

DUAL FUNCTIONING IMMUNE MODULATING COMPOUNDS, FORMULATIONS, AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/594,277, filed October 30, 2023, the content of which is herein incorporated by reference in its entirety.

FIELD

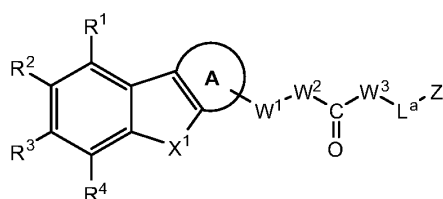
[0002] The present disclosure provides dual functioning compounds, compositions, formulations, and methods for inducing or modulating an immune or inflammatory response and treating diseases or disorders (e.g., cancer, autoimmune diseases, inflammatory diseases, and infectious diseases) with the compounds or compositions thereof.

BACKGROUND

[0003] The innate immune agonist STING (Stimulator of Interferon Genes) binds its natural ligand 2'3'-cGAMP (cyclic guanosine-adenosine monophosphate) and then, by way of its signaling pathway, induces the expression of interferons, inflammatory factors, and autophagy genes. Microbial infection, tumor DNA, and self-damaging DNA are three factors that induce the activation of the cGAS-STING signaling pathway and associate STING with the etiology of cancer, and autoimmune, infectious, and inflammatory diseases. Many natural and synthetic STING agonists have entered clinical development, particularly for cancer treatment, with the first generation demonstrating safety but only modest systemic activity. As such, most STING agonists require local delivery, due to their poor stability, thereby limiting their utility.

SUMMARY

[0004] In one aspect, disclosed herein is a compound of formula (I):



(I)

or a pharmaceutically acceptable salt thereof, wherein:

R¹, R², R³, and R⁴ are each independently selected from hydrogen, C₁-C₆ alkoxy, halo, and C₁-C₆ alkyl;

X^1 is selected from S and NH;

A is selected from a five- or six-membered cycloalkyl, heterocyclyl, heteroaryl, or aryl, each of which is optionally substituted with 1 or 2 oxo groups;

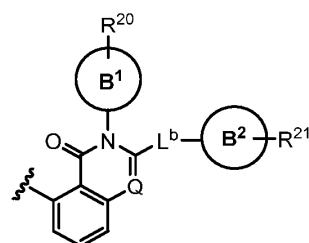
W^1 is a bond or O;

W^2 is selected from C_1 - C_6 alkylene, C_3 - C_6 cycloalkylene, C_3 - C_6 cycloalkylene- C_1 - C_6 alkylene, arylene, and hydroxy- C_1 - C_6 -alkylene;

W^3 is O or NH;

L^a is a linker; and

Z is



wherein:

Q is CH or N;

B^1 is aryl or a 5- or 6-membered monocyclic heteroaryl having 1, 2, 3, or 4 heteroatoms independently selected from N, O, S, and P;

R^{20} is selected from hydrogen, halo, C_1 - C_4 alkyl, C_3 - C_6 cycloalkyl, C_1 - C_4 haloalkyl, $-OR^{a1}$, $-N(R^{a2})(R^{a3})$, $-SO_2R^{a4}$, $-SO_2N(R^{a5})(R^{a6})$, and $-NHSO_2R^{a7}$, wherein R^{a1} , R^{a2} , R^{a3} , R^{a4} , R^{a5} , R^{a6} , and R^{a7} are each independently selected from hydrogen, C_1 - C_4 alkyl, and C_1 - C_4 haloalkyl;

R^{21} is selected from hydrogen and a group $-L^{21}-E$, wherein:

L^{21} is a bond, C_1 - C_2 alkylene, $-CH=CH-$, $-C\equiv C-$, $-C(O)-$, $-O-$, $-NH-$, $-S-$, $-C(O)O-$, $-C(O)NH-$, $-C(O)S-$, arylene, cycloalkylene, heteroarylene, or heterocyclylene, or wherein L^{21} comprises a combination of any two of such groups;

E is a bicyclic heterocyclyl or bicyclic heteroaryl, each of which is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from halo, C_1 - C_4 alkyl, C_3 - C_6 cycloalkyl, C_3 - C_6 -cycloalkyl- C_1 -4-alkyl, C_1 - C_4 haloalkyl, oxo, $-OR^{b1}$, $-N(R^{b2})(R^{b3})$, $-SO_2R^{b4}$, $-SO_2N(R^{b5})(R^{b6})$, and $-NHSO_2R^{b7}$, wherein R^{b1} , R^{b2} , R^{b3} , R^{b4} ,

R^{b5} , R^{b6} , and R^{b7} are each independently selected from hydrogen, C_1 - C_4 alkyl, and C_1 - C_4 haloalkyl;

L^b is $-(CR^{c1}R^{c2})_m-G^b-$, wherein:

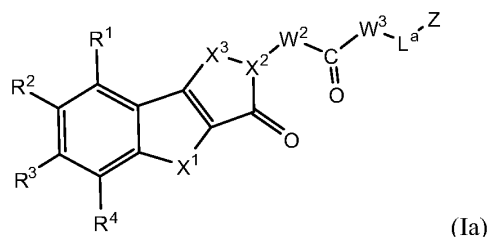
R^{c1} and R^{c2} are independently selected from hydrogen and C_1 - C_4 alkyl;

m is 0, 1, or 2; and

G^b is a bond, $-NHC(O)-$, $-NH-$, $-O-$, or $-S-$; and

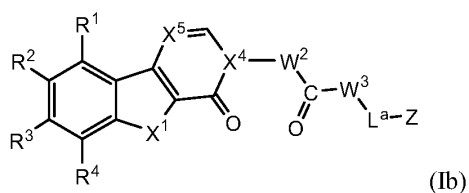
B^2 is a bicyclic heteroaryl or bicyclic heterocyclyl, each of which is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from halo, C_1 - C_4 alkyl, C_3 - C_6 cycloalkyl, C_1 - C_4 haloalkyl, optionally substituted aryl, $-OR^{d1}$, $-N(R^{d2})(R^{d3})$, $-SO_2R^{d4}$, $-SO_2N(R^{d5})(R^{d6})$, and $-NHSO_2R^{d7}$, wherein R^{d1} , R^{d2} , R^{d3} , R^{d4} , R^{d5} , R^{d6} , and R^{d7} are each independently selected from hydrogen, C_1 - C_4 alkyl, and C_1 - C_4 haloalkyl.

[0005] In some embodiments, the compound is a compound of formula (Ia):



or a pharmaceutically acceptable salt thereof. In some embodiments, X^2 is CH or N, and X^3 is CH_2 or CO. In some embodiments, X^2 is CH. In some embodiments, X^2 is N. In some embodiments, X^3 is CH_2 . In some embodiments, X^3 is CO.

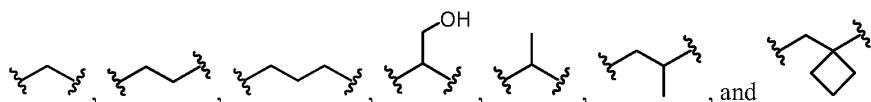
[0006] In some embodiments, the compound is a compound of formula (Ib):



or a pharmaceutically acceptable salt thereof, wherein X^4 and X^5 are independently selected from N and CH. In some embodiments, X^4 is N. In some embodiments, X^5 is N. In some embodiments, X^5 is CH.

[0007] In some embodiments, X^1 is S. In some embodiments, X^1 is NH.

[0008] In some embodiments, W^2 is selected from C_1 - C_4 alkylene, C_4 - C_5 cycloalkylene, C_4 - C_5 cycloalkylene- C_1 - C_2 alkylene, phenylene, and hydroxy- C_1 - C_3 -alkylene. In some embodiments, wherein W^2 is selected from:



[0009] In some embodiments, R^5 is selected from hydrogen, C_1 - C_{40} alkyl, C_2 - C_{40} alkenyl, C_1 - C_4 -alkylamino, di- C_1 - C_6 -alkylamino- C_1 - C_6 -alkyl, and heterocyclyl- C_1 - C_6 -alkyl.

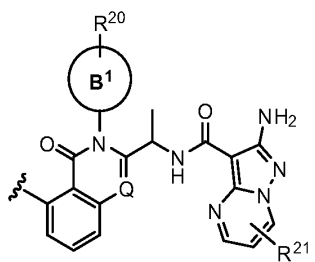
[0010] In some embodiments, W^3 is O. In some embodiments, W^3 is NH.

[0011] In some embodiments, R^2 and R^3 are each independently C_1 - C_6 alkoxy. In some embodiments, R^2 and R^3 are each methoxy.

[0012] In some embodiments, R^1 and R^4 are each independently halo or hydrogen. In some embodiments, R^1 is halo and R^4 is hydrogen.

[0013] In some embodiments, R^1 is halo, R^2 and R^3 are each independently C_1 - C_6 alkoxy, and R^4 is hydrogen. In some embodiments, R^1 is chloro or bromo, and R^2 and R^3 are methoxy. In some embodiments, R^1 and R^4 are hydrogen, and R^2 and R^3 are methoxy.

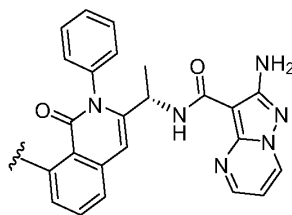
[0014] In some embodiments, Z is:



[0015] In some embodiments, B^1 is phenyl. In some embodiments, R^{20} is hydrogen.

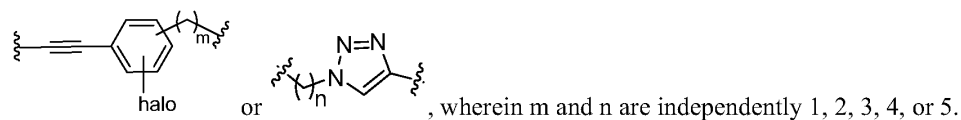
[0016] In some embodiments, Q is CH.

[0017] In some embodiments, Z is:



[0018] In some embodiments, L^a comprises one or more groups independently selected from $-C(R')_2-$, $-CH=CH-$, $-C\equiv C-$, $-O-$, $-NR'-$, $-BR'-$, $-S-$, $-C(O)-$, $-C(NR')-$, $-S(O)-$, $-S(O)_2-$,

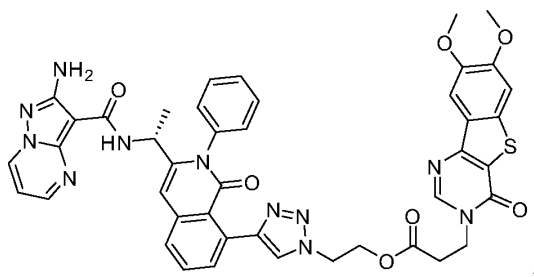
arylene, heteroarylene, cycloalkylene, and heterocyclylene, wherein each R' is independently selected from hydrogen, C₁-C₈₀ alkyl, C₂-C₈₀ alkenyl, C₂-C₈₀ alkynyl, aryl, arylalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, and heteroarylalkyl, and wherein each alkyl, arylene, heteroarylene, cycloalkylene, and heterocyclylene is independently unsubstituted or substituted with 1, 2, or 3 substituents. In some embodiments, L^a comprises one or more groups independently selected from -C(R')₂-, -C≡C-, -O-, -NH-, -C(O)-, and heteroarylene, wherein each R' is independently selected from hydrogen, C₁-C₄₀ alkyl, phenyl, and -CH₂-heterocyclyl (e.g., wherein the heterocyclyl is a 6-membered heterocyclyl having 1 or 2 heteroatoms independently selected from N, O, and S), wherein the phenyl and the heterocyclyl are each independently unsubstituted or substituted with 1 or 2 substituents. In some embodiments, L^a comprises one or more groups independently selected from -CH₂-, -C≡C-, arylene, and heteroarylene, wherein the arylene is unsubstituted or substituted with 1 or 2 halo groups. In some embodiments, L^a comprises one or more groups independently selected from -CH₂- and heteroarylene. In some embodiments, L^a is

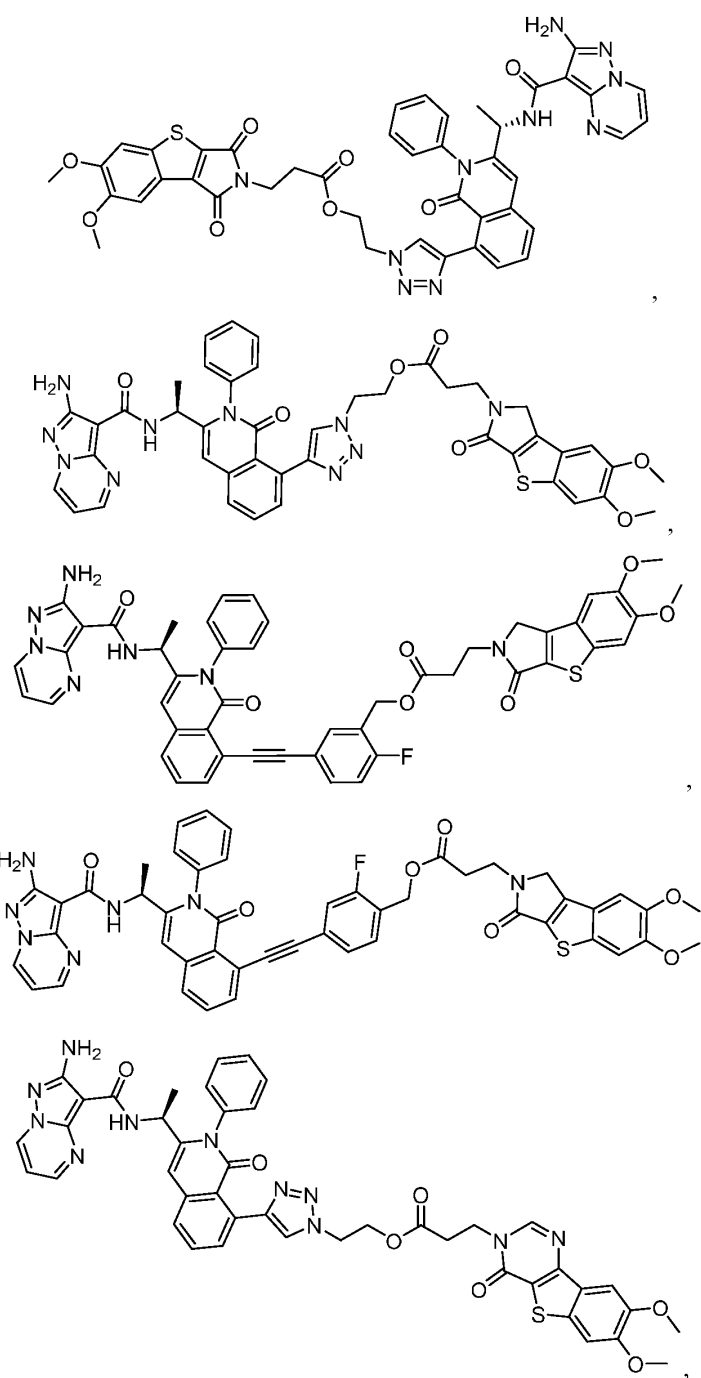


In some embodiments, L^a is

, or

[0019] In some embodiments, the compound is selected from:





and pharmaceutically acceptable salts thereof.

[0020] In another aspect, disclosed herein is a pharmaceutical composition comprising an effective amount of a compound disclosed herein (e.g., a compound of formula (I)) or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

[0021] In some embodiments, the composition comprises albumin nanoparticles, liposomes, micelles, or lipid nanoparticles.

[0022] In some embodiments, the composition further comprises at least one additional therapeutic agent. In some embodiments, the at least one additional therapeutic agent comprises an immune modulator, a chemotherapeutic agent, a nucleic acid, a decongestant, a steroid, an analgesic, an antimicrobial agent, an immunotherapy, or a combination thereof. In some embodiments, the at least one additional therapeutic agent comprises an RNA selected from the group consisting of a small interfering RNA (siRNA), an asymmetrical interfering RNA (aiRNA), a microRNA (miRNA), a Dicer-substrate RNA (dsRNA), a small hairpin RNA (shRNA), a messenger RNA (mRNA), and mixtures thereof. In some embodiments, the at least one additional therapeutic agent is selected from a chemotherapeutic agent, an IDO inhibitor, a Stat3 inhibitor, a TLR agonist, PD-1 or PD-L1 antibody, and a PI3K inhibitor. In some embodiments, the composition further comprises one or more cell targeting epitopes. In some embodiments, the one or more cell targeting epitopes are covalently attached or directly conjugated to an albumin. In some embodiments, the cell targeting epitopes comprise an immune cell epitope. In some embodiments, the composition further comprises one or more epitopes from a microbiological agent.

[0023] In another aspect, disclosed herein is a vaccine comprising an effective amount of: a compound disclosed herein (e.g., a compound of formula (I), or a pharmaceutically acceptable salt thereof), or a pharmaceutical composition disclosed herein (e.g., a pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof); and an antigen or a nucleic acid encoding thereof.

[0024] In some embodiments, the antigen is a tumor antigen, a self-antigen, or an infectious disease derived antigen. In some embodiments, the nucleic acid is messenger RNA (mRNA).

[0025] In another aspect, disclosed herein is a method of treating or preventing a disease or disorder comprising administering an effective amount of a compound disclosed herein (e.g., a compound of formula (I), or a pharmaceutically acceptable salt thereof), or a pharmaceutical composition disclosed herein (e.g., a pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof), or a vaccine disclosed herein (e.g., a vaccine comprising a compound of formula (I), or a pharmaceutically

acceptable salt thereof), to a subject in need thereof. In some embodiments, the disease or disorder comprises cancer, an autoimmune disease, an inflammatory disease, or an infectious disease. In some embodiments, the disease or disorder is cancer. In some embodiments, the subject has cancer, has had cancer, is predisposed to cancer, or has a family history of cancer. In some embodiments, the cancer comprises a solid tumor. In some embodiments, the cancer is metastatic cancer. In some embodiments, the method suppresses or eliminates cancer metastasis, decreases tumor growth, prevents tumor recurrences, or any combination thereof. In some embodiments, the cancer is pancreatic cancer. In some embodiments, the administering comprises an initial administration and at least one subsequent administration.

