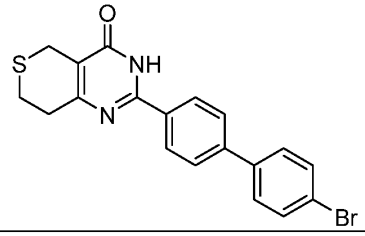
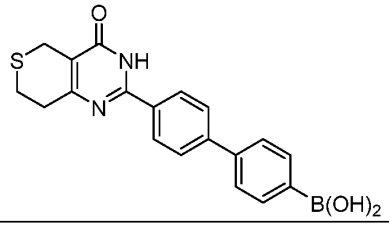
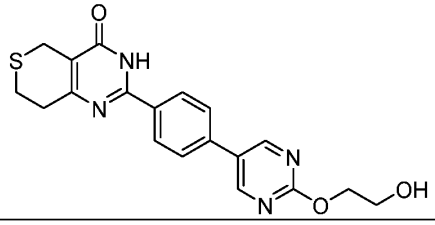
[00753] The Tankyrase 2 (TNKS2) enzyme on ice was thawed. The tube containing the enzyme was spun briefly to recover the full content of the tube. The amount of TNKS2 enzyme required for the assay was calculated and the enzyme was diluted to 0.5 ng/μL with 1x PARP assay buffer with DTT. 16) The final concentration of TNKS2 enzyme in the assay was 2 nM. The reaction was initiated by adding 10 μL of diluted TNKS2 enzyme to each well designated "Positive Control" and "Test Inhibitor." To each well designated as "Blank," 10 μL of 1x PARP assay buffer with DTT was added. The plate was incubated at room temperature for 1 hour. The plate was washed three times with 100 μL of PBST buffer and the plate was tapped onto a clean paper towel.

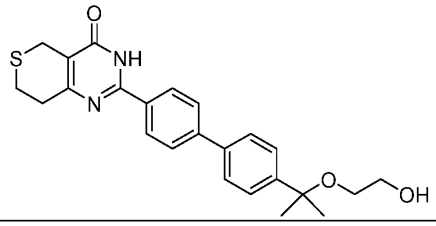
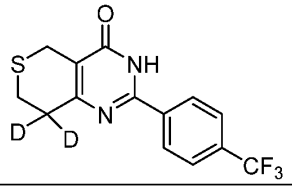
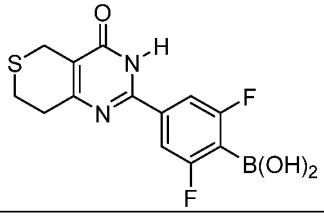
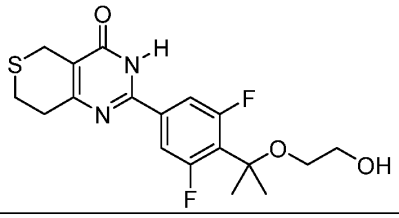
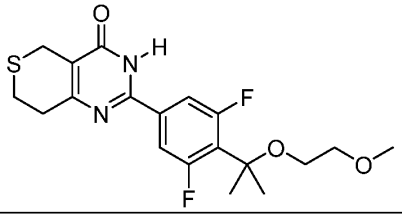
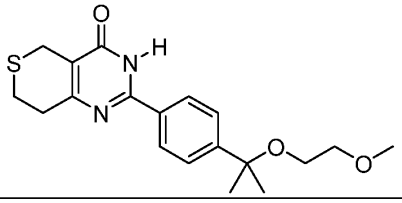
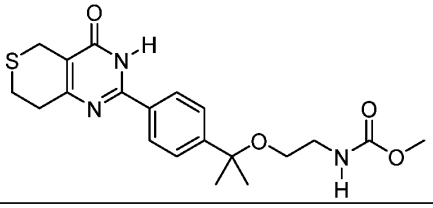
[00754] **Step 3:** Detection.

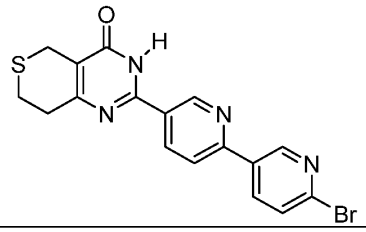
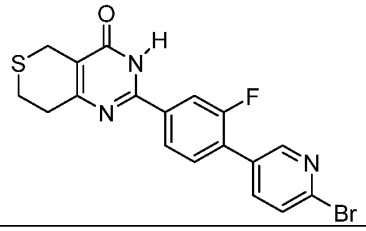
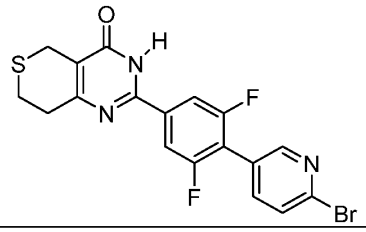
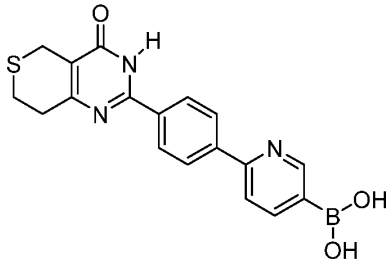
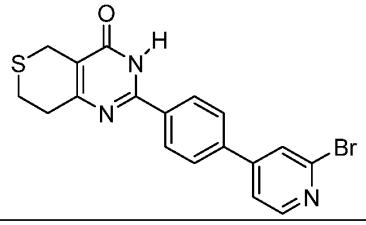
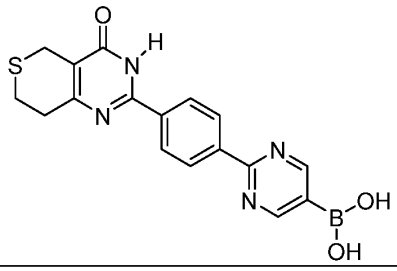
[00755] 1) Streptavidin-HRP was diluted 1:50 in Blocking buffer (purchased from BPS Bioscience) 3.50 μL of diluted Streptavidin-HRP was added to each well and incubated for 30 minutes at room temperature. The plate was washed three times with 100 μL of PBST buffer and tapped onto a clean paper towel. Just before use, 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B were mixed on ice; and 100 μL was added to each well. Immediately, the plate was read in a luminometer or microtiter-plate reader capable of reading chemiluminescence. The "Blank" value was subtracted from all other values. The results are shown in Table A. "NT", such as in Table A, means that this combination was not tested.

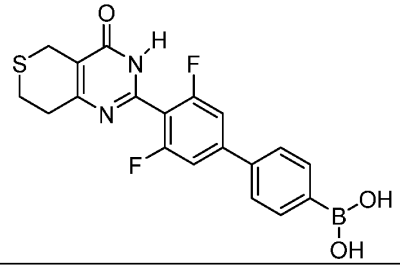
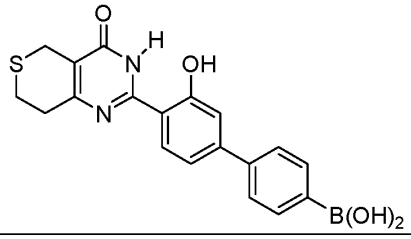
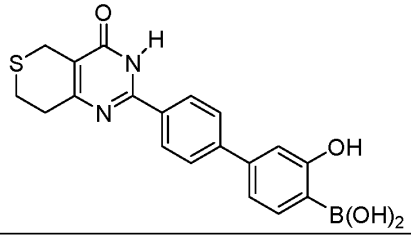
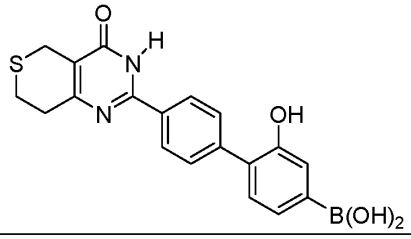
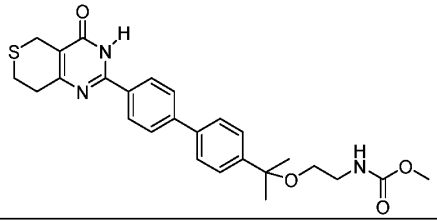
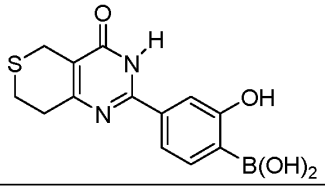
Table A. Assay Results (WNT inhibitors)

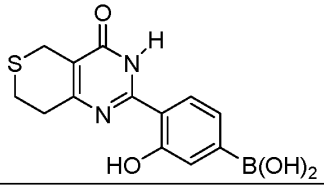
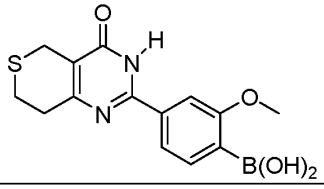
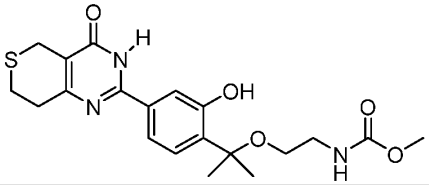
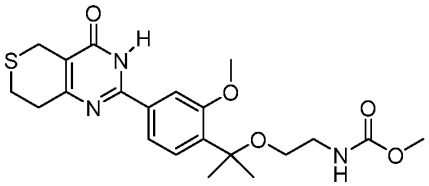
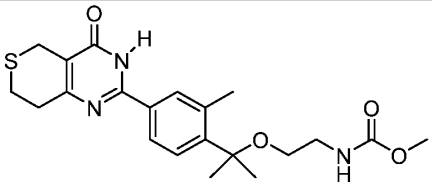
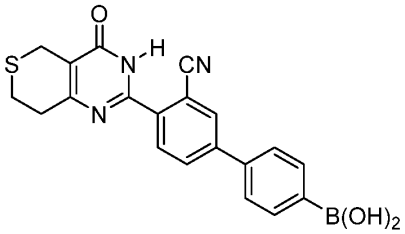
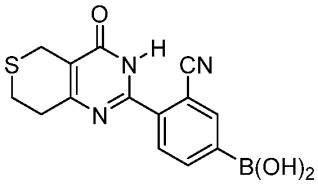
Cmpd No.	Structure	HEK CELL Wnt IC_{50} (nM)	Binding Assay (TNK2) IC_{50} (nM)
XAV939		50 nM	6 nM
1		2,000 nM	26 nM
2		60% inhibition @ >10,000 nM	NT
3		7,500 nM	28 nM
4		>10,000 nM	13 nM
5		5,000 nM	NT

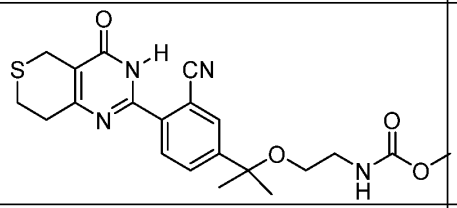
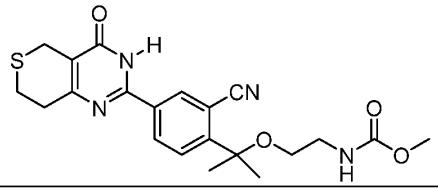
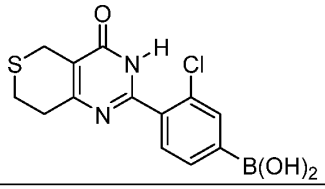
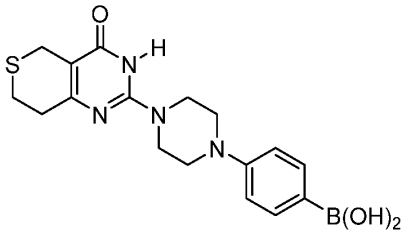
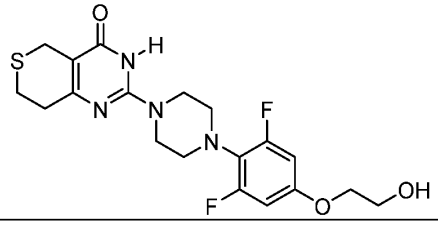
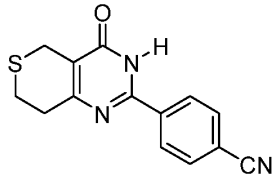
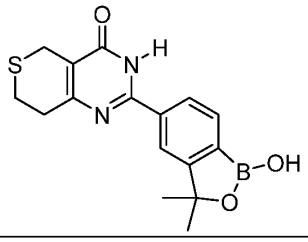
Cmpd No.	Structure	HEK CELL Wnt IC_{50} (nM)	Binding Assay (TNK2) IC_{50} (nM)
6		2,000 nM	2500 nM
7		17 nM	5 nM
8		4.4 nM	3.16 nM
9		100 nM	NT
10		5 nM	3.29 nM
11		100 nM (solubility issue)	21 nM

Cmpd No.	Structure	HEK CELL Wnt IC_{50} (nM)	Binding Assay (TNK2) IC_{50} (nM)
12		3 nM	6.5 nM
13		4 nM	3 nM
14		8,000 nM	11 nM
15		NT	90 nM
16		400 nM	50 nM
17		60 nM	3 nM
18		6 nM	4 nM

Cmpd No.	Structure	HEK CELL Wnt IC_{50} (nM)	Binding Assay (TNK2) IC_{50} (nM)
19		2,500 nM	40 nM
20		400 nM	4 nM
21		2,000 nM	12 nM
22		250 nM	4 nM
23		80 nM	14 nM
24		500 nM	3 nM

Cmpd No.	Structure	HEK CELL Wnt IC_{50} (nM)	Binding Assay (TNK2) IC_{50} (nM)
25		2,500 nM	63 nM
26		652 nM	24 nM
27		36 nM	2 nM
28		2 nM	2 nM
29		100 nM	1 nM
30		NT	20 nM

Cmpd No.	Structure	HEK CELL Wnt IC_{50} (nM)	Binding Assay (TNK2) IC_{50} (nM)
31		NT	3534 nM
32		NT	111 nM
33		NT	12 nM
34		NT	164 nM
35		NT	13 nM
36		NT	29 nM
37		NT	16 nM

Cmpd No.	Structure	HEK CELL Wnt IC_{50} (nM)	Binding Assay (TNK2) IC_{50} (nM)
38		NT	72 nM
39		NT	650 nM
40		NT	443 nM
41		NT	8.7 nM
42		NT	9.7 nM
43		1,000 nM	NT
44		NT	630 nM

[00756] Biological Example C (WNT inhibitor)

[00757] *Demonstration of Wnt inhibition by a composition of Formula (I)*

[00758] In order to determine the effect of the compounds of Formula (I) on Wnt inhibition, an *in vitro* assay using HEK293 SuperTOPFlash reporter cells, with the Wnt promoter driving a luciferase reporter gene, were utilized. 40,000 HEK293 SuperTOPFlash reporter cells were seeded, per well, in a 48 well plate for 24 hours, prior to administration of serial dilutions of the compounds of Formula (I) to individual wells. Serial dilutions ranging from 10 μm to 1 nM of compounds of Formula (I) were added with or without 50 ng/mL $Wnt_{3\alpha}$ (Time Biosciences) and allowed to incubate with the reporter cells for 18 hours. Reporter cells with no treatment were used as negative control. Following the 18 hour incubation, luciferase activity was measured using Steady-Glo® Luciferase Assay System (Promega) and a Luminometer, with luciferase activity correlated to Wnt activation, and 50% inhibition of the Wnt promoter (IC_{50}) calculated.