[0026] In another aspect, disclosed herein are methods of inducing or modulating an immune or inflammatory response, reducing or eliminating regulatory B cells (B_{regs}), and inhibiting PI3K γ in a subject comprising administering a compound disclosed herein (e.g., a compound of formula (I), or a pharmaceutically acceptable salt thereof), or a pharmaceutical composition disclosed herein (e.g., a pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof), or a vaccine disclosed herein (e.g., a vaccine comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof), to a subject in need thereof. In some embodiments, the subject is human. In some embodiments, the method further comprises administering at least one additional therapeutic agent. In some embodiments, the at least one additional therapeutic agent comprises an immune modulator, a chemotherapeutic agent, a nucleic acid, a decongestant, a steroid, an analgesic, an antimicrobial agent, an immunotherapy, or a combination thereof.

[0027] In another aspect, disclosed herein is a use of compound disclosed herein (e.g., a compound of formula (I), or a pharmaceutically acceptable salt thereof), or a pharmaceutical composition disclosed herein (e.g., a pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof), in the manufacture of a medicament for the treatment or prevention a disease or disorder. In some embodiments, the disease or disorder comprises cancer, an autoimmune disease, an inflammatory disease, or an infectious disease.

[0028] Other aspects and embodiments of the disclosure will be apparent in light of the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIG. 1 is graphs of THP1-Blue™ ISG cells assay to test the activity of the indicated dual-function compounds compared to MSA-2 and the STING agonist not in dual-function form (ZSA-52).

[0030] FIG. 2 is a graph of the in vivo efficacy, as measured by tumor volume, of the indicated dual-function compounds (S269 is SH-269 and S273 is SH-273) with and without anti-PD-1 administration.

[0031] FIGS. 3A-3B is flow cytometry analysis of regulatory (IL10+ or IL35+) B cells between transgenic KPC mice and normal B6 mice in tumors (pancreas; FIG. 3A) and lymph nodes (FIG. 3B). FIGS. 3C-3D is flow cytometry analysis of M2 macrophages between transgenic KPC mice and normal B6 mice in tumors (pancreas; FIG. 3C) and lymph nodes (FIG. 3D).

[0032] FIG. 4A shows different phosphorylation of IRF3 at B cells, bone marrow derived dendritic cells (BMDC), CD4 T cells, THP-1 ISG cells and bone marrow derived macrophages (BMDM) after incubation with sting agonist and PI3K γ inhibitor by western blot. FIGS. 4B-4C show in vitro binding affinity of STING from the dual functional compound (ZSA-52; FIG. 4B) and PI3K γ (FIG. 4C). FIGS. 4D-4E are flow cytometry analysis of in vitro regulatory B cell (IL35⁺ or IL10⁺) induction after STING agonist (MSA-2) and dual functional compound incubation at normal B cells (FIG. 4D) and Tmem173^{-/-} B cells (FIG. 4E). FIG. 4F is flow cytometry analysis of in vitro IL35 and IL10 induction after sting agonist and dual functional compound incubation at normal BMDC, BMDM, CD4 T cells, THP-1 WT cells and THP-1 R232 cells.

[0033] FIGS. 5A-5B show STING agonist and PI3K γ inhibitor combination having synergistic effects on STING activation at STING-TBK1-IRF3 (FIG. 5A) and STING-IKK α /β-NF-κb (FIG. 5B) pathways. FIGS. 5C-5E show that the dual functional compound increased STING pathway activation compared to STING agonist at bone marrow derived dendritic cells (BMDC) (FIG. 5C), THP-1 R232 (FIG. 5D) compounds-1 ISG blue (FIG. 5E) cell lines. FIG. 5F shows dual functional compound increased STING pathway activation is STING dependent. FIG. 5G is flow cytometry analysis BMDC activation comparing STING agonist and dual functional compound incubation. FIGS. 5H-5K are flow cytometry analysis (FIG. 5H) and ELISA measurement (FIG. 5I) of macrophages polarization after different compounds incubation at BMDM (bone marrow derived macrophages) and RAW264.7 cell line (FIGS 5U and 5K for flow cytometry analysis and ELISA measurement, respectively).

[0034] FIGS. 6A and 6B are DLS and TEM measurements characterizing the size of SH273 albumin nanoformulation. FIG. 6C is transgenic KPC mice survival time after indicated treatments. FIG. 6D is xenograft KPC mice survival time after indicated treatments. FIG. 6E shows cell clustering analysis for tumor samples from FIG. 6D by single cell sequencing analysis. FIG. 6F shows cell clustering percentages plots from single cell sequencing analysis (FIG. 6D). FIG. 6G shows B Cell clustering analysis for tumor samples from FIG. 6D by single cell sequencing analysis. FIG. 6H shows B Cell clustering percentages plots from single cell sequencing analysis.

[0035] FIG. 7A shows the structure of SH-273 with the IC₅₀ values of each of the moieties when functioning individually. FIG. 7B shows the size and morphology of the SH273 nanoformulation (Nano-273). FIG. 7C shows the in vivo efficacy of Nano-273 in transgenic KPC mice.

[0036] FIG. 8 shows SH-273 simultaneously enhanced STING's function in myeloid cells and overcame STING's resistance by reducing B_{regs} through an opposite mechanism in the two cell types.

[0037] FIG. 9A is a schematic showing the localization of injections used for FIG. 9B. FIG. 9B is graphs showing Nano-273 inhibition for local and distal pancreatic cancer. FIG. 9C is CyTOF analysis of immune profiling in tumors. FIG. 9D shows that Nano-273 enhanced M1 macrophage ratio in tumor. FIG. 9E shows Nano-273 (IV) eliminated B_{regs} in pancreatic tumor.

[0038] FIGS. 10A-10C show STING agonists expand Breg cells in PDAC Mice. FIG. 10A shows the antitumor efficacy of STING agonists in C57BL/6 mice inoculated with pancreatic tumor (KPC 6422 cell line). Average tumor volumes change after treatment with PBS (control), anti-PD1 antibody (100 µg, i.p.), diABZi (i.t., 20 ug/mouse or i.v., 1.5 mg/kg) with or without anti-PD1 antibody (100 µg, i.p.) or diABZi (i.v., 1.5 mg/kg) with or without anti-PD1 antibody (i.p., 100 µg). FIGS. 10B-10C show quantification of representative flow cytometry analysis of IL35⁺ and IL10⁺ Breg cells in tumor (FIG. 10B) and lymph node (FIG. 10C) from mice inoculated with KPC 6422 cell after treatment with PBS (control), diABZi (i.t., 20 ug/mouse) plus anti-PD1 antibody (i.p., 100 µg), or diABZi (i.v., 1.5 mg/kg) plus anti-PD1 antibody (i.p., 100 µg). The data represents two or three independent experiments with mean ± SD, and n = 3 (FIG. 10B-10C), and n = 10 (FIG. 10A). Statistical comparisons are based on one-way analysis of variance (FIG. 10B-10C) or two-way analysis of variance (FIG. 10A). *P* values are indicated.

[0039] FIGS. 11A-11M show that PI3K γ inhibition abolished STING-induced IRF3 phosphorylation to eliminate STING-induced Breg cells expansion, while PI3K γ inhibition sustained STING-induced IRF3 phosphorylation to preserve STING function in myeloid cells. FIG. 11A shows pathway enrichment analysis between B cells from KPC transgenic mice treated with or without MSA-2 (5 μ g/mL). FIG. 11B shows IRF3 and IRF3 phosphorylation in splenic B cells derived from KPC PDAC mice after treatments with MSA-2 (5 μ g/mL) or combination of MSA-2 with different inhibitors including, SB 590885 (Raf inhibitor, 1 μ M), Binimetinib (MEK inhibitor, 1 μ M), Temuterkib (ERK inhibitor, 1 μ M), Cyclosporin A (PP2B inhibitor, 1 μ M), Xestospongins C (IP3R inhibitor, 1 μ M) or IPI-549 (PI3K γ inhibitor, 1 μ M). FIG. 11C shows phosphorylation of IRF3 at human B cells isolated from PBMCs of healthy donor after treatments with MSA-2 (10 μ g/mL) or combination of MSA-2 (10 μ g/mL) with PI3K α (Alpelisib, 2 μ M), PI3K β (TGX-211, 2 μ M), PI3K γ (IPI-549, 10 μ M), PI3K δ (TGR-1202, 5 μ M) inhibitors. FIGS. 11D-11E shows quantitation of flow cytometry analysis of IL35 $^{+}$ and IL10 $^{+}$ Breg cells after pretreated with anti-IgM& anti-CD40 (5 μ g/mL) and then treated with MSA-2 (5 μ g/mL) or MSA-2 (5 μ g/mL) and IPI-549 (2 μ M) in B cells from PBMCs of PDAC patients (FIG. 11D) and splenic B cells from KPC transgenic mice (FIG. 11E). FIG. 11F shows quantitation of mouse interferon- β concentration of bone marrow derived dendritic cells (BMDCs) derived from C57BL/6 mice after treatments with or without MSA-2 (2.95 μ g/mL) or IPI-549 (10 μ M). FIG. 11G shows quantitation of human interferon- β concentration from THP-1 R232 hSTING^{R232} after treatments with or without MSA-2 (2.95 μ g/mL) or IPI-549 (10 μ M). FIG. 11H shows quantitation of STING activation by reporter assay from THP1-BlueTM ISG by measuring optical density at 655 nm after treatments with or without MSA-2 (2.95 μ g/mL) or IPI-549 (10 μ M). FIG. 11I shows quantitation of human Interferon- β concentration from THP-1 hSTING^{KO} after treatments with or without MSA-2 (2.95 μ g/mL) or IPI-549 (10 μ M). FIGS. 11J-11K shows quantitation of CD86 geometric mean fluorescent intensity of bone marrow derived dendritic cells (BMDCs, FIG. 11J), bone marrow derived macrophages (BMDMs, FIG. 11K) derived from C57BL/6 mice after treatments with or without MSA-2 (2.95 μ g/mL) or IPI-549 (10 μ M). FIG. 11L shows quantitation of RAW264.7 M1 polarization ratios after treatments with or without MSA-2 (2.95 μ g/mL) or IPI-549 (10 μ M). FIG. 11M shows quantitation of TNF- α concentration from RAW264.7 after treatments with or without MSA-2 (2.95 μ g/mL) or IPI-549 (10 μ M). The data represents two or three independent experiments with mean $\pm$ SD, and n = 3 (FIGS. 11A, 11D-11M). Statistical

comparisons are based on one-way analysis of variance (FIGS. 11D-11M). *P* values are indicated.

[0040] FIGS. 12A-12G show that dual targeting compound SH-273 and its albumin nanoformulation eliminate Breg cells through abolishing STING-induced IRF3 phosphorylation. FIG. 12A shows Nano-273, SH-273, MSA-2 IC₅₀ of STING activation by reporter assay from THP1-BlueTM ISG by measuring optical density at 655 nm. FIG. 12B shows phosphorylation of AKT (473) at BMDC after treatments with different concentrations of PI3K δ inhibitor TGR-1202 (1 μ M, 150 nM, from left to right), SH-273 (2 μ M, 400 nM, from left to right) or PI3K γ inhibitor IPI-549 (1 μ M, 150 nM, from left to right) in the presence of SDF-1 α for 1.5 hours. FIG. 12C shows phosphorylation of IRF3 at splenic B cells derived from KPC PDAC mice after treatments with MSA-2 (5 μ g/mL), IPI-549 (5 μ M) or SH-273 (5 μ M), respectively. FIG. 12D shows quantitation of flow cytometry analysis of IL35⁺ and IL10⁺ Breg cells after pretreated with anti-IgM& anti-CD40 (5 μ g/mL) and then treated with MSA-2 (5 μ g/mL), MSA-2 (5 μ g/mL) plus IPI-549 (2 μ M), MSA-2 (5 μ g/mL) plus SH-273 (2 μ M) in B cells from PBMCs of human PDAC patients and FIG. 12E shows the same from a healthy donor. FIGS. 12F-12G show quantitation of flow cytometry analysis of IL35⁺ and IL10⁺ Breg cells from KPC B cells (FIG. 12F) or STING knock out B cells (**FIG. 12G**) after treatments with MSA-2 (5 μ g/mL), IPI-549 (5 μ M), MSA-2 (5 μ g/mL) plus IPI-549 (5 μ M) or SH-273 (5 μ M). The data represents two or three independent experiments with mean $\pm$ SD, and *n* = 3 (FIGS. 12A, 12D-12G). Statistical comparisons are based on one-way analysis of variance (FIGS. 12D-12G). *P* values are indicated.

[0041] FIGS. 13A-13I show Nano-273 extended median survival to 201 days in KPC PDAC mice via activating systemic immunity without exhibiting toxicity. FIG. 13A shows antitumor efficacy of Nano-273 in transgenic KPC (LSL-KrasG12D, LSL-Trp53R172H/+, Pdx1cre/+) PDAC mice in comparison with different treatments. Survival rate of KPC mice after treatment with PBS (control), anti-PD1 antibody (i.p., 100 μ g), Paclitaxel in albumin nano formulation (intravenous injection, 11.7 μ mol/kg) plus anti-PD1 antibody (i.p., 100 μ g), MSA-2 (i.v. 34.0 μ mol/kg) plus anti-PD1 antibody (i.p., 100 μ g), and Nano-273 (i.v. 17.6 μ mol/kg) plus anti-PD1 antibody (i.p., 100 μ g). FIG. 13B shows pathological staining of lung tissues from FIG. 13A to evaluate pancreatic cancer metastasis or invasion. Blue arrows and circles indicated metastasis or invasion foci. FIG. 13C shows antitumor efficacy in C57BL/6 mice inoculated with KPC 6422 tumor after different treatments. Average tumor volume after treatment with PBS (control), MSA-2 (i.v. 34.0 μ mol/kg) with or without anti-PD1 antibody (i.p., 100 μ g), Nano-273 (i.v. 17.6 μ mol/kg) with or without anti-PD1 antibody (i.p., 100 μ g).

FIGS. 13D-13G show dendritic cells (DC) activation and macrophage polarization in KPC transgenic mice treated with anti-PD1 antibody (i.p., 100 μ g), MSA-2 (i.v. 34.0 μ mol/kg) with anti-PD1 antibody (i.p., 100 μ g), Nano-273 (i.v. 17.6 μ mol/kg) with anti-PD1 antibody (i.p., 100 μ g) for three doses. The lymph nodes were harvested to analysis the dendritic cells activation (FIG. 13D) and M1 and M2 macrophages ratios (FIG. 13E). cytokines levels in serum of KPC after different treatment in FIG. 13D FIGS. 13F-13G show quantitation of flow cytometry analysis of dendritic cells activation, as measured by CD86 intensity after the treatment in FIG. 13D. Quantification and representative flow cytometry analysis of M1 macrophage ratios from lymph node of KPC transgenic mice after the treatment (FIG. 13E). The serum concentration of IFN β and IFN α (FIG. 13F), IL35 and IL10 (FIG. 13G) of KPC mice after various treatments in FIG. 13D. FIG. 13H-13I show systemic antitumor efficacy and immune response of Nano-273 in xenograft model (with KPC 6422 cells) by different administration routes. C57BL/6 mice inoculated with two KPC 6422 tumors. Local tumor is referred to the tumor with intra-tumor injection or near s.c. injection site. Distal tumor is referred to no intra-tumor injection or far from s.c. injection site. Mice were treated with PBS (control) and Nano-273 (17.6 μ mol/kg) after intratumorally (i.t), subcutaneous (s.c.) or intravenous (i.v.) injection, in combined with anti-PD1 antibody (i.p., 100 μ g) (FIG. 13H). FIG. 13I shows quantitation and representative flow cytometry analysis of M1 macrophage ratios at distal tumor and lymph node after treatments from FIG. 9B. The data represents two or three independent experiments with mean $\pm$ SD, and n = 10 (FIGS. 13A, 13C), n = 3 (FIGS. 13D, 13E, 13I), n = 4 (FIGS. 13F-13G). Statistical comparisons are based on log-rank (Mantel–Cox) test (FIGS. 13A), one-way analysis of variance (FIGS. 13D-13G, 13I) or two-way analysis of variance (FIG. 13C). *P* values are indicated.

[0042] FIGS. 14A-14I show Nano-273 eliminated STING-induced Breg cells expansion and remodeled the immune microenvironment in tumor and lymph nodes for systemic anticancer immunity. FIG. 14A shows the quantitation of tumor immune infiltration by flow cytometry from the residual tumors of the mice at 10 days after final dose. C57BL/6 mice inoculated with KPC 6422 tumor were treated with PBS (control), MSA-2 (i.v. 34.0 μ mol/kg) plus anti-PD1 antibody (i.p., 100 μ g), SH-273 (i.v. 17.6 μ mol/kg) plus anti-PD1 antibody (i.p., 100 μ g) and Nano-273 (i.v. 17.6 μ mol/kg) plus anti-PD1 antibody (i.p., 100 μ g). FIG. 14B shows different immune cell populations among tumor infiltrating immune cells by single cell RNA-seq analysis from anti-tumor efficacy study in mice at 9 days after final dose in FIG. 14A. FIG. 14C shows the percentage of different subpopulations of B cells among total B cells by single cell RNA-seq analysis. FIG. 14D shows t-distributed Stochastic

Neighbor Embedding (t-SNE) plots of B cells by single cell RNA-seq analysis from mice following the treatment in FIG. 14A. FIG. 14E shows the quantitation of flow cytometry analysis of IL35⁺ and IL10⁺ Breg cells at tumor and lymph node in mice from anti-tumor efficacy study at FIG. 13C. FIG. 13F shows antitumor efficacy in C57BL/6 mice inoculated with LL/2 tumor with treatments of MSA-2 (i.v. 34.0 μ mol/kg) plus anti-PD1 antibody (i.p., 100 μ g), Nano-273 (i.v. 17.6 μ mol/kg), or Nano-273 (i.v. 17.6 μ mol/kg) plus anti-PD1 antibody (i.p., 100 μ g). FIG. 14G shows the quantitation of flow cytometry analysis of IL35⁺ Bregs at lymph node and tumor from FIG. 14F. FIG. 14H shows the antitumor efficacy in C57BL/6 mice with or without NK cell depletion inoculated with KPC 6422 tumor with Nano-273 (i.v. 17.6 μ mol/kg) plus anti-PD1 antibody (i.p., 100 μ g) treatment. FIG. 14I shows the quantitation of flow cytometry analysis of GzmB⁺ NK cells in lymph node from KPC transgenic mice after treatments with anti-PD1 antibody (i.p., 100 μ g), MSA-2 (i.v. 34.0 μ mol/kg) plus anti-PD1 antibody (i.p., 100 μ g), or Nano-273 (i.v. 17.6 μ mol/kg) plus anti-PD1 antibody (i.p., 100 μ g). The data represents two or three independent experiments with mean $\pm$ SD, and n = 3 (FIGS. 14A, 14G, 14I), n = 4 (FIG. 14E), n = 8 (FIG. 14F), and n = 10 (FIG. 14H). Statistical comparisons are based on one-way analysis of variance (FIGS. 14A, 14E, 14G, 14I) or two-way analysis of variance (FIGS. 14F, 14H). *P* values are indicated.