[00759] **FIG. 32** presents the results of IC_{50} assessment of **Compound 1** (a compound of Formula (I)) in DMSO and of GO-HA + **Compound 1** (GO-HA/Compound 1) in an aqueous formulation as further described in Pharmaceutical Composition Example 1. IC_{50} of Wnt activity was calculated as 2 μM for **Compound 1** and 500 nM for GO-HA + **Compound 1**, demonstrating that the suspension of **Compound 1** in aqueous GO-HA and **Compound 1** with DMSO as the solvent both demonstrate inhibition of Wnt activity.

[00760] **FIG. 33** presents the results of IC_{50} assessment of a compound of Formula (I), **Compound 7**, in DMSO, and of GO-HA + **Compound 7** (GO-HA/Compound 7) in an aqueous formulation as further described in Pharmaceutical Composition Example 2. IC_{50} of Wnt activity was calculated as 5-10 nM for **Compound 7** and 4 nM for GO-HA + **Compound 7**, demonstrating that the suspension of **Compound 7** in aqueous GO-HA and **Compound 7** with DMSO as the solvent both demonstrate inhibition of Wnt activity.

[00761] **FIG. 34** presents the results of IC_{50} assessment of **Compound 8** (a compound of Formula (I)) in DMSO, and of GO-HA + **Compound 8** (GO-HA/Compound 8) in an aqueous formulation as further described in Pharmaceutical Composition Example 3. IC_{50} of Wnt activity was calculated as 5-10 nM for **Compound 8** and less than 1 nM for GO-HA + **Compound 8**, demonstrating that the suspension of **Compound 8** (a compound of Formula (I)) in aqueous GO-HA and **Compound 8** (a compound of Formula (I)) with DMSO as the solvent both demonstrate inhibition of Wnt activity.

Biological Examples: Compositions

[00762] Biological Example 1A

[00763] *Full thickness excision wound and wound healing effect following administration of a pharmaceutical composition described in Example 1 (Formulation 1).*

[00764] *Animal Study 1*

[00765] Formulation 1 was tested in the treatment of a full thickness excision wound. The objective of this study was to observe the wound healing effect of a composition of the present disclosure (Formulation 1) in a dermal full thickness injury rat model (elliptical excision wound) following topical administration. The preparation of Formulation 1 is described in Example 1.

[00766] *Test Article*

[00767] Formulation 1 was used as a pharmaceutical composition according to one or more embodiments herein. Vehicle: 96 vol% water and 4 vol% PEG-400.

[00768] *Animal Husbandry*

[00769] 16 Sprague-Dawley rats at age of about 6-8 weeks were used. Animal room was set to maintain a temperature of 23±2°C, humidity of 40-70%, and a 12-hour light/12-hour dark cycle. SPF Rat Growth Breeding Feed was provided ad libitum throughout the in-life portion of the study. Reverse Osmosis water was available to the animals *ad libitum*. Animals were free to access both food and water during the whole course of study.

[00770] *Animal Grouping and Test Procedure*

[00771] Animals were housed 5 rats per cage and acclimated for 5-7 days. They were then anesthetized with pentobarbital sodium (45mg/kg, ip., 2% in saline) and shaved on both sides of the back with an electric clipper, disinfected by applying betadine followed by 70% ethanol wiping. A (3 to 4 cm^2) full thickness of elliptical excision wound was created using toothed forceps, Acupunch® (12mm, Acuderm Inc, USA) and scissors, skin was removed from the underlying muscle.

[00772] The animals were allocated to two groups of 8 animals apiece, according to the wound areas and body weight. Vehicle group: G1 (G1-1, G1-2, G1-3, G1-4, G1-5, G1-6, G1-7, G1-8); Formulation 1 group: G2 (G2-1, G2-2, G2-3, G2-4, G2-5, G2-6, G2-7, G2-8). The grouping is summarized in Table 12, below.

Table 12. Animal study 1 groupings, dosing schedule

Group	Number of animals	Treatment	Dosage (μL /rat)	Route of Adm.	Dosing Schedule
1	8	Vehicle	100	Topical	QD×14d

Group	Number of animals	Treatment	Dosage (μL /rat)	Route of Adm.	Dosing Schedule
2	8	Formulation 1	100	Topical	QD×14d

QD = same time interval every day; QDx14d = every day for 2 weeks

[00773] Formulation 1 and vehicle were topically administered to the animals respectively via a syringe to drip the formulation directly into the wound bed to create an even and consistent layer over the wound to cover the full wound area. The treatment was continued daily for 2 weeks (14 days) following injury.

[00774] Body weights of the animals were measured twice weekly. Wound area boundary was drawn on a transparency paper with a permanent marker on Day 1, Day 5, Day10 and Day 15 and the area will be measured with ImagePro Premier®. Photos of the wounds of the rats were taken on Day 1, Day 5, Day 10, and Day 15.

[00775] For staining: two 5 μm slides per wound were stained with H&E and Masson's trichrome and digitally scanned for pathology analysis. The pathology analysis included: (1) Evaluation of Scar resolution and rete ridges formation (dermal-epidermal junctions) in healing skin: Masson's trichrome stained sections of skin were created from the full-thickness excisional wounds and evaluated for "linear" extracellular matrix (scar-like) for the articles. (2) Evaluation of fiber thickness of healing skin by Masson's trichrome stained sections imaged with circular polarized light for the articles. (3) Evaluation of rete-ridge for the articles by H&E staining.

[00776] *Pathology testing*

[00777] Materials: skin samples, 10% formalin, hematoxylin, eosin staining solution, acid fuchsin, aniline blue, dimethylbenzene, 70% alcohol, 95% alcohol, 100% alcohol, etc.

[00778] Instruments: tissue hydroextractor (Shandon Excelsior ES™ Tissue Processor Fisher/Thermo A78400006); tissue embedder (Shandon Histocentre™ 3 Tissue Embedding Center Fisher/Thermo B64100010); Leica automatic slicing machine (Leica RM2255); Automatic staining machine (Automatic Slide Stainer Fisher/Thermo A74200010, Shandon Varistain® 24-4).

[00779] Tissue processing & slicing was as follows: (1) Tissue processing was by tissue hydroextractor: gradient alcohol dehydration, transparentizing was by dimethylbenzene, paraffin infiltration, embedding.

[00780] (2) The samples were cut into 5 μm thick slides by Leica automatic slicing machine, followed by (3) H&E staining, Masson's trichrome staining.

[00781] Evaluation parameters: re-epithelialization, neovascularization, rete ridge, scabbing, granulation. Scoring criteria (results or scores) are summarized in Table 13.

Table 13. Evaluation parameters (scores, results)

1. Re-epithelialization		
New epithelium attempting to bridge over the wound gap	0	No ingrowth of epithelium into the wound gap
	1	Small stumps of growth from the edge
	2	Large gap between the bridging epithelium
	3	Small gap between the bridging epithelium
	4	Complete bridging over the wound
2.Neovascularization		
Newly formed vascular network in the wound bed	0	Relatively normal
	1	Mildly increased in number in the wound bed
	2	Moderately increased in number in wound bed
	3	Moderately to markedly increased in number in wound bed
	4	Markedly increased in number in wound bed
3.Rete-ridge		
Newly formed columns of epithelium extending downward to dermis of the wound bed	0	No formation of rete ridge
	1	Few columns formed
	2	Mildly increased in number
	3	Moderately increased in number
	4	Markedly increased in number
4.Scabbing		
Formation of scab on the cornified layer or on the wound bed	0	Relatively normal
	1	Small amount of scabbing
	2	Moderate amount of scabbing
	3	Moderate to marked amount of scabbing
	4	Marked amount of scabbing
5.Granulation		
Accumulation of inflammatory cells in the matrix of fibrotic/scar tissues in the wound bed of dermis	0	Relatively normal
	1	Small amount scattered in the wound bed
	2	Moderate amount across the wound bed
	3	Dense fibrotic tissues with heavy infiltration of inflammation
	4	Fibrinoid formation in the dermis

[00782] *Observation & analysis*

[00783] Clinical signs: clinical signs were recorded for individual animals, once before commencement of treatment and once daily during the study; observations were performed at the same time interval every day (QD).

[00784] Terminal Studies: Animals in extremis or disposed for humane reasons and those that completed the scheduled test period were euthanized by carbon dioxide.

[00785] Statistical Analysis: The results (individual and group) were analyzed using Student's unpaired t-test. Data were given as Mean ± SD or Mean ± SEM. $P<0.05$ was considered significant.

[00786] *Results*

[00787] Clinical signs: Mortality, morbidity and the abnormal behavior were not found during the treatment period of the experiment. During the experiment, the following signs did not occur: suffering (cachexia, weakening, difficulty to move or to eat, pain, crying); toxicity (hunching, convulsions); 25% body weight loss for three consecutive days or 20% body weight loss on any day. There was no significant difference between groups in wound areas during the experiment ($p>0.05$).

[00788] On Day 15, 3 rats in each group (vehicle group: G1-4, G1-5, G1-8; Formulation 1 group: G2-3, G2-4, G2-6) with an average wound healing rate were euthanized with carbon oxide, and wound tissue were excised and fixed neutral formalin buffer then embedded in paraffin for histology.

[00789] The results (scores) of the Formulation 1 group and the vehicle group are represented in **FIG. 1**. From **FIG. 1**, it can be seen that Formulation 1 had a significant effect on re-epithelization (a component of wound healing used as a defining parameter of a successful wound closure), improved neovascularization (the natural formation of new blood vessels, usually in the form of functional microvascular networks, capable of perfusion by red blood cells, that form to serve as collateral circulation in response to local poor perfusion or ischemia), improved rete ridge formation (epithelial extensions that project into the underlying connective tissue in both skin and mucous membranes-improved rete ridge formation is a sign of tissue regeneration rather than fibrosis / scarring (reduced rete ridge formation)), decreased scabbing (wounds with scabs take longer to heal and a sign of fibrosis/scarring) and decreased granulation (granulation tissue is composed of extracellular matrix proteins such as fibrin and type III collagen that can be quickly laid down by the initial surge of fibroblasts that is recruited to the wound site-causing a scar).

[00790] The total effect of Formulation 1 toward wound healing with scar reduction was evaluated using the following formula: Total = (Re-epithelization + Neovascularization + Rete ridge formation) – (Scabbing + Granulation)), using mean measured values for each category: Formulation 1 = 4.66. Vehicle = -1.33. Under this metric, there was a 5.99-fold increase in scar reduction using Formulation 1 in this study as compared to the control group.

[00791] *Animal Study 2*

[00792] The objective of this study was to observe the wound healing effect of Formulation 1 in a dermal full thickness injury STZ induced diabetic rat model with topical administration.

[00793] *Pharmaceutical composition and preparation*

[00794] The preparation of Formulation 1 is described in Example 1. Vehicle: 96 vol% water and 4 vol% PEG-400.

[00795] *Test article preparation procedure*

[00796] Formulation 1 was admixed well by sonicating for 10 minutes before injecting via syringe 100 μL of sample onto the wound.

[00797] *Animal husbandry*

[00798] 14 male Spargue-Dawley rats, grade specific pathogen free (SPF) of an age of about 6-8 weeks were used. The rats had a blood glucose of > 16.7mM.

[00799] Weight at initiation of treatment: Within the range of 200~300g.

[00800] Acclimation period: 7 days.

[00801] Environmental controls for the animal room were set to maintain a temperature of 23±2°C, humidity of 40~70%, and a 12-hour light/12-hour dark cycle. The 12-hour dark cycle may be temporarily interrupted to accommodate study procedures.

[00802] Food and water: SPF Rat Growth Breeding Feed (BEIJING KEAO XIELI FEED CO. LTD.) was provided ad libitum throughout the in-life portion of the study. Reverse Osmosis water was available ad libitum.

[00803] Animal selection and fasting: Animals to be used in this study were selected based on overall health and acclimation to caging. Animals were free to access both food and water during the whole course of study.

[00804] *Experimental design*

[00805] Upon arrival at the animal facility, the animals were housed 5 rats per cage and acclimated for seven days. Prior to the experiments, food was withdrawn for about 16 hours. The rats were rendered diabetic by an intraperitoneal (i.p.) injection of streptozotocin (STZ) (65mg/kg) dissolved in sodium citrate buffer, pH 4.5. The rats with a final blood glucose level >16.7 mM (300 mg/dL) were included in the study. The selected rats were anesthetized with pentobarbital sodium (45mg/kg, i.p. 2% in saline) and shaved on both sides of the back with an electric clipper, disinfected by iodophor followed by 75% ethanol wiping. A (3 to 4 cm^2) full thickness of elliptical excision wound was created using Acupunch® (12 mm, Acuderm

Inc, USA). The skin was removed from the underlying muscle using scissors and toothed forceps.

[00806] The animals were divided into two groups according to the wound area and the body weight according to Table 14.

Table 14. Animal study 2 groupings, dosing schedule

Group	Number of Animals	Treatment	Dosage(μL/rat)	Route of Adm.	Dosing Schedule
1	7	Vehicle	100	Topical	QD×21d
2	7	Formulation 1	100	Topical	QD×21d

QD = same time interval every day; QDx21d = every day for 3 weeks

[00807] The wound was topically administrated (via a syringe to drip the formulation directly into the wound bed to create an even and consistent layer over the wound making sure to cover the full wound area) with Formulation 1 or vehicle (100 μL/rat). The treatment was continued daily for 21 days following injury.

[00808] **FIG. 2** is a plot showing the average area size of the wounds of the Vehicle group and the Formulation 1 group during the 21-day treatment. The results indicate that topical administration of Formulation 1 increased the rate of healing of the diabetic rat impaired healing animal model as compared to vehicle. Primarily, the rate increase was observed on days 10-18 of the 21 day study. There were no signs of overt toxicity in this study as indicated by body weight measurements.

[00809] **Biological Example 1B**

[00810] *Cartilage regrowth in mice following administration of a pharmaceutical composition described in Example 1 (Formulation 1).*

[00811] Formulation 1 was tested in the treatment of a cartilage wound. The objective of this study was to observe the wound healing effect (cartilage regeneration and regrowth) of a composition of the present disclosure in a cartilage wound injury mouse model following topical administration. The preparation of Formulation 1 is described in Example 1.

[00812] C57Bl/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). C57Bl/6J mice (at least 3 months of age) were anesthetized with 3–5% isoflurane in O_2 administered using Tabletop Laboratory Animal Anesthesia System (VetEquip Inc., Pleasanton, CA, USA). A 2 mm biopsy punch wound was made in the center of the cartilaginous region of each ear using a disposable biopsy punch (Acuderm Inc., Fort Lauderdale, FL, USA) as described previously (Rai, M.F. et al, 2021, *Arthritis Rheum.*, 64:2300-2310). The ears were treated topically with 5 μL/ear of XAV939 (Selleck Chemicals,

S1180, Houston, TX, USA), dissolved to 5 μM in dimethyl sulfoxide (DMSO) or a control solution of DMSO, every day (QD), for 30 days, resulting in a daily administration of 7.81 μg XAV939.

[00813] After 30 days of treatment, the ears were imaged using a Nikon Coolpix 8700 digital camera (Nikon Corporation, Japan). To quantify closure of the wounds, ears were excised following mouse sacrifice, fixed, and placed on microscope slides for examination by way of the digital camera being affixed to a Micromaster Inverted microscope (Thermo Fisher Scientific, Waltham, MA, USA). Fixation was by way of immersion in 10% buffered formalin for twenty-four hours, sliced longitudinally across the injury and then embedded in paraffin for sectioning. Sections of the tissue were then stained with trichome blue, imaged and analyzed by way of an Olympus DP71 microscope camera (Olympus America, Center Valley, PA, USA).

[00814] For Formulation 1 administration, STZ-induced type I diabetic C57Bl/6J mice were used. 8 to 12-week-old, diabetes induced mice, with serum glucose levels between 270-478 mg/dL were initiated into the study. The mice were anesthetized with 3% to 5% isoflurane in O_2 administered using Tabletop Laboratory Animal Anesthesia System (VetEquip Inc., Pleasanton, CA, USA). A 2 mm biopsy punch wound was made in the center of the cartilaginous region of each ear using a disposable biopsy punch (AcudermInc., Fort Lauderdale, FL, USA) as described previously (Rai, M.F. et al, 2021, *Arthritis Rheum.*, 64:2300-2310). The ears were treated topically with 50 μL of Formulation 1, prepared as described in Example 1, containing 1 mg/mL XAV939 (n=6) or a control solution of GO-HA, every other day (QOD) for 15 days. For quantification of wound closure, ears were excised after the mice were sacrificed, placed on microscope slides (Denville Scientific, South Plainfield, NJ, USA) and imaged using Nikon Coolpix 8700 affixed to Micromaster Inverted microscope (Thermo Fisher Scientific, Waltham, MA, USA). Excised ears were fixed for 24 h in 10% buffered formalin, cut longitudinally across the injury and embedded in paraffin blocks. Sections of the embedded tissue were stained with trichrome blue imaged and analyzed using an Olympus DP71 microscope camera (Olympus America, Center Valley, PA, USA).

[00815] The distance between opposing cartilage endplates was measured in four separate animal groups following treatment of the 2 mm biopsy punch wounds: XAV939 in DMSO every day (QD) for 30 days, DMSO (as a control) every day (QD) for 30 days, Formulation 1 every other day (QOD) for 15 days, and GO-HA (as a control) every other day (QOD) for 15 days. **FIG. 3** shows the distance between opposing cartilage endpoints from their 2 mm starting

point, from a 30-day treatment of biopsy punch wounds with XAV939 in DMSO, 15 day treatment of biopsy punch wounds with GO-HA, 30 day treatment of biopsy punch wounds with saline, and 15-day treatment with Formulation 1; where administration of Formulation 1 provided significant (ANOVA *$p<0.05$, unpaired t-test **$p<0.001$) quantitative improvement in cartilage regrowth with lesser dose of the active ingredient XAV939 administered for half the time as compared, with significant difference observed between Formulation 1 and DMSO (ANOVA **$p<0.0001$). There was significant improvement observed for Formulation 1 compared to GO-HA (unpaired t-test *$p<0.05$). Further, the nature and character of the cartilage regrowth in the wounds was observed as improved in the XAV939 in DMSO and Formulation 1, as compared to DMSO and GO-HA controls, respectively. **FIG. 4** shows a direct comparison of the distance between opposing cartilage endpoints, from a 30-day treatment of 2 mm biopsy punch wounds with XAV939 in DMSO and a 15-day treatment with Formulation 1. **FIG. 5** shows a direct comparison of the distance between opposing cartilage endpoints, from a 15-day treatment of 2 mm biopsy punch wounds with GO-HA and a 15-day treatment with Formulation 1.

[00816] Biological Example 2

[00817] *Cartilage regrowth in rabbit ears following administration of a pharmaceutical composition described in Example 2 (Formulation 2).*

[00818] Formulation 2 was tested in the treatment of a cartilage wound. The objective of this study was to observe the wound healing effect (cartilage regeneration and regrowth) of a composition of the present disclosure in a cartilage wound injury rabbit model following topical administration. The preparation of Formulation 2 is described in Example 2.

[00819] New Zealand White rabbits were purchased from the Western Oregon Rabbit Company. Animals were anesthetized by way of either inhalation isoflurane (1-5% Iso/ 2.0 L O_2) or cocktail of Ketamine/Zylazine administered intramuscularly, for the least amount of time possible for completing the biopsy procedures described herein. Before surgery, the hair on the ventral side of each ear was removed thoroughly with a razor or other appropriate methods (e.g. depilatory) and biopsy sites surgically scrubbed with Clorhexidine and alcohol. Wounds of 6 mm size were created via punch biopsy using a disposable biopsy punch, with the wound extending throughout the entirety of the ear. Up to 8 total wounds per animal were created, and the excised tissue discarded. Following creation of wounds, the wound bed was cleaned with sterile saline and/or gauze to remove any foreign matter/loose tissue debris, if present.

[00820] Wounds were dosed with 0.3 mL of either saline or 0.3 mL of 1 mg/mL water-based liposomal composition of Formulation 2, every other day (QOD) for 21 days. On dosing days the wound surface was cleaned to remove any blood clots, excessive exudates, dressing debris, test article residues, or tissue build up on the wound bed, using sterile materials (i.e. saline moistened sterile gauze), but care was taken to avoid disturbing the surface of the wound. The test article (saline or Formulation 2) was applied directly to the designated wound sites in a thin layer evenly over the wound site using a sterile applicator. After each dose the wound site was covered/bandaged in accordance with the study protocol or as recommended by the attending veterinarian. Images were taken on each day of dosing after the wounds were cleaned thoroughly and prior to dose administration on dosing days. The images were taken directly in front of the wound to ensure an accurate measurement and included a ruler with appropriate identifiers.

[00821] **FIG. 6** shows a direct comparison of the distance between opposing cartilage endpoints, from a 21-day treatment of 6 mm biopsy punch wounds through the rabbit ears with saline and a 21-day treatment with Formulation 2 (water-based liposomal composition). As can be seen, Formulation 2 treatment had a statistically significant improvement (ANOVA *$p<0.05$, unpaired t-test) of the regeneration of cartilage, compared to saline treatment (control).

[00822] **Biological Example 3**

[00823] *Demonstration of Wnt inhibition following administration of pharmaceutical compositions described in Example 3 (Formulations 3 to 5).*

[00824] To determine the effect of the pharmaceutical compositions on Wnt inhibition, an *in vitro* assay using HEK293 SuperTOPFlash reporter cells, with the Wnt promoter driving a luciferase reporter gene, were utilized. 40,000 HEK293 SuperTOPFlash reporter cells were seeded, per well, in a 48 well plate for 24 hours, prior to administration of serial dilutions of the Wnt inhibitors of Formula (I) to individual wells. Serial dilutions ranging from 10 μm to 1 nM of Wnt inhibitors of Formula (I) were added with or without 50 ng/mL $Wnt_{3}\alpha$ (Time Biosciences) and allowed to incubate with the reporter cells for 18 hours. Reporter cells with no treatment were used as negative control. Following the 18 hour incubation, luciferase activity was measured using Steady-Glo® Luciferase Assay System (Promega) and a Luminometer; with luciferase activity correlated to Wnt activation, and 50% inhibition of the Wnt promoter (IC_{50}) calculated.

[00825] **FIG. 7** presents the results of IC_{50} assessment of a Wnt inhibitor of Formula (I), **Compound 1**, in DMSO and of Formulation 3 (comprising GO-HA + **Compound 1** in an

aqueous formulation) as further described in Example 3. IC_{50} of Wnt activity was calculated as 2 μM for **Compound 1** and 500 nM for Formulation 3. These results demonstrate that the suspension of **Compound 1** in aqueous GO-HA (Formulation 3) and **Compound 1** with DMSO as the solvent both inhibited Wnt activity. Further, these results demonstrate that the pharmaceutical composition of Formulation 3 provided improved IC_{50} compared to **Compound 1** with solvent.

[00826] **FIG. 8** presents the results of IC_{50} assessment of a Wnt inhibitor of Formula (I), **Compound 7**, in DMSO, and of Formulation 4 (comprising GO-HA + **Compound 7** in an aqueous formulation) as further described in Example 4. IC_{50} of Wnt activity was calculated as 5-10 nM for **Compound 7** and 4 nM for Formulation 4. These results demonstrate that the suspension of **Compound 7** in aqueous GO-HA (Formulation 4) and **Compound 7** with DMSO as the solvent both inhibited Wnt activity. Further, these results demonstrate that the pharmaceutical composition of Formulation 4 provided improved IC_{50} compared to **Compound 7** with solvent.

[00827] **FIG. 9** presents the results of IC_{50} assessment of a Wnt inhibitor of Formula (I), **Compound 8**, in DMSO, and of Formulation 5 (comprising GO-HA + **Compound 8** in an aqueous formulation) as further described in Example 5. IC_{50} of Wnt activity was calculated as 5-10 nM for **Compound 8** and less than 1 nM for Formulation 5. These results demonstrate that the suspension of **Compound 8** in aqueous GO-HA (Formulation 5) and **Compound 8** with DMSO as the solvent both inhibited Wnt activity. Further, these results demonstrate that the pharmaceutical composition of Formulation 5 provided improved IC_{50} compared to **Compound 8** with solvent.

[00828] **Biological Example 4**

[00829] *Demonstration of bacteriostasis following administration of pharmaceutical compositions described in Example 4 (Formulations 6 to 13).*

[00830] To assess the log reduction capabilities of pharmaceutical compositions according to one or more embodiments herein, testing was performed on organisms that are found in acute and chronic wounds. Triplicate tubes were prepared for each bacteria using 50 μL of inoculum (about 1 x 10^6 CFU/mL), which was added to 450 μL of Formulation (or 100 μL of inoculum in 900 μL of Formulation), resulting in a final inoculum of about 1 x 10^5 CFU/mL, as shown in Table 15.

[00831] Tubes were mixed with a vortex and then incubated in a shaking incubator at 175 RPM and 35 °C to 37 °C.

[00832] Positive control in Tables 16a and 16b was 1 mL of 0.89% saline with 1 mL of the $1x10^6$ CFU/mL of the organism.

[00833] Negative controls in Table 17 were neat samples of the respective compounds or mixtures in Meuller Hinton Broth with no organism spike (one for the sample and one for the vehicle).

[00834] For Formulations 6 to 11 (with **Compounds 1**, **7**, **8**, **10**, **18**, and XAV939, respectively) in Tables 18a, 18b, 19, 20, 21a, 21b, 22, and 23: the preparation of these pharmaceutical compositions is as described in Example 4.

[00835] GO-HA vehicle in Table 24 was GO-HA (30 mg), polysorbate 20 (1.6 mL), butylene glycol (1.6 mL), phosphatidylcholine (0.66 g), ethanol (1.6 mL), ultra-pure water (24.9 mL), and hydroxypropyl cellulose (0.26 g).

[00836] For Formulation 12 (with **Compound 18** and GO-HA) in Table 26: the preparation of this pharmaceutical composition is as described in Example 4.

[00837] For Formulation 13 (with XAV939 and GO-HA) in Tables 25a and 25b: the preparation of this pharmaceutical composition is as described in Example 4.

[00838] Testing was performed at two time points: 18 to 24 hours, and 72±4 hours. Test results are shown in Tables 15 to 26.

Table 15. Inoculum Results

Organism	Positive Control 24 hours	Inoculum Count (CFU/mL)	Spiked Inoculum (CFU/mL)	Spike Inoculum Log Value
A. baumannii (derived from ATCC® 19606™)	Growth	2.2×10^6	2.2×10^5	5.3
C. acnes (derived from ATCC® 11827™)	Growth	7.8×10^5	7.8×10^4	4.9
E. coli (derived from ATCC® 8739™) (2 tests)	Growth	1.8×10^6	1.8×10^5	5.3
	Growth	2.8×10^5	2.8×10^4	4.5
E. coli (derived from ATCC® 25922™) (2 tests)	Growth	2.6×10^6	2.6×10^5	5.4
	Growth	3.8×10^5	3.8×10^4	4.6
E. coli 0157:H7 (derived from ATCC® 12900™)	Growth	1.4×10^5	1.4×10^4	4.2
E. faecalis (derived from ATCC® 19433™) (2 tests)	Growth	6.2×10^5	6.2×10^4	4.8
	Growth	1.2×10^6	1.2×10^5	5.1
K. pneumoniae (derived from ATCC® 10031™)	Growth	5.8×10^5	5.8×10^4	4.8
P. vulgaris (derived from ATCC® 8427™)	Growth	8.0×10^6	8.0×10^5	5.9
P. aeruginosa (derived from ATCC® 9027™) (2 tests)	Growth	6.0×10^5	6.0×10^4	4.8
	Growth	1.8×10^6	1.8×10^5	5.3
	Growth	2.6×10^6	2.6×10^5	5.4

Organism	Positive Control 24 hours	Inoculum Count (CFU/mL)	Spiked Inoculum (CFU/mL)	Spike Inoculum Log Value
P. aeruginosa (derived from ATCC® 15442™) (2 tests)	Growth	1.4×10^6	1.4×10^5	5.2
S. aureus (MRSA) (derived from ATCC® 33593™) (2 tests)	Growth	4.4×10^6	4.4×10^5	4.6
	Growth	6.4×10^5	6.4×10^4	4.8
S. aureus (derived from ATCC® 6538™) (2 tests)	Growth	2.2×10^6	2.2×10^5	5.3
	Growth	1.4×10^6	1.4×10^5	5.2
S. epidermidis (derived from ATCC® 14990™) (2 tests)	Growth	6.8×10^5	6.8×10^4	5.3
	Growth	8.0×10^5	8.0×10^4	4.9
S. maltophilia (derived from ATCC® 13637™)	Growth	1.2×10^6	1.2×10^5	5.1
S. pyogenes (derived from ATCC® 19615™)	Growth	3.0×10^6	3.0×10^5	5.5

Table 16a. Positive control comparison

Time Point	Positive Control			
	C. acnes (derived from ATCC® 11827™)	*E. coli* (derived from ATCC® 8739™)	*E. coli* (derived from ATCC® 25922™)	*E. faecalis* (derived from ATCC® 19433™)
24 hours	Growth	Growth	Growth	Growth
72 hours	Growth	Growth	Growth	Growth

Table 16b. Positive control comparison

Time Point	Positive Control			
	S. aureus (MRSA) (derived from ATCC® 33593™)	*P. aeruginosa* (derived from ATCC® 15442™)	*S. aureus* (derived from ATCC® 6538™)	*S. epidermidis* (derived from ATCC® 14990™)
24 hours	Growth	Growth	Growth	Growth
72 hours	Growth	Growth	Growth	Growth