[0043] FIG. 15 shows representative flow cytometry analysis of IL35⁺ and IL10⁺ Breg cells in lymph node and tumor from mice inoculated with KPC 6422 cell after treatment with PBS (control), anti-PD1 antibody (100 μ g, i.p.), diABZi (i.t., 20 μ g/mouse or i.v., 1.5 mg/kg) with or without anti-PD1 antibody (100 μ g, i.p.) or diABZi (i.v., 1.5 mg/kg) with or without anti-PD1 antibody (100 μ g, i.p.). The data represents two or three independent experiments with mean $\pm$ SD, and n = 4. Statistical comparisons are based on one-way analysis of variance. *P* values are indicated.

[0044] FIGS. 16A-16F: FIG. 16A shows pathway enrichment analysis BMDCs treated with or without MSA-2 (5 μ g/mL). FIGS. 16B and 16C show Gene expected count of Pik3cg (p110 γ), Pik3r5 (p101) and Pik3r6 (p87) in splenic B cells (FIG. 16B) and in BMDC (FIG. 16C) from KPC mice after treatment with or without treatment of MSA-2 (5 μ g/ml). FIG. 16D is western blot analysis of Pik3cg (p110 γ), Pik3r5 (p101) and Pik3r6 (p87), phosphorylated IRF3 and GADPH expression in splenic B cells from KPC transgenic mice treated with or without MSA-2 (5 μ g/mL). FIGS. 16E and 16F Gene expected count of Pik3cd (p110 β), Pik3r1 (p85 α) and Pik3r2 (p85 β) in splenic B cells (FIG. 16E) and BMDC (FIG. 16F) from KPC mice treated with or without MSA-2 (5 μ g/ml). The data represents

two or three independent experiments with mean $\pm$ SD, and $n = 3$. Statistical comparisons are based on one-way analysis of variance. P values are indicated.

[0045] FIGS. 17A-17B show Western blot analysis of PI3K γ (p110 γ), PI3K δ (p110 δ), and STING expression in lymph node tissues and pancreatic tumor whole tissues (including tumor immune infiltrated cells) from KPC transgenic mice and lymph node and pancreas from C57BL/6 mice (FIG. 17A) and B cell, BMDCs, and BMDMs (FIG. 17B) from KPC transgenic mice and C57BL/6 mice.

[0046] FIGS. 18A-18C: FIG. 18A shows western blot of phosphorylation of IRF3 at THP-1 wild type cells after treatments with MSA-2 (5 μ g/ml) or combination of MSA-2 (5 μ g/ml) with different inhibitors including PI3K α inhibitor Alpelisib (1 μ M), PI3K β inhibitor TGX-211 (1 μ M), PI3K δ inhibitor TGR-1202 (1 μ M), PI3K γ inhibitor IPI-549 (5 μ M). FIG. 18B shows phosphorylation of IRF3 at B cells from KPC transgenic mice after treatments with MSA-2 (5 μ g/ml) or combination of MSA-2 (5 μ g/ml) with IPI-549 (0.5 – 10 μ M). FIG. 18C shows phosphorylation of IRF3 at Human CD4 T cells, Human CD8 T cells and Human NK cells after treatments with MSA-2 (5 μ g/ml) or combination of MSA-2 (10 μ g/ml) with IPI-549 (10 μ M) or SH-273 (10 μ M).

[0047] FIG. 19 shows western blot of phosphorylation of IRF3 in B cells, CD4 T cells, CD8 T cells, bone marrow derived dendritic cells and bone marrow derived macrophages after treatments with MSA-2 (5 μ g/ml) or MSA-2 (5 μ g/ml) & IPI-549 (5 μ M) at different time points (0.5 – 12 hrs).

[0048] FIGS. 20A-20D: FIG. 20A shows the gating strategy for monitoring IL35 $^{+}$ or IL10 $^{+}$ Breg cells. FIG. 20B shows flow cytometry analysis of IL35 $^{+}$ and IL10 $^{+}$ Breg cells after pretreated with anti-IgM& anti-CD40 (5 μ g/mL) and then treated with MSA-2 (5 μ g/ml) or MSA-2 (5 μ g/ml) and IPI-549 (2 μ M) in B cells from PBMCs of healthy donors. FIGS. 20C-20D show flow cytometry analysis of IL35 $^{+}$ and IL10 $^{+}$ Breg cells after pretreated with anti-IgM& anti-CD40 (5 μ g/mL) and then treated with MSA-2 (5 μ g/ml) or MSA-2 (5 μ g/ml) and IPI-549 (2 μ M), or MSA-2 (5 μ g/ml) and TGR-1202 (1 μ M), or MSA-2 (5 μ g/ml) and SH-273 (5 μ M) in B cells from KPC transgenic mice splenocytes (FIG. 20C) and STING knock out mice (FIG. 20D). The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$. Statistical comparisons are based on one-way analysis of variance. P values are indicated.

[0049] FIGS. 21A-21B show flow cytometry analysis of IL35 $^{+}$ and IL10 $^{+}$ Breg cells treated with MSA-2 (5 μ g/ml), or IPI-549 (5 μ M), or MSA-2 (5 μ g/ml) and IPI-549 (5 μ M) in B cells from KPC transgenic mice splenocytes (FIG. 21A) and STING knock out mice

(FIG. 21B). The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$. Statistical comparisons are based on one-way analysis of variance. *P* values are indicated.

[0050] FIGS. 22A-22B show the quantification and representative flow cytometry analysis of IL35⁺ and IL10⁺ BMDCs (FIG. 22A) and BMDMs (from KPC mice) (FIG. 22B) after treatments with or without MSA-2 (5 μ g/ml), with or without IPI-549 (5 μ M). The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$. Statistical comparisons are based on one-way analysis of variance. *P* values are indicated.

[0051] FIGS. 23A-23B show the quantification and representative flow cytometry analysis of IL35⁺ and IL10⁺ THP-1 wild type (FIG. 23A) and THP-1R232107 (FIG. 23B) cells after treatments with or without MSA-2 (5 μ g/ml), with or without IPI-549 (5 μ M). The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$. Statistical comparisons are based on one-way analysis of variance. *P* values are indicated.

[0052] FIGS. 24A-24B: FIG. 24A is the quantification and representative flow cytometry analysis of CD80 intensity of BMDC after treatments with or without MSA-2 (2.95 μ g/mL), with or without IPI-549 (10 μ M). FIG. 24B is representative flow cytometry analysis of CD80 intensity of BMDMs after treatments with or without MSA-2 (2.95 μ g/mL), with or without IPI-549 (10 μ M). The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$. Statistical comparisons are based on one-way analysis of variance. *P* values are indicated.

[0053] FIGS. 25A-25C: FIG. 25A shows the phosphorylation of AKT (473) at BMDMs after treatments with different concentrations of TGR-1202 (1 μ M, 150 nM, from left to right lane), SH-273 (2 μ M, 400 nM, from left to right lane) or IPI-549 (1 μ M, 150 nM, from left to right lane) in the presence of SDF-1 α for 1.5 hours. FIG. 25B shows the phosphorylation of AKT (473) at BMDC or BMDM after treatments with different concentrations of IPI-549 (1 μ M, 150 nM, or 20 nM, from left to right lane) or SH-273 (2 μ M, 400 nM, or 40 nM, from left to right lane) in the presence of SDF-1 α for 1.5 hours. FIG. 25C shows the phosphorylation of AKT (473) and AKT (308) at SK-OV-3 or 786-O cells after treatments with Alpelisib (1 μ M, SK-OV-3 cells, PI3K α), TGX-211 (1 μ M, 786-O cells, PI3K β), or SH-273 (2 μ M) in the presence of SDF-1 α for 1.5 hours.

[0054] FIGS. 26A-26B: FIG. 26A shows the cellular IC₅₀ of STING activation after MSA-2 or ZSA-52 (released active moiety of SH273 to activate STING) treatments in THP-1-Blue™ ISG cells. FIG. 26B shows the phosphorylation of IRF3 in BMDCs from KPC transgenic mice after treatments with MSA-2 (2.95 μ g/mL), IPI-549 (10 μ M) or SH-273 (10

μM), respectively. The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$.

[0055] FIGS. 27A-27C: FIG. 27A shows the phosphorylation of IRF3 at B cells from KPC transgenic mice after treatments with MSA-2 (5 $\mu\text{g}/\text{ml}$) or combination of MSA-2 (5 $\mu\text{g}/\text{ml}$) with SH-273 (0.5 – 10 μM). FIGS. 27B-27C show flow cytometry analysis of IL35⁺ and IL10⁺ Breg cells after pretreated with anti-IgM&anti-CD40 (5 $\mu\text{g}/\text{mL}$) and then treated with MSA-2 (5 $\mu\text{g}/\text{ml}$) or MSA-2 (5 $\mu\text{g}/\text{ml}$) and IPI-549 (2 μM), or MSA-2 (5 $\mu\text{g}/\text{ml}$) and TGR-1202 (1 μM), or MSA-2 (5 $\mu\text{g}/\text{ml}$) and SH-273 (2 μM) in B cells from PBMCs of PDAC patients (FIG. 27B) and healthy donor (FIG. 27C). The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$. Statistical comparisons are based on one-way analysis of variance. *P* values are indicated.

[0056] FIG. 28 shows the pharmacokinetics and tissue distribution of Nano-273, SH-273, DMA01-148 (released active moiety of SH273 to inhibit PI3K) and ZSA-52 (released active moiety of SH273 to activate STING) concentrations in plasma, pancreatic tumor, lymph nodes, and liver after intravenous injection of SH-273 (17.6 $\mu\text{mol}/\text{kg}$) and Nano-273 (17.6 $\mu\text{mol}/\text{kg}$) in KPC transgenic mice. The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$. Statistical comparisons are based on two-way analysis of variance. *P* values are indicated.

[0057] FIG. 29 is confocal images of albumin nanoformulation and solvent based formulation of dual functional compound and fluorescent dye DID (magenta) or solvent based dual functional compound and DID in pancreatic tumor organoid derived from KPC mice. Scale bar is 100 μm .

[0058] FIG. 30 is confocal images of albumin nanoformulation and solvent based formulation in the lymph nodes from KPC mice. Lymph node samples were collected 5 hours after intravenous injection of albumin nanoformulation of dual functional compound and fluorescent dye PTX-OG488 (red) or solvent based dual functional compound and PTX-OG488 (red) in KPC mice. The macrophages, B cell and T cell were stained with F4/80 (white), B220 (blue), and CD3 (green)

[0059] FIGS. 31A-31D: FIG. 31A shows antitumor efficacy in transgenic KPC (LSL-KrasG12D, LSL-Trp53R172H, Pdx1cre/+) PDAC mice with different treatments. Survival rate of KPC mice after treatment with PBS (control), anti-PD1 antibody (100 μg , i.p.), Paclitaxel in albumin nano formulation (intravenous injection, 11.7 $\mu\text{mol}/\text{kg}$) plus anti-PD1 antibody (100 μg , i.p.), Paclitaxel in albumin nanoformulation (intravenous injection, 11.7 $\mu\text{mol}/\text{kg}$) and IPI-549 (intraperitoneal injection, 28.4 $\mu\text{mol}/\text{kg}$) plus anti-PD1 antibody (100

μg, i.p.), MSA-2 (intravenous injection, 34.0 μmol/kg) plus anti-PD1 antibody (100 μg, i.p.), MSA-2 (oral dose, 204 μmol/kg) and IPI-549 (oral dose, 28.4 μmol/kg) plus anti-PD1 antibody (100 μg, i.p.), SH-273 (intravenous injection, 17.6 μmol/kg) plus anti-PD1 antibody (100 μg, i.p.), and Nano-273 (intravenous injection, 17.6 μmol/kg) plus anti-PD1 antibody (100 μg, i.p.). n = 10 for each group. FIG. 31B shows antitumor efficacy in immunodeficient (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ, NSG) mice inoculated with KPC 6422 tumor with or without Nano-273 (i.v. 17.6 μmol/kg) with anti-PD1 antibody (100 μg, i.p.). FIGS. 31C-31D show the serum concentration of IL35, IL10 and IL27 (FIG. 31C) and IFNβ and IFNα (FIG. 31D) of C57BL/6 mice inoculated with KPC 6422 tumor after treatment with PBS (control), MSA-2 (i.v. 34.0 μmol/kg) with or without anti-PD1 antibody (100 μg, i.p.), Nano-273 (i.v. 17.6 μmol/kg) with or without anti-PD1 antibody (100 μg, i.p.). The data represents two or three independent experiments with mean ± SD, and n = 10 (FIGS. 31A-31B), and n = 4 (FIGS. 31C-31D). Statistical comparisons are based on log-rank (Mantel–Cox) test (FIG. 31A) or two-way analysis of variance (FIGS. 31B-31D). *P* values are indicated.

[0060] FIG. 32 is representative flow cytometry analysis of IL35+ and IL10+ Breg cells at tumor and lymph node in mice from anti-tumor efficacy study in FIG. 6D. The data represents two or three independent experiments with mean ± SD, and n = 3. Statistical comparisons are based on two-way analysis of variance. *P* values are indicated.

[0061] FIGS. 33A-33B show antitumor efficacy for pancreatic cancer (KPC 6422 cell line) with Nano-273 treatment in different administration routes, as indicated. Individual tumor volume after treatment with PBS (control) and Nano-273 (17.6 μmol/kg intratumorally, subcutaneously and intravenously injection, respectively) plus anti-PD1 antibody (100 μg, i.p.). n = 5 mice in each group.

[0062] FIGS. 34A-34E: FIGS. 34A-34B show antitumor efficacy for pancreatic cancer (KPC 6422 cell line) with Nano-273 treatment in subcutaneously injection. Individual tumor volume after treatment with or without Fingolimod (FTY720, 5mg/kg, oral, daily), or with PBS (control), Nano-273 (17.6 μmol/kg subcutaneously and intravenously injection, respectively) plus anti-PD1 antibody (100 μg, i.p.). FIGS. 34C-34E show representative flow cytometry analysis of tumor immune infiltration (FIG. 34C) and M1 macrophage ratios in uninvolved lymph node (FIG. 34D) and distal tumor (FIG. 34E) after treatments from efficacy study from FIG. 9B and 34A. The data represents two or three independent experiments with mean ± SD, and n = 5 (FIGS. 34A-34B), and n = 3 (FIGS. 34C-34E). Statistical comparisons are based on one-way analysis of variance (FIGS. 34C-34E) or two-way analysis of variance (FIG. 34A). *P* values are indicated.

[0063] FIGS. 35A-35B show liver enzymes (FIG. 35A), kidney function (FIG. 35A), and complete blood counts (CBCs) (FIG. 35B) in CD1 mice after treatments with SH-273 and Nano-273 every 3 days for 5 doses (17.6 $\mu\text{mol/kg}$) through intravenous injection. The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$. Alanine aminotransferase, ALT; aspartate aminotransferase, AST; Alkaline phosphatase, ALKP; total bilirubin, TBIL; creatine, CREA; blood urine nitrogen, BUN; albumin, ALB. White blood cells, WBC; neutrophils, NEU; lymphocytes, LYM; monocytes, MONO; eosinophils, EOS; basophils, BAS; red blood cells, RBC; hemoglobin, HGB; hematocrit, HCT; mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH; mean corpuscular hemoglobin concentration, MCHC; red cell distribution width, RDW; platelets, PLT; mean platelet volume, MPV

[0064] FIG. 36 shows histology of major organs in CD1 mice after treatments with SH-273 or Nano-273 every 3 days for 5 doses (17.6 $\mu\text{mol/kg}$) through intravenous injection.

[0065] FIGS. 37A-37E: FIG. 37A is body weight measurements of C57BL/6 mice after a single intravenous dose of Nano-273 (200 mg/kg). FIGS. 37B-37D shows body weight measurements of Es1^{-/-} mice (B6.Cg-Ces1c^{tm1.1Loc/J}) after a single intravenous dose of Nano-273 (200 mg/kg, FIG. 37B), Nano-273 (200 mg/kg, FIG. 37C) or Nano-273 (200 mg/kg, FIG. 37D). FIGS. 37E shows body weight measurements of Es1^{-/-} mice (B6.Cg-Ces1c^{tm1.1Loc/J}) after multiple intravenous doses of Nano-273. In the multiple-dose study, Nano-273 was administered intravenously at escalating doses of 15, 50, and 100 mg/kg every three days for 28 days. The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$.

[0066] FIG. 38 shows Complete blood cell count (CBC) after multiple doses of Nano-273 by IV administration (15-100 mg/kg in Es1^{-/-} mice (B6.Cg-Ces1c^{tm1.1Loc/J}) In the multiple-dose study, Nano-273 was administered intravenously at escalating doses of 15, 50, and 100 mg/kg every three days for 28 days. The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$. White blood cells, WBC; monocytes, MONO; lymphocytes, LYM; eosinophils, EOS; basophils, BAS; neutrophils, NEU; red blood cells, RBC; mean corpuscular volume, MCV; hematocrit, HCT; red cell distribution width, RDW; hemoglobin, HGB; mean corpuscular hemoglobin, MCH; mean corpuscular hemoglobin concentration, MCHC; platelets, PLT; mean platelet volume, MPV.

[0067] FIG. 39 shows Blood chemistry after multiple intravenous doses of Nano-273 (15-100 mg/kg) in Es1^{-/-} mice (B6.Cg-Ces1c^{tm1.1Loc/J}) In the multiple-dose study, Nano-273 was administered intravenously at escalating doses of 15, 50, and 100 mg/kg every three days for

28 days. The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$. Alanine aminotransferase, ALT; total bilirubin, TBIL; Alkaline phosphatase, ALKP; creatine, CREA; blood urine nitrogen, BUN; total protein, TBRO; albumin, ALB; glucose, GLUC; calcium, CA.

[0068] FIG. 40 is histology of major organs after multiple intravenous doses of Nano-273 in *Es1*^{-/-} mice (B6.Cg-Ces1^{tm1.1Loc/J}). In the multiple-dose study, Nano-273 was administered intravenously at escalating doses of 100 mg/kg every three days for 28 days.

[0069] FIGS. 41A-41C: FIG. 41A is flow cytometry analysis of IL35⁺ or IL10⁺ Bregs in the lymph node and spleen from KPC transgenic mice after treatments with PBS (control), anti-PD1 antibody (100 μ g, i.p.), MSA-2 (i.v. 34.0 μ mol/kg) plus anti-PD1 antibody (100 μ g, i.p.), or Nano-273 (i.v. 17.6 μ mol/kg) plus anti-PD1 antibody (100 μ g, i.p.). FIG. 41B is individual plots for antitumor efficacy in C57BL/6 mice inoculated with LL/2 tumor with treatments of MSA-2 (i.v. 34.0 μ mol/kg) plus anti-PD1 antibody (100 μ g, i.p.), Nano-273 (i.v. 17.6 μ mol/kg), or Nano-273 (i.v. 17.6 μ mol/kg) plus anti-PD1 antibody (100 μ g, i.p.). FIG. 41C is flow cytometry analysis of IL10+326 Bregs at lymph node and tumor from FIG. 41B. The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$ (FIGS. 41A, 41C), and $n = 8$ (FIG. 41B). Statistical comparisons are based on one-way analysis of variance. *P* values are indicated.

[0070] FIGS. 42A-42D: FIGS. 42A shows the antitumor efficacy in C57BL/6 mice inoculated with MC-38 tumor with treatments of MSA-2 (i.v. 34.0 μ mol/kg) plus anti-PD1 antibody (100 μ g, i.p.), Nano-273 (i.v. 17.6 μ mol/kg), or Nano-273 (i.v. 17.6 μ mol/kg) plus anti-PD1 antibody (100 μ g, i.p.). FIGS. 42B-42C show flow cytometry analysis of IL35⁺ and IL10+334 Bregs at lymph node (FIG. 42B) and tumor (FIGS. 42C from FIG. 42A). FIG. 42D shows the antitumor efficacy in C57BL/6 mice with or without CD8 T cell depletion inoculated with KPC 6422 tumor with Nano-273 (i.v. 17.6 μ mol/kg) plus anti-PD1 antibody (100 μ g, i.p.) treatment. The data represents two or three independent experiments with mean $\pm$ SD, and $n = 3$ (FIGS. 42B-42C), $n = 8$ (FIGS. 42A), and $n = 10$ (FIG. 42D). Statistical comparisons are based on one-way analysis of variance (FIGS. 42B-42C) and two-way analysis of variance (FIGS. 42BA, 42D). *P* values are indicated.

DETAILED DESCRIPTION

[0071] Described herein are dual functioning compounds comprising a stimulator of interferon (IFN) genes (STING) agonist moiety and a phosphatidylinositol 3-kinase (PI3K) inhibitor moiety, and compositions and formulations thereof. One portion of the disclosed

molecules acts as a STING agonist to target the cGAS-STING pathway, which is a cytosolic DNA-sensing pathway that drives activation of Type I IFN and other inflammatory cytokines. The other moiety of the dual functioning compounds acts as a PI3K inhibitor, such that the dual functioning compounds stimulate the immune response.

[0072] One exemplary dual functional compound, SH-273, is shown to stimulate STING and inhibit PI3K γ , preserving STING function to activate myeloid cells and eliminates Breg cells to overcome STING resistance. An albumin nanoformulation of SH-273 (Nano-273) was prepared for intravenous administration to decrease Breg cells and remodel microenvironment in tumors and lymph nodes, not attainable through local intratumorally injection of STING agonists. Nano-273 (15 mg/kg) extended median survival to 201 days in transgenic pancreatic cancer mouse model (KPC, KrasG12D, P53R172H, Pdx1-Cre), and showed excellent efficacy in xenograft models with pancreatic cancer, lung cancer, and colon cancer. Nano-273 is a candidate for pancreatic cancer immunotherapy, acting to eliminate Breg cells to overcome STING resistance and counteract suppressive microenvironment in both tumors and lymph nodes.

[0073] Section headings as used in this section and the entire disclosure herein are merely for organizational purposes and are not intended to be limiting.

1. Definitions

[0074] The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms “a,” “and” and “the” include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments “comprising,” “consisting of,” and “consisting essentially of,” the embodiments or elements presented herein, whether explicitly set forth or not.

[0075] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0076] Unless otherwise defined herein, scientific, and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear; in the event, however of any latent ambiguity, definitions provided herein take precedent over any

dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety.

[0077] As used herein, “treat,” “treating,” and the like means a slowing, stopping, or reversing of progression of a disease or disorder when provided a compound or composition described herein to an appropriate control subject. The term also means a reversing of the progression of such a disease or disorder to a point of eliminating or greatly reducing the symptoms. As such, “treating” means an application or administration of the compositions described herein to a subject, where the subject has a disease or a symptom of a disease, where the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease or symptoms of the disease.

[0078] A “subject” or “patient” may be human or non-human and may include, for example, animal strains or species used as “model systems” for research purposes, such a mouse model as described herein. Likewise, patient may include either adults or juveniles (e.g., children). Moreover, patient may mean any living organism, preferably a mammal (e.g., humans and non-humans) that may benefit from the administration of compositions contemplated herein. Examples of mammals include, but are not limited to, any member of the Mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. Examples of non-mammals include, but are not limited to, birds, fish, and the like. In one embodiment, the mammal is a human.

[0079] As used herein, the terms “providing,” “administering,” and “introducing,” are used interchangeably herein and refer to the placement of the compounds or compositions of the disclosure into a subject by a method or route which results in at least partial localization of the compounds or composition to a desired site. The compounds or compositions can be administered by any appropriate route which results in delivery to a desired location in the subject.

[0080] The term “vaccine,” as used herein, refers to any pharmaceutical composition containing at least one antigenic or immunogenic peptide or other immunogen or at least one nucleic acid encoding at least one antigenic or immunogenic peptide or other immunogen, which can be used to prevent or treat a disease or condition in a subject.

[0081] The term “immunization,” as used herein, refers to a process that increases an organisms' reaction to antigen and therefore improves its ability to resist or overcome infection.

[0082] “Polynucleotide” or “oligonucleotide” or “nucleic acid,” as used herein, means at least two nucleotides covalently linked together. The polynucleotide may be DNA, both genomic and cDNA, RNA, or a hybrid, where the polynucleotide may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. The nucleic acid, whether DNA or RNA may comprise non-natural nucleotides, modified nucleotides, and/or non- nucleotide building blocks that can exhibit the same function as natural nucleotides (e.g., “nucleotide analogs”). Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods. Polynucleotides may be single- or double-stranded or may contain portions of both double stranded and single stranded sequence. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof.

[0083] A “peptide” or “polypeptide” is a linked sequence of two or more amino acids linked by peptide bonds. Peptides and polypeptides include proteins such as binding proteins, receptors, and antibodies. The terms “polypeptide” and “protein,” are used interchangeably herein.

[0084] As used herein, “nucleic acid” or “nucleic acid sequence” refers to a polymer or oligomer of pyrimidine and/or purine bases, preferably cytosine, thymine, and uracil, and adenine and guanine, respectively (See Albert L. Lehninger, Principles of Biochemistry, at 793-800 (Worth Pub. 1982)). The present technology contemplates any deoxyribonucleotide, ribonucleotide, or peptide nucleic acid component, and any chemical variants thereof, such as methylated, hydroxymethylated, or glycosylated forms of these bases, and the like. The polymers or oligomers may be heterogenous or homogenous in composition and may be isolated from naturally occurring sources or may be artificially or synthetically produced. In addition, the nucleic acids may be DNA or RNA, or a mixture thereof, and may exist permanently or transitionally in single-stranded or double-stranded form, including homoduplex, heteroduplex, and hybrid states. In some embodiments, a nucleic acid or nucleic acid sequence comprises other kinds of nucleic acid structures such as, for instance, a DNA/RNA helix, peptide nucleic acid (PNA), morpholino nucleic acid (see, e.g., Braasch

and Corey, *Biochemistry*, 41(14): 4503-4510 (2002)) and U.S. Pat. No. 5,034,506), locked nucleic acid (LNA; see Wahlestedt et al., *Proc. Natl. Acad. Sci. U.S.A.*, 97: 5633-5638 (2000)), cyclohexenyl nucleic acids (see Wang, *J. Am. Chem. Soc.*, 122: 8595-8602 (2000)), and/or a ribozyme. Hence, the term “nucleic acid” or “nucleic acid sequence” may also encompass a chain comprising non-natural nucleotides, modified nucleotides, and/or non-nucleotide building blocks that can exhibit the same function as natural nucleotides (e.g., “nucleotide analogs”); further, the term “nucleic acid sequence” as used herein refers to an oligonucleotide, nucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin, which may be single or double-stranded, and represent the sense or antisense strand. The terms “nucleic acid,” “polynucleotide,” “nucleotide sequence,” and “oligonucleotide” are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof.

[0085] Definitions of specific functional groups and chemical terms are described in more detail below. For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics*, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in Sorrell, *Organic Chemistry*, 2nd edition, University Science Books, Sausalito, 2006; Smith, *March's Advanced Organic Chemistry: Reactions, Mechanism, and Structure*, 7th Edition, John Wiley & Sons, Inc., New York, 2013; Larock, *Comprehensive Organic Transformations*, 3rd Edition, John Wiley & Sons, Inc., New York, 2018; and Carruthers, *Some Modern Methods of Organic Synthesis*, 3rd Edition, Cambridge University Press, Cambridge, 1987; the entire contents of each of which are incorporated herein by reference.

[0086] The term “alkyl,” as used herein, means a straight or branched, saturated hydrocarbon chain. Representative examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, 4,4-dimethylpentan-2-yl, n-heptyl, n-octyl, n-nonyl, n-decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl and icosyl.

[0087] The term “alkylene,” as used herein, refers to a divalent group derived from a straight or branched, saturated hydrocarbon chain. Representative examples of alkylene include, but are not limited to, -CH₂-, -CH₂CH₂-, -CH(CH₃)-, -CH₂CH₂CH₂-, -CH₂CH(CH₃)-,

-CH₂CH₂CH₂CH₂-, -CH₂CH(CH₃)CH₂-, -CH₂CH₂CH(CH₃)-, -CH₂CH₂CH₂CH₂CH₂-, -CH₂CH(CH₃)CH₂CH₂-, -CH(CH₃)CH₂CH₂CH₂-, -CH₂CH₂CH₂CH₂CH₂CH₂-, -CH₂CH₂CH(CH₃)CH₂CH₂-, -CH₂CH(CH₃)CH₂CH₂CH₂-, and -CH(CH₃)CH₂CH₂CH₂CH₂-.

[0088] The term “alkenyl,” as used herein, means a straight or branched hydrocarbon chain containing at least one carbon-carbon double bond. The double bond(s) may be located at any positions within the hydrocarbon chain. Representative examples of alkenyl include, but are not limited to, ethenyl, 2-propenyl, 2-methyl-2-propenyl, 3-butenyl, 4-pentenyl, 5-hexenyl, 2-heptenyl, 2-methyl-1-heptenyl, and 3-decenyl.

[0089] The term “alkynyl,” as used herein, means a straight or branched hydrocarbon chain containing at least one carbon-carbon triple bond. The triple bond(s) may be located at any positions with the hydrocarbon chain. Representative examples of alkynyl include, but are not limited to, ethynyl, propynyl, and butynyl.

[0090] The term “alkoxy,” as used herein, refers to an alkyl group, as defined herein, appended to the parent molecular moiety through an oxygen atom. Representative examples of alkoxy include, but are not limited to, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, and *tert*-butoxy.

[0091] The term “amino,” as used herein, refers to an -NH₂ group. The term “alkylamino,” as used herein, refers to a group -NHR, wherein R is an alkyl group as defined herein. The term “dialkylamino,” as used herein, refers to a group -NR₂, wherein each R is independently an alkyl group as defined herein.

[0092] As used herein, the term “aryl” refers to a radical of a monocyclic, bicyclic, or tricyclic 4n+2 aromatic ring system (e.g., having 6, 10, or 14 π electrons shared in a cyclic array) having 6-14 ring carbon atoms and zero heteroatoms (“C₆-C₁₄ aryl”). In some embodiments, an aryl group has six ring carbon atoms (“C₆ aryl,” i.e., phenyl). In some embodiments, an aryl group has ten ring carbon atoms (“C₁₀ aryl,” e.g., naphthyl such as 1-naphthyl and 2-naphthyl). In some embodiments, an aryl group has fourteen ring carbon atoms (“C₁₄ aryl,” e.g., anthracenyl and phenanthrenyl).

[0093] As used herein, the term “arylene” refers to a divalent aryl radical.

[0094] The term “cycloalkyl,” as used herein, refers to a saturated carbocyclic ring system containing three to ten carbon atoms and zero heteroatoms. The cycloalkyl may be monocyclic, bicyclic, bridged, fused, or spirocyclic. Representative examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, adamantyl, bicyclo[2.2.1]heptanyl, bicyclo[3.2.1]octanyl, and bicyclo[5.2.0]nonanyl.

[0095] The term “cycloalkylene,” as used herein, refers to a divalent cycloalkyl group.

[0096] The term “halogen” or “halo,” as used herein, means F, Cl, Br, or I.

[0097] The term “haloalkyl,” as used herein, means an alkyl group, as defined herein, in which at least one hydrogen atom (e.g., one, two, three, four, five, six, seven or eight hydrogen atoms) is replaced with a halogen. In some embodiments, each hydrogen atom of the alkyl group is replaced with a halogen. Representative examples of haloalkyl include, but are not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, 2-fluoroethyl, 2,2,2-trifluoroethyl, and 3,3,3-trifluoropropyl.

[0098] The term “heteroalkyl,” as used herein, means an alkyl group, as defined herein, in which one or more of the carbon atoms (and any associated hydrogen atoms) are each independently replaced with a heteroatom group such as -NH-, -O-, -S-, -S(O)-, -S(O)₂-, -O-P(O)(O)O-, or the like. By way of example, 1, 2, 3, 4, 5, 6, or more carbon atoms may be independently replaced with the same or different heteroatom group. A heteroalkyl group can also include one or more carbonyl moieties (i.e., wherein a carbon atom of the alkyl group is oxidized to a -C(O)- group).

[0099] The term “heteroalkenyl,” as used herein, refers to an alkenyl group, as defined herein, in which one or more of the carbon atoms (and any associated hydrogen atoms) are each independently replaced with a heteroatom group such as -NH-, -O-, -S-, -S(O)-, -S(O)₂-, or the like. By way of example, 1, 2, 3, 4, 5, 6, or more carbon atoms may be independently replaced with the same or different heteroatom group. A heteroalkenyl group can also include one or more carbonyl moieties (i.e., wherein a carbon atom of the alkyl group is oxidized to a -C(O)- group).

[0100] The term “heteroalkynyl,” as used herein, refers to an alkynyl group, as defined herein, in which one or more of the carbon atoms (and any associated hydrogen atoms) are each independently replaced with a heteroatom group such as -NH-, -O-, -S-, -S(O)-, -S(O)₂-, or the like. By way of example, 1, 2, 3, 4, 5, 6, or more carbon atoms may be independently replaced with the same or different heteroatom group. A heteroalkynyl group can also include one or more carbonyl moieties (i.e., wherein a carbon atom of the alkyl group is oxidized to a -C(O)- group).

[0101] As used herein, the term “heteroaryl” refers to a radical of a 5-10 membered monocyclic or bicyclic 4n+2 aromatic ring system (e.g., having 6 or 10 π electrons shared in a cyclic array) having ring carbon atoms and 1-4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen and sulfur (“5-10 membered heteroaryl”). In heteroaryl groups that contain one or more nitrogen atoms, the point of attachment can be a

carbon or nitrogen atom, as valency permits. Heteroaryl bicyclic ring systems can include one or more heteroatoms in one or both rings. "Heteroaryl" also includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more aryl groups wherein the point of attachment is either on the aryl or heteroaryl ring, and in such instances, the number of ring members designates the number of ring members in the fused (aryl/heteroaryl) ring system. Bicyclic heteroaryl groups wherein one ring does not contain a heteroatom (e.g., indolyl, quinolinyl, and the like) the point of attachment can be on either ring, i.e., either the ring bearing a heteroatom (e.g., 2-indolyl) or the ring that does not contain a heteroatom (e.g., 5-indolyl). Exemplary 5-membered heteroaryl groups containing one heteroatom include, without limitation, pyrrolyl, furanyl and thiophenyl. Exemplary 5-membered heteroaryl groups containing two heteroatoms include, without limitation, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, and isothiazolyl. Exemplary 5-membered heteroaryl groups containing three heteroatoms include, without limitation, triazolyl, oxadiazolyl, and thiadiazolyl. Exemplary 5-membered heteroaryl groups containing four heteroatoms include, without limitation, tetrazolyl. Exemplary 6-membered heteroaryl groups containing one heteroatom include, without limitation, pyridinyl. Exemplary 6-membered heteroaryl groups containing two heteroatoms include, without limitation, pyridazinyl, pyrimidinyl, and pyrazinyl. Exemplary 6-membered heteroaryl groups containing three or four heteroatoms include, without limitation, triazinyl and tetrazinyl, respectively. Exemplary 7-membered heteroaryl groups containing one heteroatom include, without limitation, azepinyl, oxepinyl, and thiopinyl. Exemplary 5,6-bicyclic heteroaryl groups include, without limitation, indolyl, isoindolyl, indazolyl, benzotriazolyl, benzothiophenyl, isobenzothiophenyl, benzofuranyl, benzoisofuranyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzoxadiazolyl, benzthiazolyl, benzisothiazolyl, benzthiadiazolyl, indoliziny, and purinyl. Exemplary 6,6-bicyclic heteroaryl groups include, without limitation, naphthyridinyl, pteridinyl, quinolinyl, isoquinolinyl, cinnolinyl, quinoxalinyl, phthalazinyl, and quinazolinyl.