Table 17. Negative control comparison

Time Point	Negative Control				
	Compound 1	Compound 10	XAV939 + GO-HA	Compound 18	Compound 18 + GO-HA
24 hours	No Growth	No Growth	No Growth	No Growth	No Growth
72 hours	No Growth	No Growth	No Growth	No Growth	No Growth

Table 18a. Formulation 6 (Compound 1) results, test 1

Formulation 6 (Compound 1)								
		E. coli (derived from ATCC® 8739™)	*E. coli* (derived from ATCC® 25922™)	*E. faecalis* (derived from ATCC® 19433™)	*S. aureus* (MRSA) (derived from ATCC® 33593™)	*P. aeruginosa* (derived from ATCC® 15442™)	*S. aureus* (derived from ATCC® 6538™)	*S. epidermidis* (derived from ATCC® 14990™)
24 h	Average result	1.3×10^4	<200	1.1×10^3	9.3×10^3	<200	8.7×10^4	3.1×10^5
	Log Value	4.1	<2.3	3.0	4.0	<2.3	4.9	5.5
	Log Reduction	1.2	>3.1	1.8	0.6	>3.1	0.4	No reduction
72 h	Average result	<200	<200	1.1×10^4	4.0×10^4	<200	<800	<200
	Log Value	<2.3	<2.3	4.1	4.6	<2.3	<2.9	<2.3
	Log Reduction	>3.0	>3.1	0.7	No reduction	>3.1	>2.4	>2.5

Table 18b. Formulation 6 (Compound 1) results, test 2

Formulation 6 (Compound 1)								
		A. baumannii (ATCC 19606® ™)	*E. coli* 0157:H7 (ATCC® 12900 ™)	*K. pneumoniae* (ATCC® 10031 ™)	*P. aeruginosa* (ATCC® 9027 ™)	*P. vulgaris* (ATCC® 8427 ™)	*S. maltophilia* (ATCC® 13637 ™)	*S. pyogenes* (ATCC® 19615 ™)
24 h	Average result	<200	5.5×10^3	1.1×10^3	<200	<200	<200	<200
	Log Value	<2.3	3.7	3.0	<2.3	<2.3	<2.3	<2.3
	Log Reduction	>3.0	0.5	1.8	>3.0	>3.6	>2.8	>3.2
72 h	Average result	<200	<200	<200	<200	<200	<200	<200
	Log Value	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
	Log Reduction	>3.0	>1.9	>2.5	>3.0	>3.6	>2.8	>3.2

Table 19. Formulation 7 (Compound 7) results

Formulation 7 (Compound 7)								
		E. coli (derived from ATCC® 8739™)	*E. coli* (derived from ATCC® 25922™)	*E. faecalis* (derived from ATCC® 19433™)	*S. aureus* (MRSA) (derived from ATCC® 33593™)	*P. aeruginosa* (derived from ATCC® 15442™)	*S. aureus* (derived from ATCC® 6538™)	*S. epidermidis* (derived from ATCC® 14990™)
24 h	Average result	7.9×10^4	3.2×10^5	9.3×10^5	2.7×10^3	<200	7.3×10^2	2.8×10^4
	Log Value	4.9	5.5	6.0	3.4	<2.3	2.9	4.4
	Log Reduction	0.4	No reduction	No reduction	1.2	>3.1	2.4	0.4

Formulation 7 (Compound 7)								
		E. coli (derived from ATCC® 8739™)	*E. coli* (derived from ATCC® 25922™)	*E. faecalis* (derived from ATCC® 19433™)	*S. aureus* (MRSA) (derived from ATCC® 33593™)	*P. aeruginosa* (derived from ATCC® 15442™)	*S. aureus* (derived from ATCC® 6538™)	*S. epidermidis* (derived from ATCC® 14990™)
72 h	Average result	>3.6 x 10^6	2.7 x 10^4	6.9 x 10^5	1.4 x 10^5	<200	<200	<200
	Log Value	>6.6	4.4	5.8	5.1	<2.3	<2.3	<2.3
	Log Reduction	No reduction	1.0	No reduction	No reduction	>3.1	>3.0	>2.5

Table 20. Formulation 8 (Compound 8) results

Formulation 8 (Compound 8)								
		E. coli (derived from ATCC® 8739™)	*E. coli* (derived from ATCC® 25922™)	*E. faecalis* (derived from ATCC® 19433™)	*S. aureus* (MRSA) (derived from ATCC® 33593™)	*P. aeruginosa* (derived from ATCC® 15442™)	*S. aureus* (derived from ATCC® 6538™)	*S. epidermidis* (derived from ATCC® 14990™)
24 h	Average result	3.1 x 10^3	200	3.4 x 10^5	3.3 x 10^6	7.3 x 10^2	5.4 x 10^4	6.0 x 10^2
	Log Value	3.5	2.3	5.5	6.5	2.9	4.7	2.8
	Log Reduction	1.8	3.1	No reduction	No reduction	2.5	0.6	2.0
72 h	Average result	6.8 x 10^4	<200	6.5 x 10^4	1.0 x 10^6	<200	<7.4 x 10^3	<200
	Log Value	4.8	<2.3	4.8	6.0	<2.3	<3.9	<2.3
	Log Reduction	0.5	>3.1	No reduction	No reduction	>3.1	>1.4	>2.5

Table 21a. Formulation 9 (Compound 10) results, test 1

Formulation 9 (Compound 10)								
		E. coli (derived from ATCC® 8739™)	*E. coli* (derived from ATCC® 25922™)	*E. faecalis* (derived from ATCC® 19433™)	*S. aureus* (MRSA) (derived from ATCC® 33593™)	*P. aeruginosa* (derived from ATCC® 15442™)	*S. aureus* (derived from ATCC® 6538™)	*S. epidermidis* (derived from ATCC® 14990™)
24 h	Average result	<200	<200	2.9 x 10^4	3.1 x 10^4	<200	1.5 x 10^5	1.1 x 10^4
	Log Value	<2.1	<2.3	4.5	4.5	<2.3	5.2	4.0
	Log Reduction	>3.2	>3.1	0.3	0.1	>3.1	0.1	0.8
72 h	Average result	6.0 x 10^2	<200	<200	2.7 x 10^3	<200	6.7 x 10^3	3.3 x 10^2
	Log Value	2.8	<2.3	<2.3	3.4	<2.3	3.8	2.5
	Log Reduction	2.5	>3.1	>2.5	1.2	>3.1	1.5	2.3

Table 21b. Formulation 9 (Compound 10) results, test 2

Formulation 9 (Compound 10)								
		A. baumannii (ATCC® 19606™)	*E. coli* 0157:H7 (ATCC® 12900™)	*K. pneumoniae* (ATCC® 10031™)	*P. aeruginosa* (ATCC® 9027™)	*P. vulgaris* (ATCC® 8427™)	*S. maltophilia* (ATCC® 13637™)	*S. pyogenes* (ATCC® 19615™)
24 h	Average result	<200	<200	<200	<200	<200	<200	<200
	Log Value	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
	Log Reduction	>3.0	>1.9	>2.5	>3.0	>3.6	>2.8	>3.2
72 h	Average result	<200	<200	<200	<200	<200	<200	<200
	Log Value	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
	Log Reduction	>3.0	>1.9	>2.5	>3.0	>3.6	>2.8	>3.2

Table 22. Formulation 10 (Compound 18) results

Formulation 10 (Compound 18)								
		A. baumannii (ATCC® 19606™)	*C. acnes* (ATCC® 8739™)	*E. coli* (ATCC® 8739™)	*E. coli* (ATCC® 25922™)	*E. coli* 0157:H7 (ATCC® 12900™)	*E. faecalis* (ATCC® 19433™)	*K. pneumonia* (ATCC® 10031™)
24 h	Average result	<200	10.7×10^4	5.3×10^2	<200	<200	2.7×10^5	<200
	Log Value	<2.3	4.2	2.7	<2.3	<2.3	5.4	<2.3
	Log Reduction	>3.0	0.7	1.8	>2.3	>1.9	No reduction	>2.5
72 h	Average result	<200	6.5×10^3	<200	<200	<200	1.9×10^5	<200
	Log Value	<2.3	3.8	<2.3	<2.3	<2.3	5.3	<2.3
	Log Reduction	>3.0	1.1	>2.2	>2.3	>1.9	No reduction	>2.5

Table 22 (continued). Formulation 10 (Compound 18) results

Formulation 10 (Compound 18)									
		P. aeruginosa (ATCC® 9027)	*P. aeruginosa* (ATCC® 15442)	*P. vulgaris* (ATCC® 8427)	*S. aureus* (ATCC® 6538)	*S. aureus* (MRSA) (ATCC® 33593)	*S. epidermidis* (ATCC® 14990)	*S. maltophilia* (ATCC® 13637)	*S. pyogenes* (ATCC® 19615)
24 h	Avg result	<200	<200	<200	4.7×10^4	1.5×10^3	8.0×10^2	<200	<200
	Log Value	<2.3	<2.3	<2.3	4.7	3.2	2.9	<2.3	<2.3
	Log Redn	>2.5	>2.9	>3.6	0.5	1.6	2.0	>2.8	>3.2
72 h	Avg result	<200	<200	<200	9.3×10^2	<200	<200	<200	<200
	Log Value	<2.3	<2.3	<2.3	3.0	<2.3	<2.3	<2.3	<2.3
	Log Redn	>2.5	>2.9	>3.6	2.2	>2.5	>2.6	>2.8	>3.2

Table 23. Control Formulation 11 (XAV939) results (control)

Control Formulation 11 (XAV939) (control)								
		E. coli (derived from ATCC® 8739™)	*E. coli* (derived from ATCC® 25922™)	*E. faecalis* (derived from ATCC® 19433™)	*S. aureus* (MRSA) (derived from ATCC® 33593™)	*P. aeruginosa* (derived from ATCC® 15442™)	*S. aureus* (derived from ATCC® 6538™)	*S. epidermidis* (derived from ATCC® 14990™)
24 h	Average result	2.7×10^3	2.8×10^4	2.6×10^5	8.7×10^4	<200	2.5×10^3	5.5×10^4
	Log Value	3.4	4.4	5.4	4.9	<2.3	3.4	4.7
	Log Reduction	1.9	1.0	No reduction	No reduction	>3.1	1.9	0.1
72 h	Average result	<200	<200	1.7×10^5	$<6.8 \times 10^3$	<200	1.7×10^5	<200
	Log Value	<2.3	<2.3	5.1	<3.8	<2.3	5.2	<2.3
	Log Reduction	>3.0	>3.1	No reduction	>0.8	>3.1	0.1	>2.5

Table 24. GO-HA results (control)

GO-HA (control)								
		E. coli (derived from ATCC® 8739™)	*E. coli* (derived from ATCC® 25922™)	*E. faecalis* (derived from ATCC® 19433™)	*S. aureus* (MRSA) (derived from ATCC® 33593™)	*P. aeruginosa* (derived from ATCC® 15442™)	*S. aureus* (derived from ATCC® 6538™)	*S. epidermidis* (derived from ATCC® 14990™)
24 h	Average result	3.0×10^3	1.9×10^3	1.0×10^5	8.4×10^5	<200	3.9×10^5	8.2×10^3
	Log Value	3.5	3.3	5.0	5.9	<2.3	5.6	3.9
	Log Reduction	1.8	2.1	No reduction	No reduction	>3.1	No reduction	0.9
72 h	Average result	$<7.4 \times 10^3$	<200	1.1×10^5	6.9×10^4	<200	4.0×10^5	<200
	Log Value	<3.9	<2.3	5.0	4.8	<2.3	5.6	<2.3
	Log Reduction	>1.4	>3.1	No reduction	No reduction	>3.1	No reduction	>2.5

Table 25a. Formulation 13 (Compound XAV939 + GO-HA) results, test 1

Formulation 13 (XAV939 + GO-HA)								
		E. coli (derived from ATCC® 8739™)	*E. coli* (derived from ATCC® 25922™)	*E. faecalis* (derived from ATCC® 19433™)	*S. aureus* (MRSA) (derived from ATCC® 33593™)	*P. aeruginosa* (derived from ATCC® 15442™)	*S. aureus* (derived from ATCC® 6538™)	*S. epidermidis* (derived from ATCC® 14990™)
24 h	Average result	<200	2.7×10^2	7.9×10^4	2.2×10^6	<200	1.3×10^5	5.0×10^4
	Log Value	<2.1	2.4	4.9	6.4	<2.3	5.1	4.7
	Log Reduction	>3.2	3.0	No reduction	No reduction	>3.1	0.2	0.1
72 h	Average result	9.3×10^3	<200	2.9×10^3	6.5×10^3	<200	8.7×10^4	<200
	Log Value	4.0	<2.3	3.5	3.8	<2.3	4.9	<2.3

Formulation 13 (XAV939 + GO-HA)								
		E. coli (derived from ATCC® 8739™)	*E. coli* (derived from ATCC® 25922™)	*E. faecalis* (derived from ATCC® 19433™)	*S. aureus* (MRSA) (derived from ATCC® 33593™)	*P. aeruginosa* (derived from ATCC® 15442™)	*S. aureus* (derived from ATCC® 6538™)	*S. epidermidis* (derived from ATCC® 14990™)
	Log Reduction	1.3	>3.1	1.3	0.8	>3.1	0.4	>2.5

Table 25b. Formulation 13 (Compound XAV939 + GO-HA) results, test 2

Formulation 13 (XAV939 + GO-HA)								
		A. baumannii (ATCC® 19606™)	*E. coli* 0157:H7 (ATCC® 12900™)	*K. pneumoniae* (ATCC® 10031™)	*P. aeruginosa* (ATCC® 9027™)	*P. vulgaris* (ATCC® 8427™)	*S. maltophilia* (ATCC® 13637™)	*S. pyogenes* (ATCC® 19615™)
24 h	Average result	<200	1.1×10^5	<200	<200	<200	<200	<200
	Log Value	<2.3	5.0	<2.3	<2.3	<2.3	<2.3	<2.3
	Log Reduction	>3.0	No reduction	>2.5	>3.0	>3.6	>2.8	>3.2
72 h	Average result	<200	2.7×10^2	<200	<200	<200	<200	<200
	Log Value	<2.3	2.4	<2.3	<2.3	<2.3	<2.3	<2.3
	Log Reduction	>3.0	1.8	>2.5	>3.0	>3.6	>2.8	>3.2

Table 26. Formulation 12 (Compound 18 + GO-HA) results

Formulation 12 (Compound 18 + GO-HA)								
		A. baumannii (ATCC® 19606™)	*C. acnes* (ATCC® 8739™)	*E. coli* (ATCC® 8739™)	*E. coli* (ATCC® 25922™)	*E. coli* 0157:H7 (ATCC® 12900™)	*E. faecalis* (ATCC® 19433™)	*K. pneumonia* (ATCC® 10031™)
24 h	Average result	<200	2.9×10^4	3.7×10^3	<200	<200	4.9×10^4	3.0×10^2
	Log Value	<2.3	4.5	3.6	<2.3	<2.3	4.7	2.5
	Log Reduction	>3.0	0.4	0.9	>2.3	>1.9	0.4	2.3
72 h	Average result	<200	6.5×10^3	<200	<200	<200	1.3×10^4	<200
	Log Value	<2.3	3.8	<2.3	<2.3	<2.3	4.1	<2.3
	Log Reduction	>3.0	1.1	>2.2	>2.3	>1.9	1.0	>2.5

Table 26 (continued). Formulation 12 (Compound 18 + GO-HA) results

Formulation 12 (Compound 18 + GO-HA)									
		P. aeruginosa (ATCC® 9027™)	*P. aeruginosa* (ATCC® 15442™)	*P. vulgaris* (ATCC® 8427™)	*S. aureus* (ATCC® 6538™)	*S. aureus* (MRSA) (ATCC® 33593™)	*S. epidermidis* (ATCC® 14990™)	*S. maltophilia* (ATCC® 13637™)	*S. pyogenes* (ATCC® 19615™)
24 h	Average result	<200	<200	<200	4.9×10^4	<200	<200	<200	<200
	Log Value	<2.3	<2.3	<2.3	4.7	<2.3	<2.3	<2.3	<2.3
	Log Reduction	>2.5	>2.9	>3.6	0.5	>2.5	>2.6	>2.8	>3.2
72 h	Average result	<200	<200	<200	9.3×10^2	<200	<200	<200	<200
	Log Value	<2.3	<2.3	<2.3	3.0	<2.3	<2.3	<2.3	<2.3
	Log Reduction	>2.5	>2.9	>3.6	2.2	>2.5	>2.6	>2.8	>3.2

[00839] Formulation 6 (**Compound 1**) inhibited the growth of 6 of 7 bacteria in test 1 (common skin pathogenic bacteria) at 72 hours (Table 18a). Formulation 6 (**Compound 1**) did not affect MRSA growth in test 1 at 72 hours. Formulation 6 (**Compound 1**) inhibited the growth of 7 of 7 bacteria in test 2 at 72 hours (Table 18b). Formulation 7 (**Compound 7**) inhibited the growth of 4 of 7 bacteria at 72 hours (Table 19). Formulation 8 (**Compound 8**) inhibited the growth of 5 of 7 bacteria at 72 hours (Table 20). Formulation 9 (**Compound 10**) inhibited the growth of 7 of 7 bacteria in test 1 at 72 hours (Table 21a). Formulation 9 (**Compound 10**) also inhibited the growth of 7 of 7 bacteria in test 2 at 72 hours (Table 21b). Formulation 10 (**Compound 18**) inhibited the growth of 14 of 15 bacteria tested at 72 hours (Table 22). Control Formulation 11 (XAV939) inhibited the growth of 6 of 7 bacteria at 72 hours (Table 23). Formulation 13 (XAV939 + GO-HA) inhibited the growth of 14 of 14 bacteria in tests 1 and 2 at 72 hours (Tables 25a and 25b). Formulation 12 (**Compound 18** + GO-HA) inhibited the growth of 14 of 14 bacteria at 72 hours (Table 26).

[00840] As shown in Biological Example 4, the strong bacteriostatic properties of pharmaceutical compositions herein enhance the wound healing process by minimizing the complicating effects of bacteria, for example, during surgical wound repair.

[00841] Biological Example 5

[00842] *Elastic cartilage regeneration with pharmaceutical compositions described in Example 5 (Formulations 14 and 15).*

[00843] Pharmaceutical compositions according to one or more embodiments herein were tested and compared to saline control in elastic cartilage regeneration tests. Rabbit ears were punched through and through with an 8 mm punch. 4 holes were punched in each ear of the specimen (four positions L1, L2, L3, and L4 in the left ear and four positions R5, R6, R7, and R8 in right ear). Saline was applied via a spray on the control specimen, Formulation 14 (with **Compound 8**) was applied via a phospholipid spray on another specimen, and Formulation 15 (with **Compound 18**) was applied via a phospholipid spray on yet another specimen. The phospholipid sprays (Formulations 14 and 15) are as described in Example 5.

[00844] **FIG. 10** provides photographs of the 8 mm punch wounds after treatment with saline, Formulation 14 (with **Compound 8**), or Formulation 15 (with **Compound 18**) on Days 0 and 23. Ear punch area related to wound closure was measured at 2- to 3-day intervals from day 0 to day 39. Results of the rate of ear closure from day 1-21 is shown in **FIG. 11A**, and from day 21-39 is shown in **FIG. 11B**. Additional results from a separate test are shown in **FIG. 12** (day 14 to 39).

[00845] Results of the rate of ear closure show that pharmaceutical compositions (Formulations 14 and 15) improved the rate of ear closure over the control in these tests after day 4. **FIG. 11B** shows that pharmaceutical compositions according to one or more embodiments herein provided up to 2-3 times the rate of wound closure (0.01-0.02 cm^2 area) at day 21 compared to the control (0.055 cm^2 area).

[00846] At day 45, the tests were stopped and the tissue was processed. An embed cut was made across the center point of the healed wound in each site (**FIG. 13**, top). A slide was used to obtain sectioned tissue sample for staining and imaging (**FIG. 13**, bottom). Representative examples of cross sectioned and stained tissues are shown in **FIGS. 15-17**.

[00847] After staining with either Safronin O or trichrome blue, the distance between the opposing cartilage endplates was measured. From the original 8 mm wound margin distance, the saline control averaged 6 mm of distance between opposing cartilage endplates (**FIG. 14**), or 2 mm of average cartilage growth upon closure of the wound (about 25% average cartilage tissue regrowth). Pharmaceutical compositions (Formulation 14 with **Compound 8** and Formulation 15 with **Compound 18**) provided about 2-3 mm average distance between opposing cartilage endplates (**FIG. 14**), or 5.5-6 mm of average cartilage growth upon closure of the wound (about 68-75% average cartilage tissue regrowth).

[00848] **FIGS. 15-17** show expanded views of the cartilage regeneration site where chondrocytes are newly formed within the wound margin. The gray square at the middle image in **FIGS. 15-17** represents the image shown at the bottom image (magnified). Comparing **FIG. 16** (Formulation 14 with **Compound 8**) and **FIG. 17** (Formulation 15 with **Compound 18**) to **FIG. 15** (saline control), cartilage regeneration using pharmaceutical compositions according to one or more embodiments of the present disclosure was confirmed. Within the wound margin, newly formed chondrocytes are more numerous and larger than in the control and cartilage regrowth is shown in samples that were treated with pharmaceutical compositions Formulation 14 and Formulation 15 (dark tissue at center of the cross sections in the figures represents cartilage tissue).

[00849] As seen in Biological Example 5, pharmaceutical compositions according to one or more embodiments of the present disclosure were effective in wound healing and as small molecule elastic cartilage regenerators. Formulations 14 and 15 provided increased rates of wound healing up to 2-3 times the rate compared to a saline control at 21 days. These pharmaceutical compositions also provided up to 90-95% cartilage regeneration.

[00850] **Biological Example 6**

[00851] *Full thickness open excisional wound healing, closed excisional wound healing, burn studies, physical properties of healed wounds, rete ridges, and collagen organization following administration of pharmaceutical compositions described in Example 6 (Formulations 16 to 27).*

[00852] *Full thickness open excisional wound healing procedure*

[00853] A 21-day full thickness open excisional wound healing study was conducted on Yucatan™ Miniature Swine (Sinclair BioResources, Auxvasse, Missouri, USA) to evaluate the efficacy of healing and local tissue reaction of pharmaceutical compositions and controls were topically administered on full thickness open excisional wounds once every other day (QOD, total 11 doses).

[00854] The control for this study was a saline control (0.9% NaCl, aqueous).

[00855] For experiments herein, where the pharmaceutical composition according to one or more embodiments (Formulation) included GO-HA, the respective GO-HA vehicle (also called GO-HA or GO-HA control) was compared to the Formulation comprising GO-HA, along with the separate control (saline) comparison. Where the pharmaceutical composition according to one or more embodiments (Formulation) did not comprise GO-HA, the respective serum formulation (also called serum formulation control) was compared as a vehicle, along with the separate control (saline) comparison.

[00856] The test compositions for these studies are described as follows.

[00857] The test composition in the full thickness open excisional wound studies for pharmaceutical compositions without GO-HA (no GO-HA added) was a mixture with an overall concentration of 0.25 to 0.27 mg/mL of the respective Wnt inhibitor in serum formulation described in Example 6 (Formulations 16 and 17).

[00858] The test composition in the full thickness open excisional wounds studies for pharmaceutical compositions with GO-HA was a mixture with an overall concentration of 1.0 mg/mL of the respective Wnt inhibitor and 1.0 mg/mL of GO-HA in serum formulation as described in Example 6 (Formulations 18 and 19).

[00859] Miniature swine used in this study are a well-accepted non-rodent model for dermal wound healing studies due to the anatomical similarities between humans and swine, including dermal thickness, subcutaneous tissues, and underlying muscular structures. For this reason, Yucatan™ Miniature Swine were chosen as an appropriate species to evaluate dermal materials and wound healing properties of the pharmaceutical compositions.

[00860] The dermal wounds were generated to mimic a full-thickness skin lesion consistent with severe clinical scenarios. The number of animals (6) ensured sufficient endpoints from 12 total wounds per animal for the designated test articles, which were collected for evaluation from the treatment groups (**FIG. 18**).

[00861] The dose volume related to the full thickness open excisional wounds was selected based on the wound size and intended clinical application to have the wound surface covered and effective. The dose concentration and volume (0.2 mL/site per dose) were chosen for the wounds at the specified size.

[00862] Full-thickness 60-day burn wound healing study. Full-thickness, 3rd degree burn wounds of 3x3 cm^2 size were created on the back of the Yucatan™ Miniature Swine (5 wounds on both sides of the spine, 10 total per swine) by using a brass cylinder (circular cross-section, 3 cm diameter) that was preheated in an oven at 100°C and applied to the dorsal skin with light pressure for 30 seconds. Debridement of the wounds occurred day 7 post-dose. Pharmaceutical compositions were dosed topically three times a week (0.24 mL) for thirty days. At 30 day time-point, the wounds were photographed once a week and no dosing of pharmaceutical compositions were conducted from day 31-60. On day 60, animals were sacrificed and tissue prepared for analysis.

[00863] The animals were fasted overnight (or at least 4 hours) prior to anesthesia. The animals were given analgesic treatments for surgery procedures on dosing phase (day 1), and dressing changes when needed.

[00864] The frequency of wound creation was once on dosing phase day 1. The frequency of dose administration was once on dosing phase days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21. Each animal had twelve 5 cm x 0.5 cm full thickness fusiform (spindle shaped) wound sites, spaced at least 3 cm apart to the appropriate depth and in a staggered pattern to reduce central cross-sectional wound tension.

[00865] *Closed excisional wound healing procedure*

[00866] Once homeostasis was obtained from the full thickness open excisional wound healing studies, the wounds were sutured closed using non-absorbable suture, the simple interrupted closure technique is shown in **FIG. 19**. Each wound had 5 simple interrupted sutures with a 0.5 to 1 cm interval between sutures.

[00867] The negative control for this study was a saline control (0.9% NaCl, aqueous). The test compositions for these studies are described as follows.

[00868] The test composition in the closed excisional wound and burn studies for pharmaceutical compositions without GO-HA (no GO-HA added) was a mixture with an overall concentration of 0.20 to 0.25 mg/mL of the respective Wnt inhibitor in serum formulation as described in Example 6 (Formulations 20 to 23).

[00869] The test composition in the closed excisional wound and burn studies for pharmaceutical compositions with GO-HA was a mixture with an overall concentration of 1.0 mg/mL of the respective Wnt inhibitor and 0.5 mg/mL of GO-HA in serum formulation as described in Example 6 (Formulations 24 to 27).

[00870] Pharmaceutical compositions and controls were applied directly to the wound site in a generous layer evenly over the sutured wound using a sterile applicator as appropriate. After application, the wound sites were covered with suitable sterile dressing materials.

[00871] Dressings were changed every dosing phase day at day 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, and sutures were removed on dosing phase day 15. After dose administration on each dosing day, the wounds were covered with dressings as described above.

[00872] The animals were scheduled to be terminated on dosing phase day 22. At termination, the wound sites (dose sites) were removed and collected separately for each site. The collection included at least 0.5 cm margin skin around a single wound site and a depth below the wound bed of a single wound site.

[00873] *Full thickness open excisional wound healing study results*

[00874] **FIG. 20A** and **FIG. 20B** show the resulting full thickness excisional wound area (cm^2) from this study for pharmaceutical compositions: Formulations 16 and 17 (with **Compound 7** and **Compound 8**, respectively), and Formulations 18 and 19 (with

Compound 7 + GO-HA and **Compound 8** + GO-HA, respectively) compared to the GO-HA vehicle (previously described), and the control (saline).

[00875] In the full thickness excisional wound tests, the pharmaceutical compositions provided improved wound healing from day 5 to day 15 compared to the GO-HA vehicle alone and the saline control. In some tests, the pharmaceutical compositions provided improved wound healing throughout the test (day 3 to day 22), such as Formulation 16 (with **Compound 7**), Formulation 17 (with **Compound 8**), and Formulation 19 (with **Compound 8** + GO-HA).

[00876] *Closed excisional wound healing study results (cosmesis)*

[00877] Results of the closed excisional wound healing study are shown in **FIGS. 25 to 29**. Formulation 20 (with **Compound 1**) resulted in 7 of 8 wounds with improved cosmesis (appearance such as greater amount of visible wound closure and less scaring or smaller scar size) than the control; Formulation 24 (with **Compound 1** + GO-HA) resulted in 3 of 8 wounds with improved visible appearance than the control; Formulation 23 (with **Compound 10**) resulted in 3 of 8 wounds with improved visible appearance than the control; and Formulation 22 (with **Compound 8**) resulted in 2 of 8 wounds with improved visible appearance than the control.

[00878] **FIG. 25** shows results of the closed excisional wound study for saline control, serum formulation (also called serum formulation control or serum control) as a vehicle, and GO-HA vehicle, from day 1 to day 21. The sutures were removed at day 13. Serum formulation control was butylene glycol (0.80 mL), polysorbate 20 (0.40 mL), ultra-pure water (18 mL), and hydroxypropyl cellulose (0.12 g). GO-HA vehicle was PEG-400 (0.8 mL), GO-HA (9 mg), ultra-pure water (18 mL), and hydroxypropyl cellulose (0.12 g).

[00879] **FIG. 26** shows that pharmaceutical compositions (Formulations 20, 22, and 23) provided improved wound closure with less scaring compared to the saline control and respective vehicle (GO-HA vehicle or serum formulation without GO-HA as a vehicle) (**FIG. 25**) at day 21.