[0102] As used herein, the term "heterocyclyl" refers to a radical of a 3- to 10-membered non-aromatic ring system having ring carbon atoms and 1 to 4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, sulfur, boron, phosphorus, and silicon ("3-10 membered heterocyclyl"). In heterocyclyl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. A heterocyclyl group can either be monocyclic ("monocyclic heterocyclyl") or a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic heterocyclyl"), and can be saturated or can be partially unsaturated. Heterocyclyl bicyclic ring systems can include one

or more heteroatoms in one or both rings. "Heterocyclyl" also includes ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more cycloalkyl groups wherein the point of attachment is either on the cycloalkyl or heterocyclyl ring, or ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups, wherein the point of attachment is on the heterocyclyl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heterocyclyl ring system. A heterocyclyl group may be described as, e.g., a 3-7-membered heterocyclyl, wherein the term "membered" refers to the non-hydrogen ring atoms, i.e., carbon, nitrogen, oxygen, sulfur, boron, phosphorus, and silicon, within the moiety. Exemplary 3-membered heterocyclyl groups containing one heteroatom include, without limitation, azirdinyl, oxiranyl, and thiorenlyl. Exemplary 4-membered heterocyclyl groups containing one heteroatom include, without limitation, azetidiny, oxetanyl, and thietanyl. Exemplary 5-membered heterocyclyl groups containing one heteroatom include, without limitation, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothiophenyl, dihydrothiophenyl, pyrrolidinyl, dihydropyrrolyl, and pyrrolyl-2,5-dione. Exemplary 5-membered heterocyclyl groups containing two heteroatoms include, without limitation, dioxolanyl, oxasulfuranyl, disulfuranyl, and oxazolidin-2-one. Exemplary 5-membered heterocyclyl groups containing three heteroatoms include, without limitation, triazolinyl, oxadiazolinyl, and thiadiazolinyl. Exemplary 6-membered heterocyclyl groups containing one heteroatom include, without limitation, piperidinyl (e.g., 2,2,6,6-tetramethylpiperidinyl), tetrahydropyranyl, dihydropyridinyl, pyridinonyl (e.g., 1-methylpyridin-2-onyl), and thianyl. Exemplary 6-membered heterocyclyl groups containing two heteroatoms include, without limitation, piperazinyl, morpholinyl, pyridazinonyl (2-methylpyridazin-3-onyl), pyrimidinonyl (e.g., 1-methylpyrimidin-2-onyl, 3-methylpyrimidin-4-onyl), dithianyl, dioxanyl. Exemplary 6-membered heterocyclyl groups containing two heteroatoms include, without limitation, triazinanyl. Exemplary 7-membered heterocyclyl groups containing one heteroatom include, without limitation, azepanyl, oxepanyl and thiepanyl. Exemplary 8-membered heterocyclyl groups containing one heteroatom include, without limitation, azocanyl, oxecanyl and thiocanyl. Exemplary 5-membered heterocyclyl groups fused to a C₆ aryl ring (also referred to herein as a 5,6-bicyclic heterocyclyl ring) include, without limitation, indolinyl, isoindolinyl, dihydrobenzofuranyl, dihydrobenzothienyl, benzoxazolinonyl, and the like. Exemplary 5-membered heterocyclyl groups fused to a heterocyclyl ring (also referred to herein as a 5,5-bicyclic heterocyclyl ring) include, without limitation, octahydropyrrolopyrrolyl (e.g., octahydropyrrolo[3,4-c]pyrrolyl), and the like. Exemplary 6-

membered heterocyclyl groups fused to a heterocyclyl ring (also referred to as a 4,6-membered heterocyclyl ring) include, without limitation, diazaspirononanyl (e.g., 2,7-diazaspiro[3.5]nonanyl). Exemplary 6-membered heterocyclyl groups fused to an aryl ring (also referred to herein as a 6,6-bicyclic heterocyclyl ring) include, without limitation, tetrahydroquinoliny, tetrahydroisoquinoliny, and the like. Exemplary 6-membered heterocyclyl groups fused to a cycloalkyl ring (also referred to herein as a 6,7-bicyclic heterocyclyl ring) include, without limitation, azabicyclooctanyl (e.g., (1,5)-8-azabicyclo[3.2.1]octanyl). Exemplary 6-membered heterocyclyl groups fused to a cycloalkyl ring (also referred to herein as a 6,8-bicyclic heterocyclyl ring) include, without limitation, azabicyclononanyl (e.g., 9-azabicyclo[3.3.1]nonanyl).

[0103] The term “hydroxy,” as used herein, refers to an -OH group.

[0104] The term “hydroxyalkyl,” as used herein, refers to an alkyl group, as defined herein, in which at least one hydrogen atom (e.g., one hydrogen atom) is replaced with a hydroxy group.

[0105] As used herein, the term “substituent” refers to a group substituted on an atom of the indicated group.

[0106] When a group or moiety can be substituted, the term “substituted” indicates that one or more (e.g., 1, 2, 3, 4, 5, or 6; in some embodiments 1, 2, or 3; and in other embodiments 1 or 2) hydrogen atoms on the group indicated in the expression using “substituted” can be replaced with a selection of recited indicated groups or with a suitable substituent group known to those of skill in the art (e.g., one or more of the groups recited below), provided that the designated atom’s normal valence is not exceeded. Substituent groups include, but are not limited to, alkyl, alkenyl, alkynyl, alkoxy, acyl, amino, amido, amidino, aryl, azido, carbamoyl, carboxyl, carboxyl ester, cyano, cycloalkyl, cycloalkenyl, guanidino, halo, haloalkyl, haloalkoxy, heteroalkyl, heteroaryl, heterocyclyl, hydroxy, hydrazino, imino, oxo, nitro, phosphate, phosphonate, sulfonic acid, sulfonamido, thiol, thione, thioxo, or combinations thereof.

[0107] As used herein, in chemical structures the indication:



represents a point of attachment of one moiety to another moiety.

[0108] In some instances, the number of carbon atoms in a hydrocarbyl substituent (e.g., alkyl alkenyl) is indicated by the prefix “C_x-C_y”, wherein x is the minimum and y is the

maximum number of carbon atoms in the substituent. Thus, for example, “C₁-C₃ alkyl” refers to an alkyl substituent containing from 1 to 3 carbon atoms.

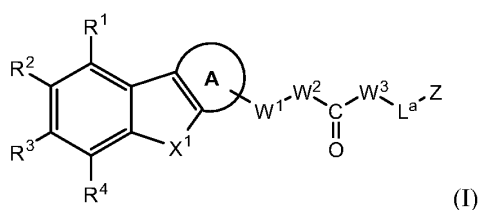
[0109] For compounds described herein, groups and substituents thereof may be selected in accordance with permitted valence of the atoms and the substituents, such that the selections and substitutions result in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

[0110] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they optionally encompass substituents resulting from writing the structure from right to left, e.g., -CH₂O- is intended to encompass -OCH₂-, and -C(O)NH- is intended to encompass -NHC(O)-.

[0111] Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present disclosure. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

2. Compounds

[0112] In one aspect, disclosed is a compound of formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

R¹, R², R³, and R⁴ are each independently selected from hydrogen, C₁-C₆ alkoxy, halo, and C₁-C₆ alkyl;

X¹ is selected from S and NH;

A is selected from a five- or six-membered cycloalkyl, heterocyclyl, heteroaryl, or aryl, each of which is optionally substituted with 1 or 2 oxo groups;

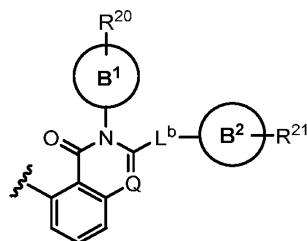
W¹ is a bond or O;

W² is selected from C₁-C₆ alkylene, C₃-C₆ cycloalkylene, C₃-C₆ cycloalkylene-C₁-C₆ alkylene, arylene, and hydroxy-C₁-C₆-alkylene;

W³ is O or NH;

L^a is a linker; and

Z is



wherein:

Q is CH or N;

B¹ is aryl or a 5- or 6-membered monocyclic heteroaryl having 1, 2, 3, or 4 heteroatoms independently selected from N, O, S, and P;

R²⁰ is selected from hydrogen, halo, C₁-C₄ alkyl, C₃-C₆ cycloalkyl, C₁-C₄ haloalkyl, -ORᵃ¹, -N(Rᵃ²)(Rᵃ³), -SO₂Rᵃ⁴, -SO₂N(Rᵃ⁵)(Rᵃ⁶), and -NHSO₂Rᵃ⁷, wherein Rᵃ¹, Rᵃ², Rᵃ³, Rᵃ⁴, Rᵃ⁵, Rᵃ⁶, and Rᵃ⁷ are each independently selected from hydrogen, C₁-C₄ alkyl, and C₁-C₄ haloalkyl;

R²¹ is selected from hydrogen and a group -L²¹-E, wherein:

L²¹ is a bond, C₁-C₂ alkylene, -CH=CH-, -C≡C-, -C(O)-, -O-, -NH-, -S-, -C(O)O-, -C(O)NH-, -C(O)S-, arylene, cycloalkylene, heteroarylene, or heterocyclylene, or wherein L²¹ comprises a combination of any two of such groups;

E is a bicyclic heterocyclyl or bicyclic heteroaryl, each of which is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from halo, C₁-C₄ alkyl, C₃-C₆ cycloalkyl, C₃-C₆-cycloalkyl-C₁-4-alkyl, C₁-C₄ haloalkyl, oxo, -ORᵇ¹, -N(Rᵇ²)(Rᵇ³), -SO₂Rᵇ⁴, -SO₂N(Rᵇ⁵)(Rᵇ⁶), and -NHSO₂Rᵇ⁷, wherein Rᵇ¹, Rᵇ², Rᵇ³, Rᵇ⁴, Rᵇ⁵, Rᵇ⁶, and Rᵇ⁷ are each independently selected from hydrogen, C₁-C₄ alkyl, and C₁-C₄ haloalkyl;

Lᵇ is -(CRᶜ¹Rᶜ²)ₘ-Gᵇ-, wherein:

Rᶜ¹ and Rᶜ² are independently selected from hydrogen and C₁-C₄ alkyl;

m is 0, 1, or 2; and

Gᵇ is a bond, -NHC(O)-, -NH-, -O-, or -S-; and

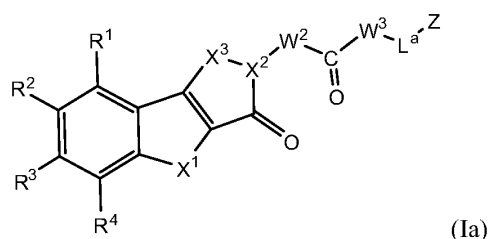
B² is a bicyclic heteroaryl or bicyclic heterocyclyl, each of which is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected

from halo, C₁-C₄ alkyl, C₃-C₆ cycloalkyl, C₁-C₄ haloalkyl, optionally substituted aryl, -OR^{d1}, -N(R^{d2})(R^{d3}), -SO₂R^{d4}, -SO₂N(R^{d5})(R^{d6}), and -NHSO₂R^{d7}, wherein R^{d1}, R^{d2}, R^{d3}, R^{d4}, R^{d5}, R^{d6}, and R^{d7} are each independently selected from hydrogen, C₁-C₄ alkyl, and C₁-C₄ haloalkyl.

[0113] In some embodiments, A is a 5- or 6-membered cycloalkyl. In some embodiments, A is a 5-membered cycloalkyl. In some embodiments, A is a 5- or 6-membered heterocyclyl having 1 or 2 nitrogen atoms. In some embodiments, A is a 5-membered heterocyclyl having 1 nitrogen atom. In some embodiments, A is a 6-membered heterocyclyl having 1 or 2 nitrogen atoms. In some embodiments, A is a 5- or 6-membered heteroaryl having 1 or 2 nitrogen atoms. In some embodiments, A is a 6-membered heteroaryl having 2 nitrogen atoms. In some embodiments, A is substituted with 1 oxo group. In some embodiments, A is substituted with 2 oxo groups.

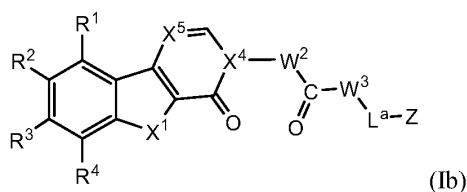
[0114] In some embodiments, W¹ is a bond. In some embodiments, W¹ is O.

[0115] In some embodiments, the compound of formula (I) is a compound of formula (Ia):



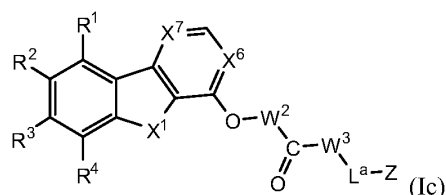
wherein X² is CH or N, and X³ is CH₂ or CO. In some embodiments, X² is CH. In some embodiments, X² is N. In some embodiments, X³ is CH₂. In some embodiments, X³ is CO. In some embodiments, X² is CH and X³ is CH₂. In some embodiments, X² is N and X³ is CH₂. In some embodiments, X² is N and X³ is CO.

[0116] In some embodiments, the compound of formula (I) is a compound of formula (Ib):



wherein X⁴ and X⁵ are independently selected from N and CH. In some embodiments, X⁴ is N. In some embodiments, X⁵ is N. In some embodiments, X⁵ is CH. In some embodiments, X⁴ and X⁵ are N. In some embodiments, X⁴ is N and X⁵ is CH.

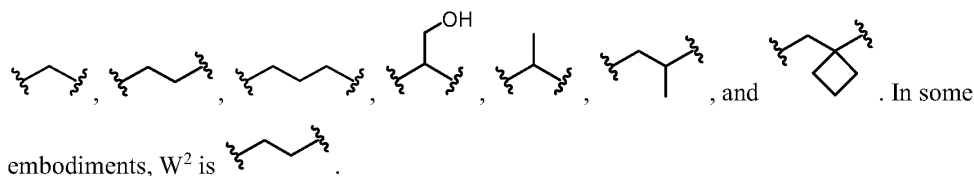
[0117] In some embodiments, the compound of formula (I) is a compound of formula (Ic):



wherein X^6 and X^7 are independently selected from CH and N. In some embodiments, X^6 is N. In some embodiments, X^7 is N. In some embodiments, X^6 and X^7 are N.

[0118] In some embodiments, X^1 is S. In some embodiments, X^1 is NH.

[0119] In some embodiments, W^2 is selected from C_1 - C_4 alkylene, C_4 - C_5 cycloalkylene, C_4 - C_5 cycloalkylene- C_1 - C_2 alkylene, phenylene, and hydroxy- C_1 - C_3 -alkylene. In some embodiments, W^2 is selected from C_1 - C_4 alkylene, C_4 - C_5 cycloalkylene- C_1 - C_2 alkylene, and hydroxy- C_1 - C_3 -alkylene. In some embodiments, W^2 is selected from:



[0120] In some embodiments, W^3 is O. In some embodiments, W^3 is NR^w , wherein R^w is selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, arylalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, and heteroarylalkyl. In some embodiments, W^3 is NH.

[0121] In some embodiments, R^1 , R^2 , R^3 , and R^4 are each independently selected from hydrogen, C_1 - C_6 alkoxy, and halo. In some embodiments, R^1 , R^2 , R^3 , and R^4 are each independently selected from hydrogen, methoxy, chloro, and bromo.

[0122] In some embodiments, R^2 and R^3 are each independently C_1 - C_6 alkoxy. In some embodiments, R^2 and R^3 are each methoxy.

[0123] In some embodiments, R^1 and R^4 are each independently halo or hydrogen. In some embodiments, R^1 and R^4 are each independently chloro, bromo, or hydrogen. In some embodiments, R^1 is halo and R^4 is hydrogen. In some embodiments, R^1 and R^4 are each hydrogen. In some embodiments, R^1 is chloro or bromo, and R^4 is hydrogen.

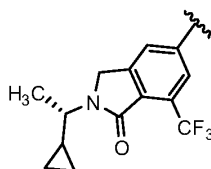
[0124] In some embodiments, R^1 and R^4 are each hydrogen, and R^2 and R^3 are each independently C_1 - C_6 alkoxy. In some embodiments, R^1 and R^4 are each hydrogen, and R^2 and R^3 are each methoxy.

[0125] In some embodiments, R^1 is halo, R^2 and R^3 are each independently C_1 - C_6 alkoxy, and R^4 is hydrogen. In some embodiments, R^1 is chloro or bromo, R^2 and R^3 are each methoxy, and R^4 is hydrogen.

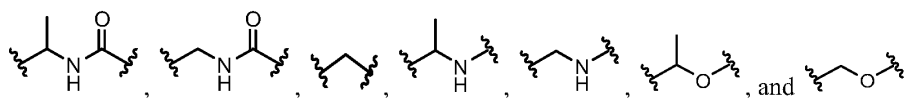
[0126] In some embodiments, B^1 is phenyl or a monocyclic heteroaryl having 1, 2, 3, or 4 heteroatoms independently selected from N, O, S, and P. In some embodiments, B^1 is phenyl or a monocyclic heteroaryl having 1, 2, 3, or 4 heteroatoms independently selected from N, O, and S. In some embodiments, B^1 is phenyl or a monocyclic heteroaryl having one heteroatom selected from N, O, and S. In some embodiments, B^1 is selected from phenyl, pyridyl, furan, and thiophene. In some embodiments, B^1 is phenyl. In some embodiments, R^{20} is hydrogen. In some embodiments, B^1 is phenyl and R^{20} is hydrogen.

[0127] In some embodiments, B^2 is a nine-membered bicyclic heteroaryl or a nine-membered bicyclic heterocyclyl having 1, 2, 3, or 4 heteroatoms independently selected from N, O, S, and P. In some embodiments, B^2 is a nine-membered bicyclic heteroaryl or a nine-membered bicyclic heterocyclyl having 1, 2, 3, or 4 heteroatoms independently selected from N, O, and S. In some embodiments, B^2 is a nine-membered bicyclic heteroaryl or a nine-membered bicyclic heterocyclyl having 1, 2, 3, or 4 nitrogen atoms. In some embodiments, B^2 is a pyrazolopyrimidine. In some embodiments, in addition to $-R^{21}$, B^2 is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from halo, C_1 - C_4 alkyl, C_1 - C_4 haloalkyl, $-OR^{e1}$, $-N(R^{e2})(R^{e3})$, $-SO_2R^{e4}$, $-SO_2N(R^{e5})(R^{e6})$, and $-NHSO_2R^{e7}$, wherein R^{e1} , R^{e2} , R^{e3} , R^{e4} , R^{e5} , R^{e6} , and R^{e7} are each independently selected from hydrogen, C_1 - C_4 alkyl, and C_1 - C_4 haloalkyl. In some embodiments, in addition to $-R^{21}$, B^2 is substituted with one substituent selected from halo, C_1 - C_4 alkyl, C_1 - C_4 haloalkyl, $-OR^{e1}$, $-N(R^{e2})(R^{e3})$, $-SO_2R^{e4}$, $-SO_2N(R^{e5})(R^{e6})$, and $-NHSO_2R^{e7}$. In some embodiments, in addition to $-R^{21}$, B^2 is substituted with one substituent selected from halo, methyl, trifluoromethyl, $-OR^{e1}$, $-N(R^{e2})(R^{e3})$, $-SO_2R^{e4}$, $-SO_2N(R^{e5})(R^{e6})$, and $-NHSO_2R^{e7/5}$, wherein R^{e1} , R^{e2} , R^{e3} , R^{e4} , R^{e5} , R^{e6} , and R^{e7} are each independently selected from hydrogen, methyl, ethyl, isopropyl, t-butyl, and trifluoromethyl.