[00880] **FIG. 27** shows results of the closed excisional wound study: pharmaceutical compositions with GO-HA, Formulation 24 (with **Compound 1** + GO-HA), Formulation 26 (with **Compound 8** + GO-HA), and Formulation 27 (with **Compound 10** + GO-HA), from day 1 to day 21. The sutures were removed at day 13.

[00881] **FIG. 28** shows results of the closed excisional wound study: cross sectional tissue samples stained with trichrome blue. Wound sites treated with saline control, serum formulation control [butylene glycol (0.80 mL), polysorbate 20 (0.40 mL), ultra-pure water (18

mL), and hydroxypropyl cellulose (0.12 g)], GO-HA formulation control (vehicle) [PEG-400 (0.8 mL), GO-HA (9 mg), ultra-pure water (18 mL), and hydroxypropyl cellulose (0.12 g)], Formulations 20, 22, and 23 (with **Compound 1**, **Compound 8**, **Compound 10**, respectively), and Formulations 24, 26, and 27 (with **Compound 1** + GO-HA, **Compound 8** + GO-HA, and **Compound 10** + GO-HA, respectively) are compared.

[00882] **FIG. 29** shows results of the closed excisional wound study: cross sectional tissue samples under polarized microscopy. Wound sites treated with saline control (0.9% NaCl, aqueous), serum formulation control [butylene glycol (0.80 mL), polysorbate 20 (0.40 mL), ultra-pure water (18 mL), and hydroxypropyl cellulose (0.12 g)], GO-HA formulation control (vehicle) [PEG-400 (0.8 mL), GO-HA (9 mg), ultra-pure water (18 mL), and hydroxypropyl cellulose (0.12 g)], Formulations 20, 22, and 23 (with **Compound 1**, **Compound 8**, **Compound 10**, respectively), and Formulations 24, 26, and 27 (with **Compound 1** + GO-HA, **Compound 8** + GO-HA, and **Compound 10** + GO-HA, respectively) are compared.

[00883] *Burn wound healing study results*

[00884] As shown in **FIGS. 21-24**, Formulation 22 (with **Compound 8)**, Formulation 21 (with **Compound 7**), Formulation 26 (with **Compound 8** + GO-HA), and Formulation 25 (with **Compound 7** + GO-HA) resulted in statistically significant improved burn wound healing rates (wound area in cm^2 over time) compared to saline control from about day 16 to day 30. Histology (H&E staining: hematoxylin and eosin) demonstrated that the pharmaceutical compositions promoted regeneration of tissue (improved organized reticular collagen and rete ridge formation) and reduced scar formation as compared to saline control and GO-HA vehicle. Results of the histological stains are shown in **FIG. 31**. GO-HA vehicle and serum formulation (without GO-HA) as a vehicle is as provided above for **FIG. 25** (controls).

[00885] *Physical properties of healed wounds*

[00886] Additional tests were performed including elasticity (Table 27); max force (Table 28); tensile strength (strain) (Table 29); energy peak (Table 30); rete-ridge formation; and collagen organization (plane polarized light) (**FIG. 30**) of the healed wound sites, described as follows.

Table 27. Elasticity of new porcine skin topically treated with pharmaceutical compositions (mechanical and measured by elastin) (day 21)

	Elasticity (N/mm)								
	A	B	C	D	E	F	G	H	J
1	13.427	22.789	37.729	27.299	37.757	55.607	59.303	52.568	23.620

	Elasticity (N/mm)								
	A	B	C	D	E	F	G	H	J
2	37.769	38.947	19.017	33.002	67.483	46.485	58.036	74.191	39.158
3	32.830	29.511	48.957	50.617	45.378	28.331	54.893	44.962	55.709
4	28.367	33.011	-	39.593	31.494	47.748	51.866	43.004	37.715
5	-	-	-	-	-	22.23	-	-	-
Mean	28.098	31.064	35.234	37.628	45.528	40.080	56.024	53.682	39.050
STDEV	10.508	6.753	15.125	10.011	15.699	14.121	3.335	14.282	13.133

[00887] A is saline (control), B is serum formulation control (vehicle), C is GO-HA (vehicle), D is Formulation 20 (with **Compound 1**); E is Formulation 24 (with **Compound 1** + GO-HA); F is Formulation 22 (with **Compound 8**); G is Formulation 26 (with **Compound 8** + GO-HA); H is Formulation 23 (with **Compound 10**); and J is Formulation 27 (with **Compound 10** + GO-HA).

[00888] Elasticity (N/mm) is the ability of a material to return to its original shape after stress has been removed. Pharmaceutical compositions according to one or more embodiments herein tested with or without GO-HA ranged from 33% to 99% improvement in elasticity compared to the saline control. Notably, Formulation 23 (with **Compound 10**) provided a 91% increase and Formulation 26 (with **Compound 8** + GO-HA) provided a 99% increase in elasticity compared to the saline control.

Table 28. Max force of new porcine skin topically treated with Wnt inhibitors of Formula (I) (day 21)

	Max Force (N)								
	A	B	C	D	E	F	G	H	J
1	158.975	239.052	323.715	290.184	229.942	404.094	371.235	406.879	211.166
2	305.553	332.215	264.140	278.868	496.001	488.094	455.581	474.825	475.377
3	184.179	216.024	405.855	250.554	383.447	273.110	370.527	344.860	330.911
4	279.416	248.901	-	273.195	234.632	330.413	261.925	335.864	264.003
5	-	-	-	-	-	169.61	-	-	-
Mean	232.031	259.048	331.237	273.200	336.005	333.196	364.817	390.607	320.364
STDEV	71.362	50.686	71.156	16.668	128.290	121.954	79.370	64.413	114.369

[00889] A is saline (control), B is serum formulation control (vehicle), C is GO-HA (vehicle), D is Formulation 20 (with **Compound 1**); E is Formulation 24 (with **Compound 1** + GO-HA); F is Formulation 22 (with **Compound 8**); G is Formulation 26 (with **Compound 8** + GO-HA); H is Formulation 23 (with **Compound 10**); and J is Formulation 27 (with **Compound 10** + GO-HA). Descriptions for saline control, serum formulation (also called serum formulation control or serum control) as a vehicle, and GO-HA vehicle are as provided above for **FIG. 25**.

[00890] Max Force (N) is the average force required to break/rupture a material. The pharmaceutical compositions tested with or without GO-HA ranged from 18% to 68%

improvement in max force compared to the saline control. Notably, Formulation 26 (with **Compound 8** + GO-HA) provided a 57% improvement and Formulation 23 (with **Compound 10**) provided a 68% improvement in max force over the saline control.

Table 29. Strain (tensile strength) of new porcine skin topically treated with pharmaceutical compositions (day 21)

	Strain (N/mm^2)								
	A	B	C	D	E	F	G	H	J
1	1.134	2.172	4.397	2.568	6.200	7.640	9.473	6.792	2.642
2	4.669	4.566	1.369	3.906	9.181	4.427	7.393	11.592	3.226
3	5.852	4.032	5.906	10.226	5.370	2.939	8.132	5.862	9.379
4	2.880	4.378	-	5.738	4.227	6.900	10.271	5.506	5.388
5	-	-	-	-	-	2.913	-	-	-
Mean	3.634	3.787	3.891	5.609	6.245	4.964	8.817	7.438	5.159
STDEV	2.066	1.099	2.310	3.341	2.118	2.208	1.296	2.822	3.051

[00891] A is saline (control), B is serum formulation (vehicle), C is GO-HA (vehicle), D is Formulation 20 (with **Compound 1**); E is Formulation 24 (with **Compound 1** + GO-HA); F is Formulation 22 (with **Compound 8**); G is Formulation 26 (with **Compound 8** + GO-HA); H is Formulation 23 (with **Compound 10**); and J is Formulation 27 (with **Compound 10** + GO-HA).

[00892] Strain (tensile strength) (N/mm^2) is the force required to pulls or stretch a material to its breaking point. A 20% improvement over saline considered good. Pharmaceutical compositions according to one or more embodiments herein tested with or without GO-HA ranged from 36% to 142% improvement in tensile strength compared to the saline control. Notably, Formulation 23 (with **Compound 10**) provided a 105% improvement and Formulation 26 (with **Compound 8** + GO-HA) provided a 142% improvement in tensile strength (strain) over the saline control.

Table 30. Energy peak of new porcine skin topically treated with pharmaceutical compositions (day 21)

	Energy Peak (N*mm)								
	A	B	C	D	E	F	G	H	J
1	1882.263	2507.653	2777.473	3084.657	1400.346	2946.121	2323.932	3149.241	1887.823
2	2471.921	2833.797	3668.901	2356.435	3645.607	5124.987	3576.311	3038.880	5771.077
3	1033.244	1581.296	3364.5383	1240.242	3240.127	2632.780	2501.057	2645.076	1965.611
4	2752.248	1876.714	-	1885.046	1748.008	2286.458	1322.721	2623.098	1848.021
5	-	-	-	-	-	1294.14	-	-	-

	Energy Peak (N*mm)								
	A	B	C	D	E	F	G	H	J
Mean	2034.919	2199.865	3270.304	2141.595	2508.522	2856.897	2431.005	2864.074	2868.133
STDEV	759.866	572.613	453.124	777.555	1100.704	1411.562	923.109	269.510	1935.912

[00893] A is saline (control), B is serum formulation (vehicle), C is GO-HA (vehicle), D is Formulation 20 (with **Compound 1**); E is Formulation 24 (with **Compound 1** + GO-HA); F is Formulation 22 (with **Compound 8**); G is Formulation 26 (with **Compound 8** + GO-HA); H is Formulation 23 (with **Compound 10**); and J is Formulation 27 (with **Compound 10** + GO-HA).

[00894] Energy Peak (N*mm) is the maximum force applied to a material over time. Pharmaceutical compositions according to one or more embodiments herein tested with or without GO-HA ranged from 5% to 41% improvement in energy peak compared to the saline control. Notably, Formulation 22 (with **Compound 8**) provided a 29% improvement and Formulation 23 (with **Compound 10**) provided a 41% improvement in energy peak over the saline control. The serum formulation (vehicle) provided an 8% improvement in energy peak over the saline control. The GO-HA vehicle provided a 61% improvement in energy peak over the saline control.

[00895] *Rete ridges*

[00896] Regarding rete ridge formation, representative example images from the full thickness wound healing study are shown in **FIG. 20A** and **FIG. 20B**. **FIGS. 20A** and **20B** show that pharmaceutical compositions herein (Formulation 16 with **Compound 7**, Formulation 17 with **Compound 8**) and pharmaceutical compositions herein with GO-HA (Formulation 18 with **Compound 7** + GO-HA, Formulation 19 with **Compound 8** + GO-HA) improved wound healing compared to the saline control (rete ridge/mm), for example, approaching the rete ridge/mm value of uninjured skin. Improved rete ridge formation is described as epithelial extensions that project into the underlying connective tissue in both skin and mucous membranes – improved rete ridge formation is a sign of tissue regeneration rather than fibrosis/scarring (reduced rete ridge formation).

[00897] *Collagen organization*

[00898] Regarding collagen organization, the number of wounds indicating collagen infiltration into the scar were identified by polarized imaging (plane polarized light). As shown in **FIG. 30**, the pharmaceutical compositions with or without GO-HA provided greater collagen

organization compared to the saline control. Notably, Formulation 23 (with **Compound 10**) and Formulation 26 (with **Compound 8** + GO-HA) showed 7 wounds indicating collagen infiltration into the scar.

[00899] In summary, in these tests pharmaceutical compositions with or without GO-HA showed improvements in wound healing (physical properties of the healed wound site) compared to the control. Wounds treated with Formulation 26 (with **Compound 8** + GO-HA) and Formulation 23 (with **Compound 10**) provided outstanding wound healing integrity for: elasticity, strain, and rete ridge formation (Formulation 26 with **Compound 8** + GO-HA); and max force (Formulation 23 with **Compound 10**), and collagen organization compared to the control. The wounds treated with GO-HA vehicle had the greatest energy peak. Wounds treated with 0.9% saline provided poor wound healing integrity for elasticity, max force, strain, and energy peak parameters.

[00900] While particular embodiments of the present invention have been described in the foregoing, it is to be understood that other embodiments are possible within the scope of the invention and are intended to be included herein. It will be clear to any person skilled in the art that modifications of and adjustments to this invention, not shown, are possible without departing from the spirit of the invention as demonstrated through one or more embodiments of the present disclosure. The invention is therefore to be considered limited solely by the scope of the appended claims.

CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition, comprising:

a Wnt inhibitor which is 2-(4-(trifluoromethyl)phenyl)-1,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound A**) preferably in an amount from about 0.01 wt% to about 2 wt%;

a graphene oxide-hyaluronic acid conjugate (GO-HA), preferably wherein the GO is linked to the HA via -CH_2-C(O)-NH-NH-C(O)-$(CH_2)_4$-C(O)NH-NH- where the HA is attached via an amide bond comprising the terminal NH of the linker and the GO is attached to the CH_2 end of the linker (in some embodiments through an ether bond with GO phenol), and preferably in an amount from about 0.01 wt% to about 2 wt%;

a glycol, preferably in an amount from about 2 wt% to about 10 wt%, or in an amount from about 3 wt% to about 6 wt%;

one or more solubilizers, preferably in an amount from about 0.5 wt% to about 10 wt%, preferably about 1 wt% to about 6 wt%;

one or more optional thickeners, and when present, preferably in an amount from about 0.5 wt% to about 2.5 wt%;

one or more phospholipids, preferably in an amount from about 0.1 wt% to about 4 wt%;

one or more carriers, preferably in an amount from about 2 wt% to about 10 wt%, preferably about 3 wt% to about 6 wt% when the carrier is ethanol;

an optional stabilizer, and when present, preferably in an amount from about 0.1 wt% to about 0.5 wt%;

an optional buffer, and when present, preferably in an amount from about 0.2 wt% to about 2.0 wt%;

an optional preservative, and when present, preferably in an amount from about 0.025 wt% to about 1.5 wt%; and

saline or water; and

wherein the wt% of the WNT inhibitor, GO-HA, glycol, one or more solubilizers, one or more optional thickeners (when present), one or more phospholipids, one or more carriers, optional stabilizer (when present), optional buffer (when present), optional preservative (when present), and saline or water is 100 wt%; or wherein the wt% of the WNT inhibitor, GO-HA, glycol, one or more solubilizers, one or more phospholipids, one or more carriers, optional stabilizer, (when present) optional

buffer (when present), optional preservative (when present), and saline or water is 100 wt%;

wherein the wt% ratio of the WNT inhibitor to GO-HA is about 0.1:1 to about 12:1.

2. The pharmaceutical composition of claim 1, wherein at least one of the stabilizer, buffer, and preservative is present; or wherein each of the stabilizer, buffer, and preservative is present.

3. A pharmaceutical composition, comprising:

a WNT inhibitor of Formula (I), preferably in an amount from about 0.01 wt% to about 2 wt%;

an optional graphene oxide-hyaluronic acid conjugate (GO-HA), and when present, preferably in an amount from about 0.01 wt% to about 2 wt%, and preferably wherein the GO is linked to the HA via -CH_2-C(O)-NH-NH-C(O)-$(CH_2)_4$-C(O)NH-NH- where the HA is attached via an amide bond comprising the terminal NH of the linker and the GO is attached to the CH_2 end of the linker (in some embodiments through an ether bond with GO phenol);

a glycol, preferably in an amount from about 2 wt% to about 10 wt%, preferably about 3 wt% to about 6 wt%;

one or more solubilizers, preferably in an amount from about 0.5 wt% to about 10 wt%, preferably about 1 wt% to about 6 wt%;

one or more optional thickeners, and when present, preferably in an amount from about 0.5 wt% to about 2.5 wt%;

one or more optional phospholipids, and when present, preferably in an amount from about 0.1 wt% to about 4 wt%;

one or more optional carriers, and when present, preferably in an amount from about 2 wt% to about 10 wt% or in an amount from about 3 wt% to about 6 wt% when the carrier is ethanol;

an optional stabilizer, and when present, preferably in an amount from about 0.1 wt% to about 0.5 wt%;

an optional buffer, and when present, preferably in an amount from about 0.2 wt% to about 2.0 wt%;

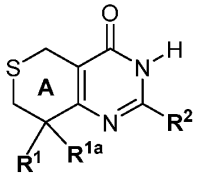
an optional preservative, and when present, preferably in an amount from about 0.025 wt% to about 1.5 wt%; and

saline or water; and

wherein the wt% of the WNT inhibitor, optional GO-HA (when present), glycol, one or more solubilizers, one or more optional thickeners (when present), one or more optional phospholipids (when present), one or more optional carriers (when present), optional stabilizer (when present), optional buffer (when present), optional preservative (when present), and water or saline is 100 wt%; or wherein the wt% of the WNT inhibitor, optional GO-HA (when present), glycol, one or more solubilizers, one or more optional phospholipids (when present), one or more optional carriers (when present), optional stabilizer (when present), optional buffer (when present), optional preservative (when present), and water or saline is 100 wt%;

wherein the wt% ratio of the WNT inhibitor to GO-HA, when present, is about 0.1:1 to about 12:1; and

wherein the WNT inhibitor of Formula (I) is according to:



(I),

or a single stereoisomer or mixture of stereoisomers thereof; a single tautomer or mixture of tautomers thereof; and/or a pharmaceutically acceptable salt thereof;

wherein:

R^1 is hydrogen, deuterium, C_1-C_3alkyl, -OH, -O-C_1-C_3alkyl, -CH_2OH, or -B(OH)$_2$;

R^{1a} is hydrogen, deuterium, or C_1-C_3alkyl;

R^2 is:

(a) phenyl substituted with **R^3** and additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups;

(b) phenyl substituted with 5- or 6-membered heteroaryl where the 5- or 6-membered heteroaryl is substituted with **R^3** and additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups and where the phenyl is additionally optionally substituted with 1, 2, or 3 **R^{3a}** groups;

(c) phenyl optionally substituted with 1, 2, or 3 **R^{3a}** groups and additionally substituted with -phenyl-**R^3** where the phenyl in -phenyl-**R^3** is optionally substituted with 1, 2, or 3 **R^{3a}** groups;

(d) 5- or 6-membered heteroaryl substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups;

(e) 5- or 6-membered heteroaryl optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups and additionally substituted with -phenyl-$\mathbf{R^3}$ where the phenyl is additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups;

(f) 5- or 6-membered heteroaryl optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups and additionally substituted with –(5- or 6-membered heteroaryl)-$\mathbf{R^3}$ where the 5- or 6-membered heteroaryl in –(5- or 6-membered heteroaryl)-$\mathbf{R^3}$ is optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups;

(g) C_3-C_6cycloalkyl substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1 or 2 $\mathbf{R^{3a}}$ groups;

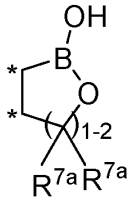
(h) C_3-C_6cycloalkyl substituted with 5- or 6-membered heteroaryl where the 5- or 6-membered heteroaryl is substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups, and where the C_3-C_6cycloalkyl is optionally substituted with 1 or 2 $\mathbf{R^{3a}}$ groups;

(j) C_3-C_6cycloalkyl substituted with phenyl where the phenyl is substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups, and where the C_3-C_6cycloalkyl is optionally substituted with 1 or 2 $\mathbf{R^{3a}}$ groups;

(k) 3- to 8-membered heterocycloalkyl substituted with phenyl or substituted with 5- or 6-membered heteroaryl, where the phenyl and the 5- or 6-membered heteroaryl are substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups; or

(m) -CH=CH-$\mathbf{R^5}$ where $\mathbf{R^5}$ is phenyl or 5- or 6- membered heteroaryl, where the phenyl and the 5- or 6-membered heteroaryl are substituted with $\mathbf{R^3}$ and additionally optionally substituted with 1, 2, or 3 $\mathbf{R^{3a}}$ groups;

$\mathbf{R^3}$ is independently selected from -$B(OH)_2$, cyano, halo, halo-C_1-C_6alkyl, -(C_0-C_6alkylene)-O-$\mathbf{R^4}$, or 5- to 10-membered heterocyclic wherein the 5- to 10-membered heterocyclic is optionally substituted with cyano; or when $\mathbf{R^2}$ is (a), then $\mathbf{R^3}$ and one $\mathbf{R^{3a}}$, when on adjacent carbons, together with the carbons to which they are attached form:



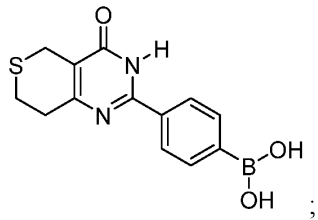
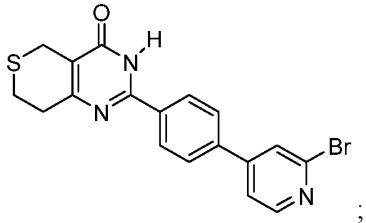
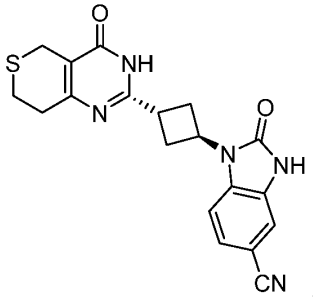
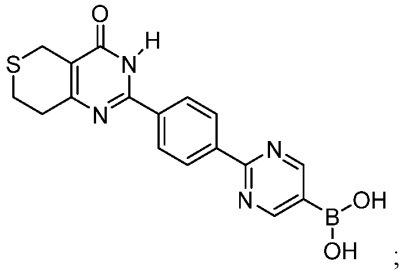
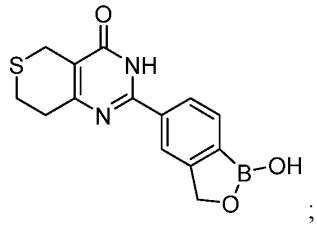
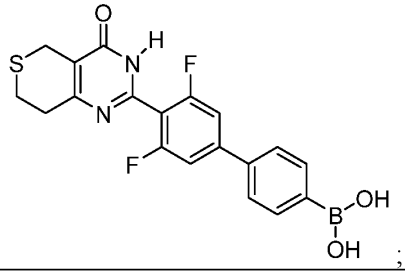
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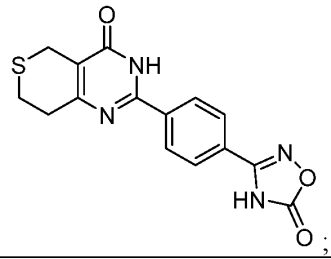
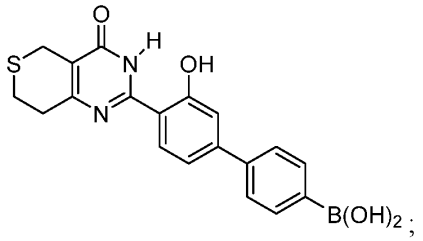
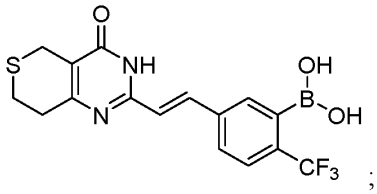
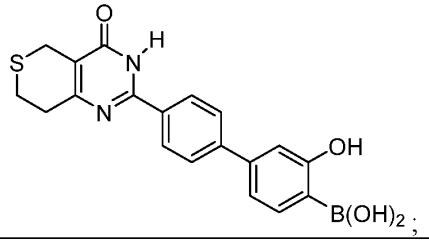
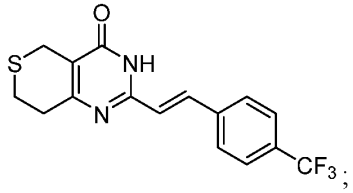
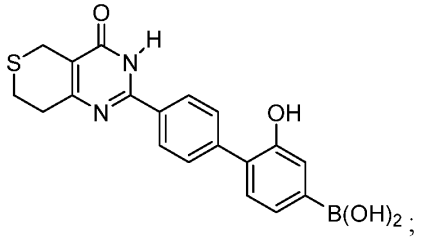
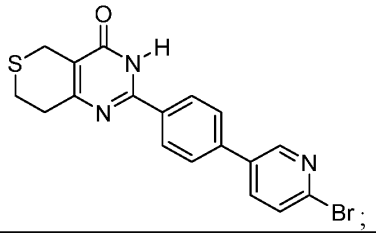
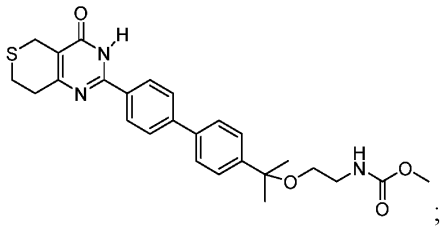
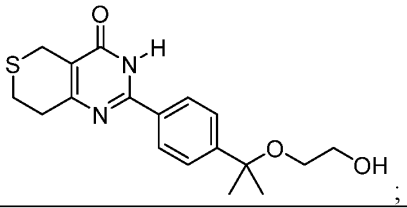
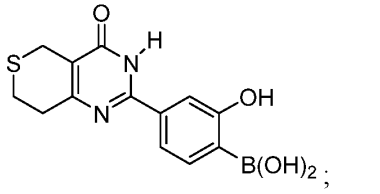
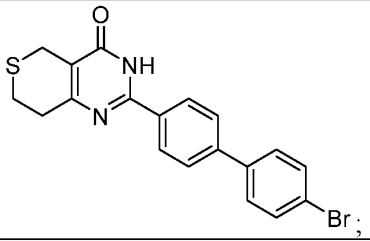
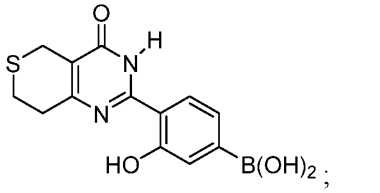
where the * indicate the carbons shared with a phenyl ring and where the remaining optional $\mathbf{R^{3a}}$ on the phenyl ring portion are as defined below, and each $\mathbf{R^{7a}}$ is independently hydrogen or C_1-C_6alkyl;

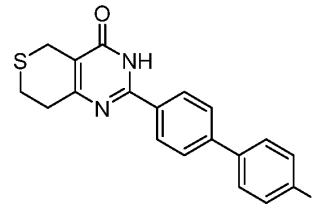
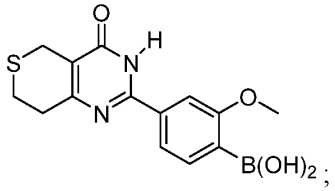
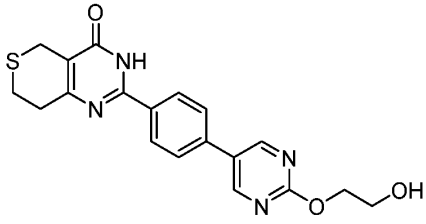
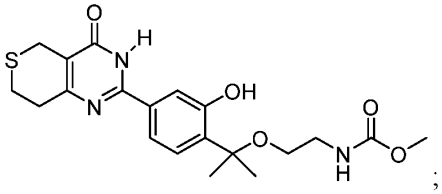
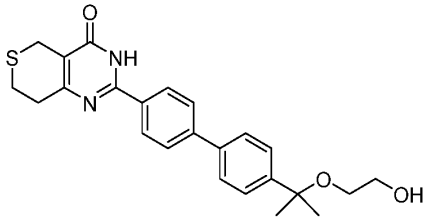
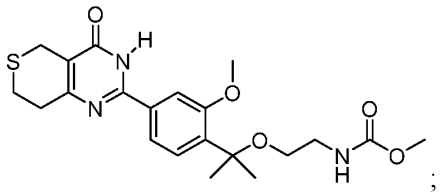
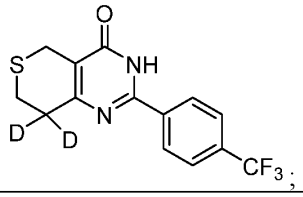
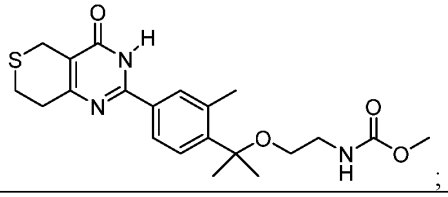
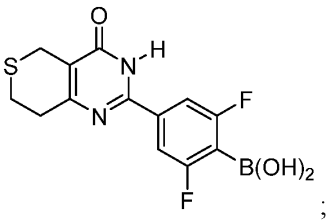
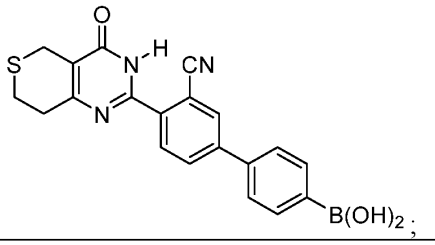
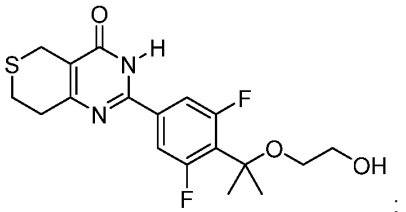
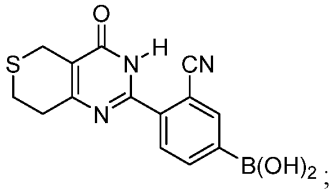
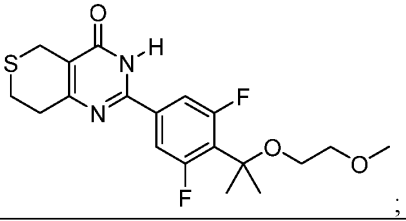
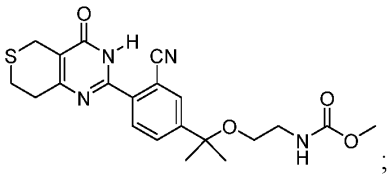
each $\mathbf{R^{3a}}$ is independently selected from cyano, halo, -OH, C_1-C_6alkyl, halo-C_1-C_6alkyl, and C_1-C_6alkoxy; and