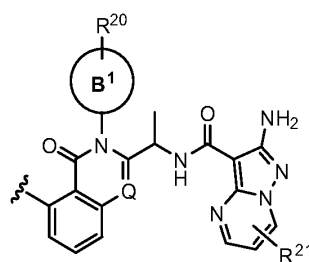
[0128] In some embodiments, R^{21} is selected from hydrogen and a group of formula:



[0129] In some embodiments, L^b is $-(CR^{c1}R^{c2})_m-G^b-$, wherein m is 0, 1, or 2, R^{c1} and R^{c2} are independently selected from hydrogen and methyl, and G^b is a bond, $-NHC(O)-$, $-NH-$, $-O-$, or $-S-$. In some embodiments, L^b has a formula selected from:

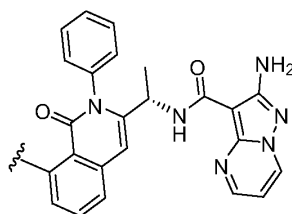


[0130] In some embodiments, Z is



wherein B^1 , Q , R^{20} , and R^{21} are any of the groups defined and described herein.

[0131] In some embodiments, Z is

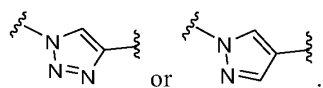


[0132] The compound of formula (I) includes L^a , which is a linker moiety. In some embodiments, L^a provides sufficient distance between two elements of the compound (i.e., the STING agonist moiety and the group Z (a PI3K agonist), to allow each to function undisturbed (or minimally disturbed) by the linkage to the other. In some embodiments, L^a separates the two groups by about 5 Å to about 1000 Å. In some embodiments, L^a separates the two groups by 5 Å, 10 Å, 20 Å, 50 Å, 100 Å, 150 Å, 200 Å, 300 Å, 400 Å, 500 Å, 600 Å, 700 Å, 800 Å, 900 Å, 1000 Å, or any suitable range therebetween (e.g., 5-100 Å, 50-500 Å, 150-700 Å, etc.). In some embodiments, L^a separates two groups by about 1-200 atoms (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, or any suitable ranges therebetween (e.g., 2-20, 10-50, etc.)).

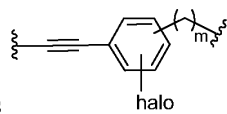
[0133] L^a can include one or more groups independently selected from $-C(R')_2-$, $-CH=CH-$, $-C\equiv C-$, $-O-$, $-NR'-$, $-BR'-$, $-S-$, $-C(O)-$, $-C(NR')-$, $-S(O)-$, $-S(O)_2-$, arylene, heteroarylene, cycloalkylene, and heterocyclylene, wherein each R' is independently selected from hydrogen, C_1 - C_{80} alkyl, C_2 - C_{80} alkenyl, C_2 - C_{80} alkynyl, aryl, arylalkyl, cycloalkyl,

cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, and a lipid moiety, and wherein each alkyl, arylene, heteroarylene, cycloalkylene, and heterocyclylene is independently unsubstituted or substituted with 1, 2, or 3 substituents. In some embodiments, L^a comprises one or more groups independently selected from $-C(R')_2-$, $-C\equiv C-$, $-O-$, $-NH-$, $-C(O)-$, arylene and heteroarylene, wherein each R' is independently selected from hydrogen, C_1 - C_{40} alkyl, phenyl, and $-CH_2$ -heterocyclyl (e.g., wherein the heterocyclyl is a 6-membered heterocyclyl having 1 or 2 heteroatoms independently selected from N, O, and S), wherein the arylene, heteroarylene, phenyl and the heterocyclyl are each independently unsubstituted or substituted with 1 or 2 substituents. In some embodiments, L^a comprises one or more groups independently selected from $-C(R')_2-$, $-C\equiv C-$, $-O-$, $-NH-$, $-C(O)-$, and heteroarylene (e.g., a five-membered heteroarylene having 1, 2, or 3 nitrogen atoms), wherein each R' is independently selected from hydrogen, C_1 - C_{40} alkyl, phenyl, and $-CH_2$ -heterocyclyl (e.g., wherein the heterocyclyl is a 6-membered heterocyclyl having 1 or 2 heteroatoms independently selected from N, O, and S), wherein the phenyl and the heterocyclyl are each independently unsubstituted or substituted with 1 or 2 substituents.

[0134] In some embodiments, L^a comprises one or more alkylene groups (e.g., $-(CH_2)_n-$, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12). In some embodiments, L^a comprises one or more branched alkylene groups. In some embodiments, L^a comprises at least one five-membered heteroarylene group having 2 or 3 nitrogen atoms (e.g., a group of formula



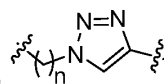
[0135] In some embodiments, L^a comprises one or more groups independently selected from $-CH_2-$, $-C\equiv C-$, arylene (e.g., phenylene), and heteroarylene (e.g., a five-membered heteroarylene group having 2 or 3 nitrogen atoms), wherein the arylene is unsubstituted or substituted with one or two halo groups. In some embodiments, L^a comprises one or more groups independently selected from $-CH_2-$ and heteroarylene.



[0136] In some embodiments, L^a is , wherein m is 1, 2, 3, 4 or 6.. In

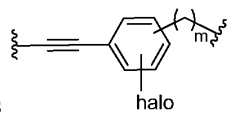
some embodiments, L^a is or . In some

embodiments, L^a is . In some embodiments, L^a is .

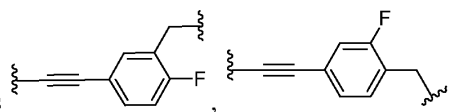


[0137] In some embodiments, L^a is and n is 1, 2, 3, 4, or 5. In some

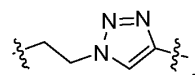
embodiments, L^a is .



[0138] In some embodiments, L^a is or , wherein m and n are independently 1, 2, 3, 4, or 5.

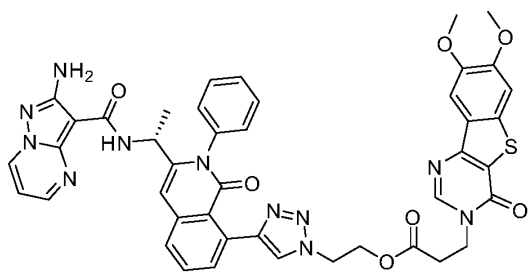


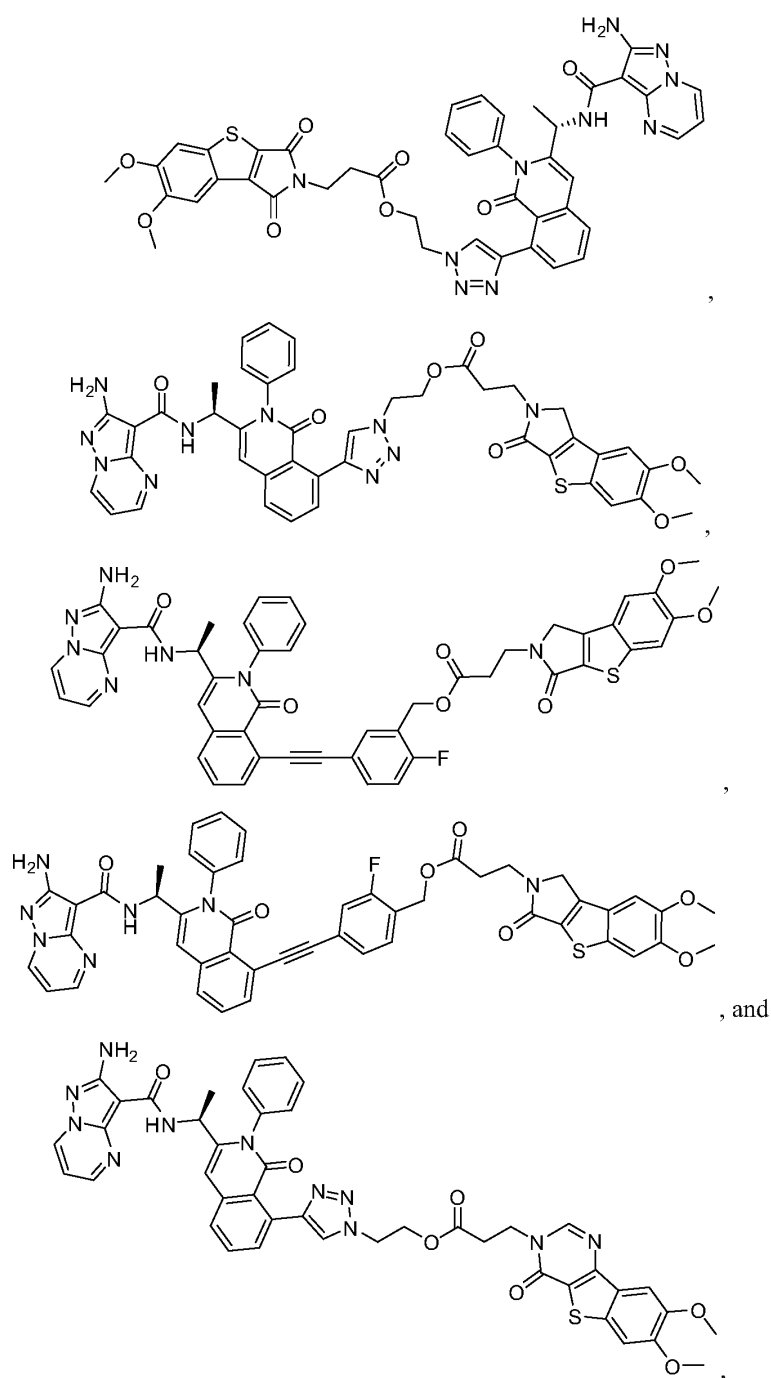
[0139] In some embodiments, L^a is , or



[0140] In some embodiments, L^a comprises one or more substituents, pendants, side chains, etc., comprising any suitable organic functional groups (e.g., -OH, -NH₂, -SH, -CN, =O, =S, halogen (e.g., -F, -Cl, -Br, -I), -COOH, -CONH₂, -CH₃, etc.).

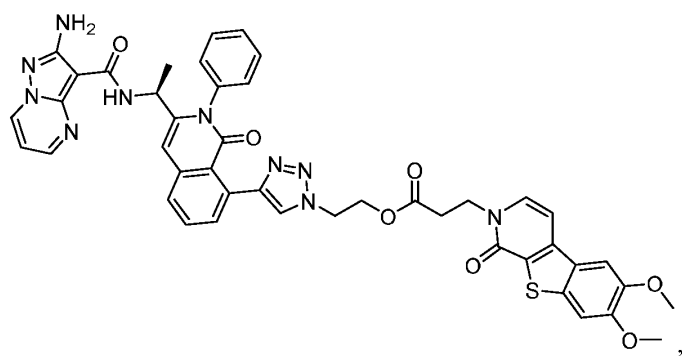
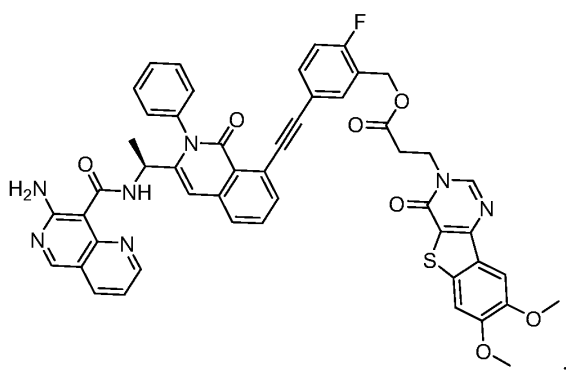
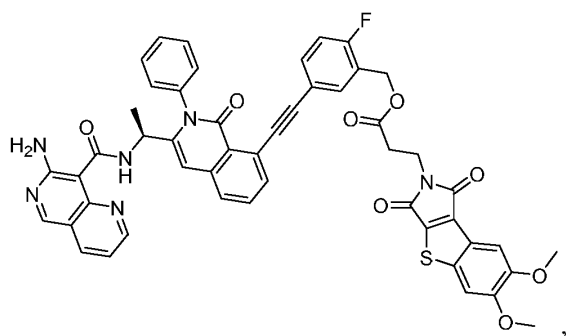
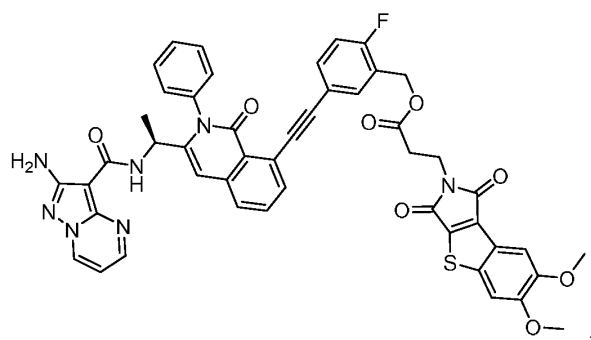
[0141] In some embodiments, the compound of formula (I) is selected from:

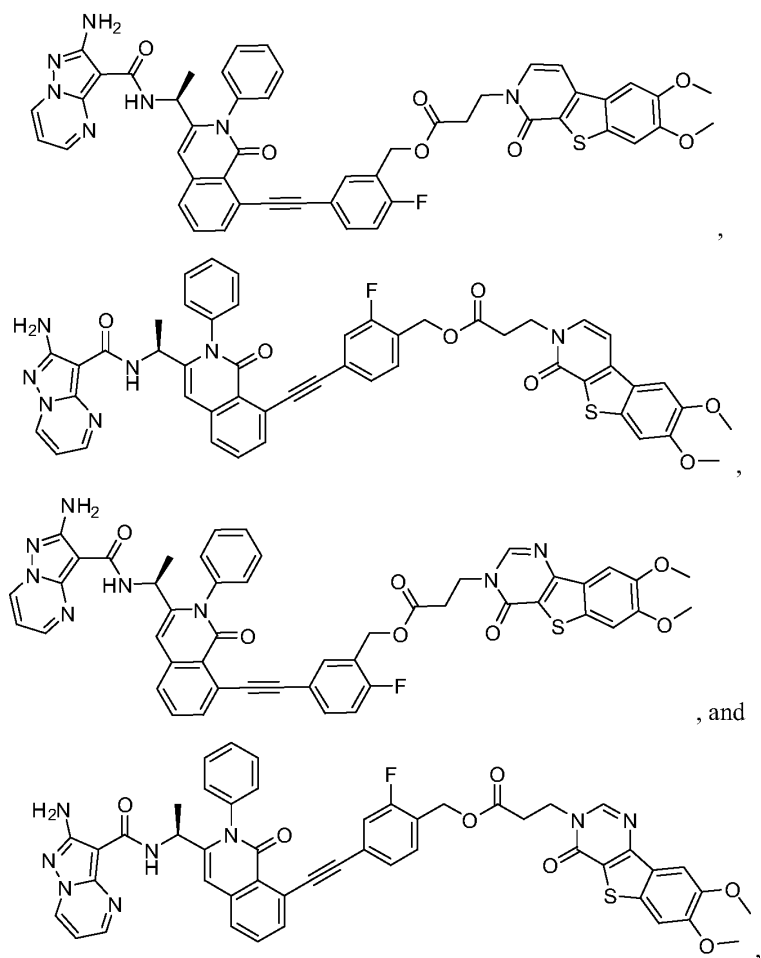




and pharmaceutically acceptable salts thereof.

[0142] Additional compounds of formula (I) include:





and pharmaceutically acceptable salts thereof.

[0143] The compounds may exist as a stereoisomer wherein asymmetric or chiral centers are present. The stereoisomer is “R” or “S” depending on the configuration of substituents around the chiral carbon atom. The terms “R” and “S” used herein are configurations as defined in IUPAC 1974 Recommendations for Section E, Fundamental Stereochemistry, in *Pure Appl. Chem.*, 1976, 45: 13-30. The disclosure contemplates various stereoisomers and mixtures thereof and these are specifically included within the scope of this disclosure. Stereoisomers include enantiomers and diastereomers, and mixtures of enantiomers or diastereomers. Individual stereoisomers of the compounds may be prepared synthetically from commercially available starting materials, which contain asymmetric or chiral centers or by preparation of racemic mixtures followed by methods of resolution well-known to those of ordinary skill in the art. These methods of resolution are exemplified by (1) attachment of a

mixture of enantiomers to a chiral auxiliary, separation of the resulting mixture of diastereomers by recrystallization or chromatography and optional liberation of the optically pure product from the auxiliary as described in Furniss, Hannaford, Smith, and Tatchell, "Vogel's Textbook of Practical Organic Chemistry," 5th edition (1989), Longman Scientific & Technical, Essex CM20 2JE, England (or more recent versions thereof), or (2) direct separation of the mixture of optical enantiomers on chiral chromatographic columns, or (3) fractional recrystallization methods.

[0144] It should be understood that the compounds may possess tautomeric forms, as well as geometric isomers, and that these also constitute embodiments of the disclosure.

[0145] The present disclosure also includes isotopically-labeled compounds, which is identical to those recited in formula (I), but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes include those for hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, and chlorine, such as, but not limited to ^2H , ^3H , ^{13}C , ^{14}C , ^{15}N , ^{18}O , ^{17}O , ^{31}P , ^{32}P , ^{35}S , ^{18}F , and ^{36}Cl , respectively. Substitution with heavier isotopes such as deuterium, for example, ^2H , can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. The compound may incorporate positron-emitting isotopes for medical imaging and positron-emitting tomography (PET) studies. Suitable positron-emitting isotopes that can be incorporated in compounds of formula (I) are ^{11}C , ^{13}N , ^{15}O , and ^{18}F . Isotopically-labeled compounds of formula (I) can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the accompanying Examples using appropriate isotopically-labeled reagent in place of non-isotopically-labeled reagent.

[0146] The disclosed compounds may exist as pharmaceutically acceptable salts. The term "pharmaceutically acceptable salt" refers to salts or zwitterions of the compounds which are water or oil-soluble or dispersible, suitable for treatment of disorders without undue toxicity, irritation, and allergic response, commensurate with a reasonable benefit/risk ratio and effective for their intended use. The salts may be prepared during the final isolation and purification of the compounds or separately by reacting an amino group of the compounds with a suitable acid. For example, a compound may be dissolved in a suitable solvent, such as but not limited to methanol and water and treated with at least one equivalent of an acid, like hydrochloric acid. The resulting salt may precipitate out and be isolated by filtration and dried under reduced pressure. Alternatively, the solvent and excess acid may be removed

under reduced pressure to provide a salt. Representative salts include acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, formate, isethionate, fumarate, lactate, maleate, methanesulfonate, naphthylenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, oxalate, maleate, pivalate, propionate, succinate, tartrate, trichloroacetate, trifluoroacetate, glutamate, para-toluenesulfonate, undecanoate, hydrochloric, hydrobromic, sulfuric, phosphoric and the like. The amino groups of the compounds may also be quaternized with alkyl chlorides, bromides and iodides such as methyl, ethyl, propyl, isopropyl, butyl, lauryl, myristyl, stearyl and the like.

[0147] Basic addition salts may be prepared during the final isolation and purification of the disclosed compounds by reaction of a carboxyl group with a suitable base such as the hydroxide, carbonate, or bicarbonate of a metal cation such as lithium, sodium, potassium, calcium, magnesium, or aluminum, or an organic primary, secondary, or tertiary amine. Quaternary amine salts can be prepared, such as those derived from methylamine, dimethylamine, trimethylamine, triethylamine, diethylamine, ethylamine, tributylamine, pyridine, N,N-dimethylaniline, N-methylpiperidine, N-methylmorpholine, dicyclohexylamine, procaine, dibenzylamine, N,N-dibenzylphenethylamine, 1-ephedrine and N,N'-dibenzylethylenediamine, ethylenediamine, ethanolamine, diethanolamine, piperidine, piperazine, and the like.

[0148] Compounds may be synthesized according to a variety of methods, including those illustrated in the Examples. Reaction conditions and reaction times for each individual step can vary depending on the particular reactants employed and substituents present in the reactants used. Specific procedures are provided in the Examples section. Reactions can be worked up in the conventional manner, e.g., by eliminating the solvent from the residue and further purified according to methodologies generally known in the art such as, but not limited to, crystallization, distillation, extraction, trituration, and chromatography. Unless otherwise described, the starting materials and reagents are either commercially available or can be prepared by one skilled in the art from commercially available materials using methods described in the chemical literature. Starting materials, if not commercially available, can be prepared by procedures selected from standard organic chemical techniques, techniques that are analogous to the synthesis of known, structurally similar compounds, or techniques that are analogous to the above described schemes or the procedures described in the synthetic examples section.

[0149] Routine experimentations, including appropriate manipulation of the reaction conditions, reagents and sequence of the synthetic route, protection of any chemical functionality that cannot be compatible with the reaction conditions, and deprotection at a suitable point in the reaction sequence of the method are included in the scope of the disclosure. Suitable protecting groups and the methods for protecting and deprotecting different substituents using such suitable protecting groups are well known to those skilled in the art; examples of which can be found in PGM Wuts and TW Greene, in Greene's book titled Protective Groups in Organic Synthesis (4th ed.), John Wiley & Sons, NY (2006), which is incorporated herein by reference in its entirety. Synthesis of the compounds of the disclosure can be accomplished by methods analogous to those described in the synthetic schemes described hereinabove and in specific examples.

[0150] When an optically active form of a disclosed compound is required, it can be obtained by carrying out one of the procedures described herein using an optically active starting material (prepared, for example, by asymmetric induction of a suitable reaction step), or by resolution of a mixture of the stereoisomers of the compound or intermediates using a standard procedure (such as chromatographic separation, recrystallization, or enzymatic resolution).

[0151] Similarly, when a pure geometric isomer of a compound is required, it can be obtained by carrying out one of the above procedures using a pure geometric isomer as a starting material, or by resolution of a mixture of the geometric isomers of the compound or intermediates using a standard procedure such as chromatographic separation.

[0152] It can be appreciated that the synthetic schemes and specific examples as described are illustrative and are not to be read as limiting the scope of the disclosure as it is defined in the appended claims. All alternatives, modifications, and equivalents of the synthetic methods and specific examples are included within the scope of the claims.

3. Compositions

[0153] The disclosed compounds may be incorporated into compositions that may be suitable for administration to a subject (such as a patient, which may be a human or non-human).

a. Pharmaceutical Compositions

[0154] The disclosed compounds may be incorporated into pharmaceutically acceptable compositions. The pharmaceutical compositions may include a "therapeutically effective amount" or a "prophylactically effective amount" of the compound(s). A "therapeutically

effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the composition may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the composition to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of a compound of the invention (e.g., a compound of formula (I)) are outweighed by the therapeutically beneficial effects. A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0155] The pharmaceutical compositions and formulations may include pharmaceutically acceptable carriers. The term “pharmaceutically acceptable carrier,” as used herein, means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material, surfactant, cyclodextrins or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as, but not limited to, lactose, glucose and sucrose; starches such as, but not limited to, corn starch and potato starch; cellulose and its derivatives such as, but not limited to, sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as, but not limited to, cocoa butter and suppository waxes; oils such as, but not limited to, peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; surfactants such as, but not limited to, cremophor EL, cremophor RH 60, Solutol HS 15 and polysorbate 80; cyclodextrins such as, but not limited to, alpha-CD, beta-CD, gamma-CD, HP-beta-CD, SBE-beta-CD; glycols; such as propylene glycol; esters such as, but not limited to, ethyl oleate and ethyl laurate; agar; buffering agents such as, but not limited to, magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as, but not limited to, sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

[0156] The route by which the disclosed compounds are administered and the form of the composition will dictate the type of carrier to be used. The composition may be in a variety of forms, suitable, for example, for systemic administration (e.g., oral, rectal, nasal, sublingual,

buccal, implants, or parenteral injections) or topical administration (e.g., dermal, pulmonary, nasal, aural, ocular, liposome delivery systems, or iontophoresis). In some embodiments, the composition is for oral administration. In some embodiments, the composition is for subcutaneous administration. In some embodiments, the composition is for intravenous administration.

[0157] Carriers for systemic administration typically include at least one of diluents, lubricants, binders, disintegrants, colorants, flavors, sweeteners, antioxidants, preservatives, glidants, solvents, suspending agents, wetting agents, surfactants, cyclodextrins combinations thereof, and others. All carriers are optional in the compositions.

[0158] Suitable diluents include sugars such as glucose, lactose, dextrose, and sucrose; diols such as propylene glycol; calcium carbonate; sodium carbonate; sugar alcohols, such as glycerin; mannitol; and sorbitol. The amount of diluent(s) in a systemic or topical composition is typically about 50 to about 90%.

[0159] Suitable lubricants include silica, talc, stearic acid and its magnesium salts and calcium salts, calcium sulfate; and liquid lubricants such as polyethylene glycol and vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma. The amount of lubricant(s) in a systemic or topical composition is typically about 5 to about 10%.

[0160] Suitable binders include polyvinyl pyrrolidone; magnesium aluminum silicate; starches such as corn starch and potato starch; gelatin; tragacanth; and cellulose and its derivatives, such as sodium carboxymethylcellulose, ethyl cellulose, methylcellulose, microcrystalline cellulose, and sodium carboxymethylcellulose. The amount of binder(s) in a systemic composition is typically about 5 to about 50%.

[0161] Suitable disintegrants include agar, alginic acid and the sodium salt thereof, effervescent mixtures, croscarmellose, crospovidone, sodium carboxymethyl starch, sodium starch glycolate, clays, and ion exchange resins. The amount of disintegrant(s) in a systemic or topical composition is typically about 0.1 to about 10%.

[0162] Suitable colorants include a colorant such as an FD&C dye. When used, the amount of colorant in a systemic or topical composition is typically about 0.005 to about 0.1%.

[0163] Suitable flavors include menthol, peppermint, and fruit flavors. The amount of flavor(s), when used, in a systemic or topical composition is typically about 0.1 to about 1.0%.

[0164] Suitable sweeteners include aspartame and saccharin. The amount of sweetener(s) in a systemic or topical composition is typically about 0.001 to about 1%.

[0165] Suitable antioxidants include butylated hydroxyanisole ("BHA"), butylated hydroxytoluene ("BHT"), and vitamin E. The amount of antioxidant(s) in a systemic or topical composition is typically about 0.1 to about 5%.

[0166] Suitable preservatives include benzalkonium chloride, methyl paraben and sodium benzoate. The amount of preservative(s) in a systemic or topical composition is typically about 0.01 to about 5%.

[0167] Suitable glidants include silicon dioxide. The amount of glidant(s) in a systemic or topical composition is typically about 1 to about 5%.

[0168] Suitable solvents include water, isotonic saline, ethyl oleate, glycerine, hydroxylated castor oils, alcohols such as ethanol, dimethyl sulfoxide, N-methyl-2-pyrrolidone, dimethylacetamide and phosphate (or other suitable buffer). The amount of solvent(s) in a systemic or topical composition is typically from about 0 to about 100%.

[0169] Suitable suspending agents include AVICEL RC-591 (from FMC Corporation of Philadelphia, Pa.) and sodium alginate. The amount of suspending agent(s) in a systemic or topical composition is typically about 1 to about 8%.

[0170] Suitable surfactants include lecithin, Polysorbate 80, and sodium lauryl sulfate, and the TWEENS from Atlas Powder Company of Wilmington, Del. Suitable surfactants include those disclosed in the C.T.F.A. Cosmetic Ingredient Handbook, 1992, pp.587-592; Remington's Pharmaceutical Sciences, 15th Ed. 1975, pp. 335-337; and McCutcheon's Volume 1, Emulsifiers & Detergents, 1994, North American Edition, pp. 236-239. The amount of surfactant(s) in the systemic or topical composition is typically about 0.1% to about 5%.

[0171] Suitable cyclodextrins include alpha-CD, beta-CD, gamma-CD, hydroxypropyl betadex (HP-beta-CD), sulfobutyl-ether β -cyclodextrin (SBE-beta-CD). The amount of cyclodextrins in the systemic or topical composition is typically about 0% to about 40%.

[0172] Although the amounts of components in the systemic compositions may vary depending on the type of systemic composition prepared, in general, systemic compositions include 0.01% to 50% of an active compound (e.g., a compound of formula (I)) and 50% to 99.99% of one or more carriers. Compositions for parenteral administration typically include 0.1% to 10% of actives and 90% to 99.9% of a carrier including a diluent and a solvent.

[0173] Compositions for oral administration can have various dosage forms. For example, solid forms include tablets, capsules, granules, and bulk powders. These oral dosage forms

include a safe and effective amount, usually at least about 5%, and more particularly from about 25% to about 50% of actives. The oral dosage compositions include about 50% to about 95% of carriers, and more particularly, from about 50% to about 75%.

[0174] Tablets can be compressed, tablet triturates, enteric-coated, sugar-coated, film-coated, or multiple-compressed. Tablets typically include an active component, and a carrier comprising ingredients selected from diluents, lubricants, binders, disintegrants, colorants, flavors, sweeteners, glidants, and combinations thereof. Specific diluents include calcium carbonate, sodium carbonate, mannitol, lactose, and cellulose. Specific binders include starch, gelatin, and sucrose. Specific disintegrants include alginic acid and croscarmellose. Specific lubricants include magnesium stearate, stearic acid, and talc. Specific colorants are the FD&C dyes, which can be added for appearance. Chewable tablets preferably contain sweeteners such as aspartame and saccharin, or flavors such as menthol, peppermint, fruit flavors, or a combination thereof.

[0175] Capsules (including implants, time release and sustained release formulations) typically include an active compound (e.g., a compound of formula (I)), and a carrier including one or more diluents disclosed above in a capsule comprising gelatin. Granules typically comprise a disclosed compound, and preferably glidants such as silicon dioxide to improve flow characteristics. Implants can be of the biodegradable or the non-biodegradable type.

[0176] The selection of ingredients in the carrier for oral compositions depends on secondary considerations like taste, cost, and shelf stability, which are not critical for the purposes of this invention.

[0177] Solid compositions may be coated by conventional methods, typically with pH or time-dependent coatings, such that a disclosed compound is released in the gastrointestinal tract in the vicinity of the desired application, or at various points and times to extend the desired action. The coatings typically include one or more components selected from the group consisting of cellulose acetate phthalate, polyvinyl acetate phthalate, hydroxypropyl methyl cellulose phthalate, ethyl cellulose, EUDRAGIT® coatings (available from Evonik Industries of Essen, Germany), waxes and shellac.

[0178] Compositions for oral administration can have liquid forms. For example, suitable liquid forms include aqueous solutions, emulsions, suspensions, solutions reconstituted from non-effervescent granules, suspensions reconstituted from non-effervescent granules, effervescent preparations reconstituted from effervescent granules, elixirs, tinctures, syrups, and the like. Liquid orally administered compositions typically include a disclosed compound

and a carrier, namely, a carrier selected from diluents, colorants, flavors, sweeteners, preservatives, solvents, suspending agents, and surfactants. Peroral liquid compositions preferably include one or more ingredients selected from colorants, flavors, and sweeteners.

[0179] Other compositions useful for attaining systemic delivery of the subject compounds include sublingual, buccal and nasal dosage forms. Such compositions typically include one or more of soluble filler substances such as diluents including sucrose, sorbitol, and mannitol; and binders such as acacia, microcrystalline cellulose, carboxymethyl cellulose, and hydroxypropyl methylcellulose. Such compositions may further include lubricants, colorants, flavors, sweeteners, antioxidants, and glidants.

[0180] The disclosed compounds can be topically administered. Topical compositions that can be applied locally to the skin may be in any form including solids, solutions, oils, creams, ointments, gels, lotions, shampoos, leave-on and rinse-out hair conditioners, milks, cleansers, moisturizers, sprays, skin patches, and the like. Topical compositions include: a disclosed compound (e.g., a compound of formula (I)), and a carrier. The carrier of the topical composition preferably aids penetration of the compounds into the skin. The carrier may further include one or more optional components.

[0181] The amount of the carrier employed in conjunction with a disclosed compound is sufficient to provide a practical quantity of composition for administration per unit dose of the compound. Techniques and compositions for making dosage forms useful in the methods of this invention are described in the following references: Modern Pharmaceutics, Chapters 9 and 10, Banker & Rhodes, eds. (1979); Lieberman et al., Pharmaceutical Dosage Forms: Tablets (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms, 2nd Ed., (1976).

[0182] A carrier may include a single ingredient or a combination of two or more ingredients. In the topical compositions, the carrier includes a topical carrier. Suitable topical carriers include one or more ingredients selected from phosphate buffered saline, isotonic water, deionized water, monofunctional alcohols, symmetrical alcohols, aloe vera gel, allantoin, glycerin, vitamin A and E oils, mineral oil, propylene glycol, PPG-2 myristyl propionate, dimethyl isosorbide, castor oil, combinations thereof, and the like. More particularly, carriers for skin applications include propylene glycol, dimethyl isosorbide, and water, and even more particularly, phosphate buffered saline, isotonic water, deionized water, monofunctional alcohols, and symmetrical alcohols.

[0183] The carrier of a topical composition may further include one or more ingredients selected from emollients, propellants, solvents, humectants, thickeners, powders, fragrances, pigments, and preservatives, all of which are optional.

[0184] Suitable emollients include stearyl alcohol, glyceryl monoricinoleate, glyceryl monostearate, propane-1,2-diol, butane-1,3-diol, mink oil, cetyl alcohol, isopropyl isostearate, stearic acid, isobutyl palmitate, isocetyl stearate, oleyl alcohol, isopropyl laurate, hexyl laurate, decyl oleate, octadecan-2-ol, isocetyl alcohol, cetyl palmitate, di-n-butyl sebacate, isopropyl myristate, isopropyl palmitate, isopropyl stearate, butyl stearate, polyethylene glycol, triethylene glycol, lanolin, sesame oil, coconut oil, arachis oil, castor oil, acetylated lanolin alcohols, petroleum, mineral oil, butyl myristate, isostearic acid, palmitic acid, isopropyl linoleate, lauryl lactate, myristyl lactate, decyl oleate, myristyl myristate, and combinations thereof. Specific emollients for skin include stearyl alcohol and polydimethylsiloxane. The amount of emollient(s) in a skin-based topical composition is typically about 5% to about 95%.

[0185] Suitable propellants include propane, butane, isobutane, dimethyl ether, carbon dioxide, nitrous oxide, and combinations thereof. The amount of propellant(s) in a topical composition is typically about 0% to about 95%.

[0186] Suitable solvents include water, ethyl alcohol, methylene chloride, isopropanol, castor oil, ethylene glycol monoethyl ether, diethylene glycol monobutyl ether, diethylene glycol monoethyl ether, dimethylsulfoxide, dimethyl formamide, tetrahydrofuran, and combinations thereof. Specific solvents include ethyl alcohol and homotopic alcohols. The amount of solvent(s) in a topical composition is typically about 0% to about 95%.

[0187] Suitable humectants include glycerin, sorbitol, sodium 2-pyrrolidone-5-carboxylate, soluble collagen, dibutyl phthalate, gelatin, and combinations thereof. Specific humectants include glycerin. The amount of humectant(s) in a topical composition is typically 0% to 95%.

[0188] The amount of thickener(s) in a topical composition is typically about 0% to about 95%.

[0189] Suitable powders include beta-cyclodextrins, hydroxypropyl cyclodextrins, chalk, talc, fullers earth, kaolin, starch, gums, colloidal silicon dioxide, sodium polyacrylate, tetra alkyl ammonium smectites, trialkyl aryl ammonium smectites, chemically-modified magnesium aluminum silicate, organically-modified montmorillonite clay, hydrated aluminum silicate, fumed silica, carboxyvinyl polymer, sodium carboxymethyl cellulose, ethylene glycol monostearate, and combinations thereof. The amount of powder(s) in a topical composition is typically 0% to 95%.

[0190] The amount of fragrance in a topical composition is typically about 0% to about 0.5%, particularly, about 0.001% to about 0.1%.