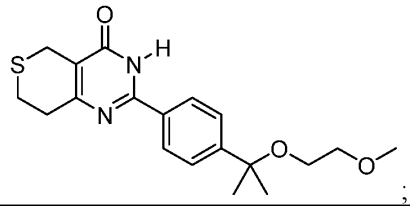
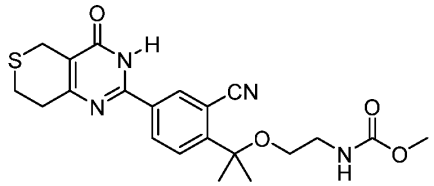
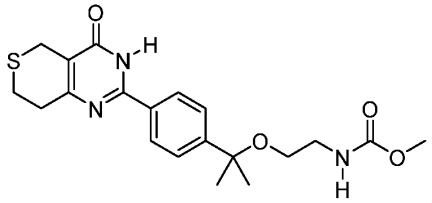
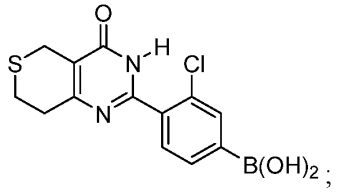
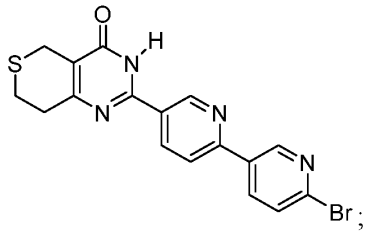
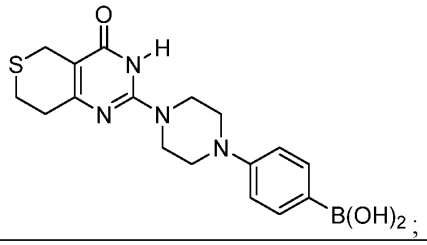
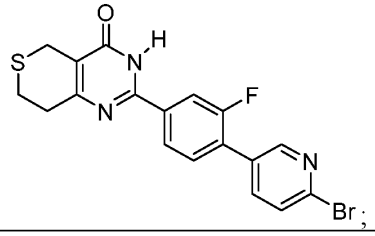
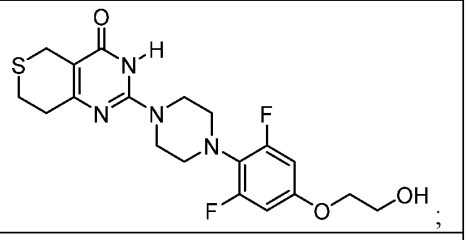
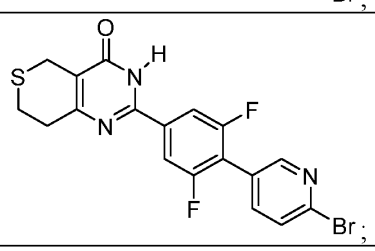
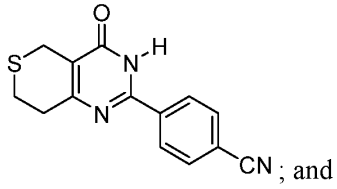
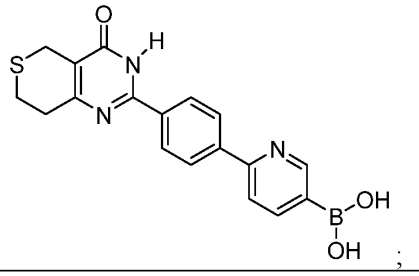
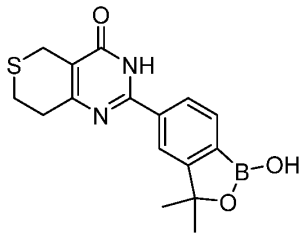
$\mathbf{R^4}$ is hydroxy-C_1-C_6alkyl, C_1-C_6alkoxy-C_1-C_6alkyl, or C_1-C_6alkoxycarbonyl-NH-C_1-C_6alkyl.

4. The pharmaceutical composition of claim 3, wherein the WNT inhibitor is selected from

1	 ;	**23**	 ;
2	 ;	**24**	 ;
3	 ;	**25**	 ;

4	 ;	**26**	 ;
5	 ;	**27**	 ;
6	 ;	**28**	 ;
7	 ;	**29**	 ;
8	 ;	**30**	 ;
9	 ;	**31**	 ;

10	 ;	**32**	 ;
11	 ;	**33**	 ;
12	 ;	**34**	 ;
13	 ;	**35**	 ;
14	 ;	**36**	 ;
15	 ;	**37**	 ;
16	 ;	**38**	 ;

17	 ;	**39**	 ;
18	 ;	**40**	 ;
19	 ;	**41**	 ;
20	 ;	**42**	 ;
21	 ;	**43**	 ; and
22	 ;	**44**	 ;

or a single stereoisomer or mixture of stereoisomers thereof; a single tautomer or mixture of tautomers thereof; and/or a pharmaceutically acceptable salt thereof.

5. The pharmaceutical composition of claim 3 or 4, wherein the WNT inhibitor is (4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)boronic acid

(**Compound 1**); a single tautomer or mixture of tautomers thereof and/or a pharmaceutically acceptable salt thereof.

6. The pharmaceutical composition of claim 3 or 4, wherein the WNT inhibitor is 2-(4-(6-bromopyridin-3-yl) phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 7**); a single tautomer or mixture of tautomers thereof and/or a pharmaceutically acceptable salt thereof.

7. The pharmaceutical composition of claim 3 or 4, wherein the WNT inhibitor is 2-(4-(2-(2-hydroxyethoxy)propan-2-yl)phenyl)-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-4-one (**Compound 8**); a single tautomer or mixture of tautomers thereof and/or a pharmaceutically acceptable salt thereof.

8. The pharmaceutical composition of claim 3 or 4, wherein the WNT inhibitor is (4'-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)-[1,1'-biphenyl]-4-yl)boronic acid (**Compound 10**); a single tautomer or mixture of tautomers thereof and/or a pharmaceutically acceptable salt thereof.

9. The pharmaceutical composition of claim 3 or 4, wherein the WNT inhibitor is methyl (2-((2-(4-(4-oxo-3,5,7,8-tetrahydro-4H-thiopyrano[4,3-d]pyrimidin-2-yl)phenyl)propan-2-yl)oxy)ethyl)carbamate (**Compound 18**); a single tautomer or mixture of tautomers thereof and/or a pharmaceutically acceptable salt thereof.

10. The pharmaceutical composition of any one of claims 1 to 9, comprising:
the WNT inhibitor in an amount from about 0.01 wt% to about 2 wt%;
optional GO-HA, and when present, in an amount from about 0.01 wt% to about 2 wt%;
a glycol in an amount from about 2 wt% to about 10 wt%;
one or more solubilizers in an amount from about 2 wt% to about 10 wt%;
one or more thickeners in an amount from about 0.5 wt% to about 2.5 wt%;
a phospholipid in an amount from about 0.1 wt% to about 4 wt%;
one or more carriers in an amount from about 2 wt% to about 10 wt%;
an optional stabilizer, and when present, preferably in an amount from about 0.1 wt% to about 0.5 wt%;
an optional buffer, and when present, preferably in an amount from about 0.2 wt% to about 2.0 wt%;
an optional preservative, and when present, preferably in an amount from about 0.025 wt% to about 1.5 wt%; and

saline or water; and

wherein the GO-HA is not optional when the WNT inhibitor is **Compound A**; and

wherein one or more embodiments, the stabilizer, buffer, and preservative are not present.

11. The pharmaceutical composition of any one of claims 1 to 10, wherein a stabilizer is present.

12. The pharmaceutical composition of claim 11, wherein the stabilizer is an antioxidant.

13. The pharmaceutical composition of claim 11 or 12, wherein the stabilizer is methionine.

14. The pharmaceutical composition of any one of claims 1 to 13, wherein a buffer is present.

15. The pharmaceutical composition of any one of claims 1 to 14, wherein the pH is in a range from about 5.5 to about 7.

16. The pharmaceutical composition of claim 14 or 15, wherein the buffer is or comprises citric acid.

17. The pharmaceutical composition of any one of claims 1 to 16, wherein a preservative is present.

18. The pharmaceutical composition of any one of claims 1 to 17, wherein the preservative is present and comprises phenoxyethanol and ethylhexylglycerin, or is potassium sorbate.

19. The pharmaceutical composition of any one of claims 1 to 18, wherein the glycol is one or more selected from the group consisting of butylene glycol and propanediol (including propylene glycol).

20. The pharmaceutical composition of any one of claims 1 to 19, wherein the one or more solubilizers comprise PPG-26 Buteth-26 and PEG-40 hydrogenated castor oil; or the one or more solubilizers are one or more selected from the group consisting of hydroxypropyl cellulose, polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80.

21. The pharmaceutical composition of any one of claims 3 to 9, and 11 to 20, wherein a phospholipid is present.

22. The pharmaceutical composition of any one of claims 1 to 21, wherein the phospholipid is phosphatidylcholine.

23. The pharmaceutical composition of any one of claims 1 to 22, wherein one or more carriers is present.

24. The pharmaceutical composition of claim 23, wherein the one or more carriers comprises or is ethanol.

25. The pharmaceutical composition of any one of claims 1 to 24, wherein the saline is from about 0.22% to about 0.9% NaCl in water.

26. The pharmaceutical composition of any one of claims 1 to 25, wherein the pharmaceutical composition comprises the WNT inhibitor, a glycol, one or two solubilizers, one or two thickeners, a phospholipid, ethanol, and water or saline; and optionally contains GO-HA; wherein the GO-HA is not optional when the WNT inhibitor is **Compound A**; and wherein the ratio of WNT inhibitor:GO-HA, when GO-HA is present, is about 1:1.

27. The pharmaceutical composition of any one of claims 1, 2, and 10 to 26 wherein the wt% ratio of WNT inhibitor:phospholipid is from about 1:1 to about 1:50, or about 1:10 to about 1:25, or about 1:10 to about 1:13.

28. The pharmaceutical composition of any one of claims 3 to 27, wherein GO-HA is present.

29. The pharmaceutical composition of any one of claims 1, 2, and 10 to 28, wherein the wt% ratio of WNT inhibitor:GO-HA is from about 1:0.1 to about 1:1.

30. The pharmaceutical composition of any one of claims 1 to 29, wherein one or more carriers and phospholipid are present and are in a wt% ratio of carrier(s):phospholipid is from about 2:1 to about 4:1.

31. The pharmaceutical composition of any one of claims 1 to 30, glycol and one or more solubilizer(s) are present in a wt% ratio of about 1:0.5 to about 1:1.5.

32. The pharmaceutical composition of any one of claims 1 to 31, wherein glycol and one or more carrier(s) are present in a wt% ratio of about 1:0.5 to about 1:1.5, wherein one or more embodiments, the one or more carrier is ethanol.

33. The pharmaceutical composition of any one of claims 3 to 9, 19 to 25, and 27 to 32, comprising:

the WNT inhibitor in an amount from about 0.01 wt% to about 2 wt%;

optional GO-HA, and when present, in an amount from about 0.01 wt% to about 2 wt%;

a glycol in an amount from about 2 wt% to about 10 wt%;

one or more thickeners in an amount from about 0.5 wt% to about 2.5 wt%;

an optional stabilizer, and when present, preferably in an amount from about 0.1 wt% to about 0.5 wt%;

an optional buffer, and when present, preferably in an amount from about 0.2 wt% to about 2.0 wt%;

an optional preservative, and when present, preferably in an amount from about 0.025 wt% to about 1.5 wt%; and

saline or water; and

wherein the GO-HA is not optional when the WNT inhibitor is **Compound A**;

wherein the one or more optional phospholipids and the one or more carriers are not present; and

wherein one or more embodiments, the stabilizer, buffer, and preservative are not present.

34. The pharmaceutical composition of any one of claims 1 to 33, wherein the pharmaceutical composition comprises the WNT inhibitor, a glycol, one or two thickeners, and water or saline; and optionally contains GO-HA; and wherein the ratio of WNT inhibitor:GO-HA, when GO-HA is present, is about 1:1.

35. The pharmaceutical composition of claim 33 or 34, wherein glycol and one or more thickener(s) are present in a wt% ratio of about 1:0.25 to about 1:1.5.

36. The pharmaceutical composition of any one of claims 1-9, 11 to 25, and 27-32, wherein the pharmaceutical composition does not comprise a thickener.

37. The pharmaceutical composition of any one of claims 1 to 35, wherein the pharmaceutical composition comprises a thickener.

38. The pharmaceutical composition of any one of claims 1 to 37, wherein the pharmaceutical composition does not comprise a thickener or comprises a thickener in an amount suitable for a spray.

39. The pharmaceutical composition of any one of claims 1 to 35 and 37, wherein the pharmaceutical composition comprises a thickener and the pharmaceutical composition is a serum.

40. The pharmaceutical composition of any one of claims 1 to 39, wherein when the GO-HA is present, the GO is linked to the HA via $-CH_2-C(O)-NH-NH-C(O)-(CH_2)_4-C(O)NH-NH-$ where the HA is attached via an amide bond comprising the terminal NH of the linker and the GO is attached to the CH_2 end of the linker (in some embodiments through an ether bond with GO phenol).

41. A method of treating a disease, disorder, or condition associated with Wnt signaling pathway activity, comprising administering topically a pharmaceutical composition of any one of claims 1 to 40 to a mammal in need thereof.

42. The method of claim 41, wherein the method is for stimulating regeneration of tissue at a wound in the mammal in need thereof and wherein the wound is contacted with a therapeutically effective amount of the pharmaceutical composition.

43. The method of claim 41, wherein the disease, disorder, or condition is selected from a chronic wound, an acute wound, an alkali-burned corneal wound, an incisional wound (open or closed), a burn, a lesion (including lesions caused by HPV and/or a virus selected from the *Poxviridae* family of viruses), an inflammatory dermatitis disease (including acne, psoriasis, rosacea, and scleroderma), cancer (including melanoma and breast cancer), a de-nerved body part in need of reinnervation, tissue in need of regeneration (including damaged elastic cartilage), bacterial growth in need of inhibition, fungal growth in need of inhibition, and tissue in need of neovascularization.

44. The method of claim 41, wherein the method is for inducing bacteriostasis associated with Wnt signaling pathway activity.

45. The method of claim 41, wherein the method is for treating an open excisional wound, and the pharmaceutical composition is according to any one of claims 1 to 35.

46. The method of claim 45, wherein the WNT inhibitor is **Compound A**.

47. The method of claim 41, wherein the method is for treating a closed excisional wound the pharmaceutical composition is according to any one of claims 1 to 25 and 33 to 37.

48. The method of claim 41, wherein the method is for treating a burn and the pharmaceutical composition is according to any one of claims 1 to 25 and 33 to 37.

49. The method of claim 41 or 42, wherein the method is for regenerating elastic cartilage and the pharmaceutical composition is according to any one of claims 1 to 37.