[0191] Suitable pH adjusting additives include HCl or NaOH in amounts sufficient to adjust the pH of a topical pharmaceutical composition.

b. Albumin Compositions

[0192] The disclosure further provides compositions comprising albumin nanoparticles. In some embodiments, a compound as disclosed herein is incorporated into compositions comprising an albumin nanoparticle. The albumin nanoparticle compositions and formulations may also include pharmaceutically acceptable carriers, as described above.

[0193] Albumins include the most abundant plasma proteins in mammals and albumins from a large and diverse number of mammals have been characterized by biochemical methods and/or by sequence information. Any natural, synthetic, or engineered albumin may be used in the context of the nanoparticle compositions described herein. In some embodiments, the albumin is human serum albumin.

[0194] In some embodiments, the albumin nanoparticles further comprise one or more cell targeting epitopes. In some embodiments, the epitopes are covalently attached or directly conjugated to the albumin. In some embodiments, the epitopes are crosslinked to the albumin. In select embodiments, the albumin nanoparticles further comprise one or more immune cell epitopes (e.g., B cell and T cell epitopes). The one or more immune cell antigens may facilitate targeting to lymphatic systems. In select embodiments, the albumin nanoparticles further comprise one or more epitopes from a microbiological agent (e.g., *Clostridioides difficile*, *Bacillus anthracis*, *Clostridium botulinum*, *Helicobacter pylori*, *Rotavirus sp.*, *Coronaviridae*).

c. Lipophilic Formulations

[0195] In some embodiments, the compounds disclosed herein are incorporated into lipophilic compositions comprising a liposome, a lipid nanoparticle, a micelle, or the like. In some embodiments, a disclosed compound is encapsulated in the liposome, the lipid nanoparticle, or the micelle. The formulations may also include pharmaceutically acceptable carriers, as described above.

[0196] In some embodiments, the disclosed compounds are incorporated into lipophilic compositions comprising one or more vesicle forming lipids. Methods of making lipophilic compositions include, for example, lipid film hydration, optionally coupled with sonication or extrusion, solvent evaporation (e.g., ethanol injection, ether injection, or reverse phase evaporation), solvent-diffusion method, hot homogenization process, detergent removal methods, or combinations thereof. The disclosed compounds can be combined with the lipid(s) before formation of the vesicles (passive loading) or after vesicle formation (active

loading). The lipophilic compositions may prolong circulation time in vivo, increase stability of the compound, and prevent degradation in the bloodstream. The lipophilic compositions may increase the distribution of the compounds within the lung, breast, pancreas, and spleen.

[0197] Any naturally occurring or synthetic vesicle forming lipid or combinations thereof can be used. The one or more vesicle forming lipids may be selected from di-aliphatic chain lipids, such as phospholipids; diglycerides; di-aliphatic glycolipids; single lipids such as sphingomyelin or glycosphingolipid; steroidal lipids; hydrophilic polymer derivatized lipids; or mixtures thereof.

[0198] Lipophilic compositions of the disclosure may include one or more cationic and/or ionizable lipids, phospholipids, neutral or non-cationic lipids, polyethylene glycol (PEG)-lipid conjugates, and/or sterols. In some embodiments, the lipid nanoparticle comprises a cationic lipid and/or ionizable lipid, a neutral or non-cationic lipid, and cholesterol.

[0199] Cationic and/or ionizable lipids include, for example, amine-containing lipids that can be readily protonated and may have a positive or partial positive charge at physiological pH due to a pKa value between pH 5 and 8. The polar headgroup of the cationic lipids preferably comprises amine derivatives such as primary, secondary, and/or tertiary amines, quaternary ammonium, various combinations of amines, amidinium salts, or guanidine and/or imidazole groups as well as pyridinium, piperazine and amino acid headgroups such as lysine, arginine, ornithine and/or tryptophan. Cationic lipids include, but are not limited to, 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine (DMEPC), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and/or 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), 1,2-dimyristoyl-3-trimethylammonium propane (DMTAP), 2,3-di(tetradecoxy)propyl-(2-hydroxyethyl)-dimethylazanium bromide (DMRIE), didodecyl(dimethyl)ammonium bromide (DDAB), 1,2-dioleyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE), 3β-[N—(N,N'-dimethylamino-ethane)carbamoyl]cholesterol (DC-Chol) or dioleoyl ether phosphatidylcholine (DOEPC). Ionizable lipids include, but are not limited to, 1,2-dioleyloxy-3-dimethylamino-propane (DODMA).

[0200] In some embodiments, the lipophilic compositions comprise a polyethylene glycol (PEG)-lipid conjugate. A PEG-lipid conjugate may include, but is not limited to, PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols, and mixtures thereof. For example, a PEG lipid may be PEG-DMG (1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol), PEG-c-DOMG (R-3-[(ω-methoxy

poly(ethylene glycol)2000)carbamoyl)]-1,2-dimyristyloxylpropyl-3-amine), PEG-DMA (PEG-dimethacrylate), PEG-DLPE (1,2-didodecanoyl-sn-glycero-3-phosphoethanolamine-PEG), PEG-DMPE (PEG- 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), PEG-DPPC (PEG-dipalmitoyl phosphatidylcholine), PEG-N,N-di(tetradecyl)acetamide, or a PEG-DSPE (1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol)) lipid. In some embodiments, the lipid nanoparticle comprises PEG-DMG and/or PEG-N,N-di(tetradecyl)acetamide.

[0201] The sterol may comprise cholesterol, fecosterol, ergosterol, campesterol, sitosterol, stigmasterol, brassicasterol, or a sterol ester, such as cholesteryl hemisuccinate, cholesteryl sulfate, or any other derivatives of cholesterol.

[0202] A neutral or non-cationic lipid may include one or more phospholipids. Phospholipids include a phospholipid moiety and one or more fatty acid moieties. A phospholipid moiety may include, but is not limited to, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, and sphingomyelin. A fatty acid moiety may include, but is not limited to, lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, phytanic acid, arachidic acid, arachidonic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid.

[0203] Phospholipids suitable for use in the compositions may include, but are not limited to, phosphatidylglycerol (PG) including dimyristoyl phosphatidylglycerol (DMPG) and 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG); phosphatidylcholine (PC), including egg yolk phosphatidylcholine, dimyristoyl phosphatidylcholine (DMPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine; phosphatidylethanolamine (PE) including 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-

phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine; phosphatidic acid (PA); phosphatidylinositol (PI); phosphatidylserine (PS); and sphingomyelin (SM).

[0204] The positively charged lipid structures described herein may also include other components typically used in the formation of vesicles (e.g., for stabilization). Examples of such other components includes, without being limited thereto, fatty alcohols, fatty acids, and/or any other pharmaceutically acceptable excipients which may affect the surface charge, the membrane fluidity and assist in the incorporation of the lipid into the lipid assembly.

[0205] The lipophilic compositions can also be targeting, e.g., contain one or more targeting moieties or biodistribution modifiers on the surface. A targeting moiety can be any agent that is capable of specifically binding or interacting with a desired target and are generally known in the art, for example ligands such as folic acid, proteins, antibody or antibody fragments, and the like). In some embodiments, the targeting moiety is an immune cell epitope (e.g., B cell and T cell epitopes). In select embodiments, the targeting moiety comprises one or more epitopes from a microbiological agent (e.g., *Clostridioides difficile*, *Bacillus anthracis*, *clostridium botulinum*, *Helicobacter pylori*, *Rotavirus sp.*, *Coronaviridae*).

[0206] The lipophilic compositions can have any structure, e.g., structures having an inner space sequestered from the outer medium by one or more lipid bilayers, or any microcapsule that has a semi-permeable membrane with a lipophilic central part where the membrane sequesters an interior. In some embodiments, the lipophilic compositions may comprise unilamellar liposomes, having a single lipid layer. The disclosed compounds may be completely or partially located in the interior space of the liposome or completely or partially within the bilayer membrane of the liposome. In some embodiments, the lipophilic compositions comprise micelles.

d. Additional Formulations

[0207] In some embodiments, the disclosed compounds are incorporated into formulations comprising PLA and/or PLGA. PLA or PLGA formulations may be prepared by various methods known in the art such as single/double emulsion-solvent evaporation technique, spray drying, spray freeze drying, supercritical fluid drying, and nanoprecipitation.

[0208] In some embodiments, the disclosed compounds are incorporated into polymeric drug delivery systems formed by polymers of naturally occurring materials (e.g., poly-arginine, chitosan, dextrin, polysaccharides, poly(glycolic acid), poly(lactic acid), and hyaluronic acid) or synthetic polymers (e.g., poly (2-hydroxyethyl methacrylate), poly(N-

isopropyl acrylamide)s, poly(ethylenimine)s, dendritic polymers, and the like). Formulations may be prepared by various methods known in the art such as a single/double emulsion-solvent evaporation technique, spray drying, spray freeze drying, supercritical fluid drying, and nanoprecipitation.

[0209] In some embodiments, the disclosed compounds are incorporated into an organic nanoparticles formed by a peptide, protein, nucleic acid, or any combination thereof.

[0210] In some embodiments, the disclosed compounds are incorporated into an inorganic nanoparticle formed by silica, gold, silver, iron, or the like.

e. Additional Therapeutic Agents

[0211] Any of the above compositions or formulations disclosed herein may further comprise at least one additional therapeutic agent. In some embodiments the at least one additional therapeutic agent comprises an immune modulator, a chemotherapeutic agent, a nucleic acid (e.g., mRNA, aptamers, antisense oligonucleotides, ribozyme nucleic acids, interfering RNAs, antisense and antigene nucleic acids), a decongestant, a steroid, an analgesic, an antimicrobial agent, an immunotherapy, or a combination thereof. In some embodiments, the at least one additional therapeutic agent is selected from a chemotherapeutic agent, an indoleamine 2,3-dioxygenase (IDO) inhibitor, a Stat3 inhibitor, a TLR agonist, PD-1 or PD-L1 antibody, and a phosphatidylinositol 3-kinase (PI3K) inhibitor (e.g., a Class I PI3K inhibitors, an isoform-selective PI3K inhibitor).

[0212] Exemplary immune modulators include: indoleamine 2,3-dioxygenase (IDO) inhibitors and analogs thereof, such as, epacadostat, BMS-986205, indoximod, PF-06840003, and analogs thereof; signal transducer and activator of transcription 3 (Stat3) inhibitors and analogs thereof, such as, SM-36 and its analogs; toll-like receptor (TLR) agonists and analogs thereof, such as, imiquimod, resiquimod, selgantolimod, gardiquimod, SM-360320, TMX-101, TMX-202, TMX-302, TMX-306, GSK2245035, CL097, 852A, AZD-8848, DSP-3025, GS-9620, RO7020531, RO6871765, ANA773, DSP-0509, NJH395, BNT411, TQ-A3334, JNJ-4964, LHC165, CV8102, VTX-1463, VTX-2337, IMO-8400, IMO-3100, IRS-954, and analogs thereof; and statins or other lipid-lowering medications and analogs thereof, such as, atorvastatin, pravastatin, fluvastatin, simvastatin, lovastatin, mevastatin, pitavastatin, rosuvastatin, and analogs thereof.

[0213] In some embodiments, the at least one additional therapeutic agent comprises at least one chemotherapeutic agent. As used herein, the term “chemotherapeutic” or “anti-cancer drug” includes any small molecule or other drug used in cancer treatment or prevention. Chemotherapeutics include, but are not limited to, cyclophosphamide,

methotrexate, 5-fluorouracil, doxorubicin, docetaxel, daunorubicin, bleomycin, vinblastine, dacarbazine, cisplatin, paclitaxel, raloxifene hydrochloride, tamoxifen citrate, abemaciclib, afinitor (Everolimus), alpelisib, anastrozole, pamidronate, anastrozole, exemestane, capecitabine, epirubicin hydrochloride, eribulin mesylate, toremifene, fulvestrant, letrozole, gemcitabine, goserelin, ixabepilone, emtansine, lapatinib, olaparib, megestrol, neratinib, palbociclib, ribociclib, talazoparib, thiotepa, toremifene, methotrexate, and tucatinib.

[0214] In some embodiments, the at least one additional therapeutic agent comprises a polynucleotide or nucleic acid (e.g., ribonucleic acid or deoxyribonucleic acid). The term “polynucleotide,” in its broadest sense, includes any compound and/or substance that is or can be incorporated into an oligonucleotide chain. Exemplary polynucleotides for use in accordance with the present disclosure include, but are not limited to, one or more of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) including messenger mRNA (mRNA), hybrids thereof, RNAi-inducing agents, RNAi agents, siRNAs, shRNAs, miRNAs, antisense RNAs, ribozymes, catalytic DNA, RNAs that induce triple helix formation, aptamers, vectors, etc.

[0215] In some embodiments, the at least one additional therapeutic agent is an RNA. RNAs useful in the compositions and methods described herein can be selected from the group consisting of, but are not limited to, shortmers, antagomirs, antisense RNAs, ribozymes, small interfering RNA (siRNA), asymmetrical interfering RNA (aiRNA), microRNA (miRNA), Dicer-substrate RNA (dsRNA), small hairpin RNA (shRNA), transfer RNA (tRNA), messenger RNA (mRNA), and mixtures thereof.

[0216] In certain embodiments, the at least one additional therapeutic agent is an mRNA. An mRNA may encode any polypeptide of interest, including any naturally or non-naturally occurring or otherwise modified polypeptide. A polypeptide encoded by an mRNA may be of any size and may have any secondary structure or activity. In some embodiments, a polypeptide encoded by an mRNA may have a therapeutic effect when expressed in a cell.

[0217] In other embodiments, the at least one additional therapeutic agent is an siRNA. An siRNA may be capable of selectively knocking down or down regulating expression of a gene of interest. For example, an siRNA could be selected to silence a gene associated with a particular disease, disorder, or condition upon administration to a subject in need thereof of a nanoparticle composition including the siRNA. An siRNA may comprise a sequence that is complementary to an mRNA sequence that encodes a gene or protein of interest. In some embodiments, the siRNA may be an immunomodulatory siRNA.

[0218] In some embodiments, the at least one additional therapeutic agent is an shRNA or a vector or plasmid encoding the same. An shRNA may be produced inside a target cell upon delivery of an appropriate construct to the nucleus. Constructs and mechanisms relating to shRNA are well known in the relevant arts.

[0219] In some embodiments, the at least one additional therapeutic agent is PI3K inhibitor. The PI3K inhibitors may target any class of PI3K, including Class I (e.g., IA and IB), Class II, or Class III. In some embodiments, the PI3K inhibitors comprises isoform-selective PI3K inhibitors, dual pan-Class I PI3K/m-TOR inhibitors, and pan-Class I PI3K inhibitors without significant m-TOR activity. PI3K inhibitors useful in the present compositions and methods include, but are not limited to, IPI-549, idelalisib, copanlisib, duvelisib, alpelisib, leniolisib, umbralisib, buparlisib, taselisib, pictilisib, PX-886, pilaralisib, BEZ235, GSK2126458, GSK2636771, AZD8186, SAR260301, gedatolisib, apitolisib, PQR309, MLN1117, and perifosine.

f. Vaccines

[0220] The compounds and compositions may also be used for vaccines. The vaccines comprise the compound or compositions disclosed and an antigen or a nucleic acid encoding thereof. Suitable antigens include microbial pathogens, bacteria, viruses, proteins, glycoproteins lipoproteins, peptides, glycopeptides, lipopeptides, toxoids, carbohydrates, and tumor-specific antigens. Mixtures of two or more antigens may be employed.

[0221] The antigen can be derived and/or isolated from essentially any desired source depending on the infectious disease, autoimmune disease, condition, cancer, pathogen, or a disease that is to be treated with a given vaccine composition.

[0222] The vaccines described herein may be capable of providing immunity against one or more conditions related to infectious diseases, including but not limited to, influenza, measles, human papillomavirus (HPV), rabies, meningitis, whooping cough, tetanus, plague, hepatitis, and tuberculosis and can include infectious disease derived antigens and/or epitopes, or nucleic acids encoding thereof.

[0223] The vaccines described herein may also direct an immune response against cancer cells and can include tumor cell derived antigens, epitopes, and/or neoepitopes, or portions thereof, or nucleic acids encoding tumor cell derived antigens, epitopes, and/or neoepitopes. Tumor antigens are surface molecules that are differentially expressed in tumor cells relative to non-tumor tissues. Tumor antigens make tumor cells immunologically distinct from normal cells and provide diagnostic and therapeutic targets for human cancers. Tumor antigens have been characterized either as membrane proteins or as altered carbohydrate

molecules of glycoproteins or glycolipids on the cell surface. Cancer cells often have distinctive tumor antigens on their surfaces, such as truncated epidermal growth factor, folate binding protein, epithelial mucins, melanoferrin, carcinoembryonic antigen, prostate-specific membrane antigen, HER2-neu, which are candidates for use in therapeutic cancer vaccines. Because tumor antigens are normal or related to normal components of the body, the immune system often fails to mount an effective immune response against those antigens to destroy the tumor cells. Illustrative cancer types for which this approach can be used include prostate, colon, breast, ovarian, pancreatic, brain, head and neck, melanoma, leukemia, lymphoma, etc.

[0224] In other embodiments, the antigen present in the vaccine composition is not a foreign antigen, but a self-antigen, e.g., the vaccine composition is directed toward an autoimmune disease. Examples of autoimmune diseases include type 1 diabetes, conventional organ specific autoimmunity, neurological disease, rheumatic diseases/connective tissue disease, autoimmune cytopenias, and related autoimmune diseases. Such conventional organ specific autoimmunity may include thyroiditis (Graves+Hashimoto's), gastritis, adrenalitis (Addison's), ovaritis, primary biliary cirrhosis, myasthenia gravis, gonadal failure, hypoparathyroidism, alopecia, malabsorption syndrome, pernicious anemia, hepatitis, anti-receptor antibody diseases and vitiligo. Such neurological diseases may include schizophrenia, Alzheimer's disease, depression, hypopituitarism, diabetes insipidus, sicca syndrome and multiple sclerosis. Such rheumatic diseases/connective tissue diseases may include rheumatoid arthritis, systemic lupus erythematosus (SLE) or Lupus, scleroderma, polymyositis, inflammatory bowel disease, dermatomyositis, ulcerative colitis, Crohn's disease, vasculitis, psoriatic arthritis, exfoliative psoriatic dermatitis, pemphigus vulgaris, Sjogren's syndrome. Other autoimmune related diseases may include autoimmune uveoretinitis, glomerulonephritis, post myocardial infarction cardiotomy syndrome, pulmonary hemosiderosis, amyloidosis, sarcoidosis, aphthous stomatitis, and other immune related diseases, as presented herein and known in the related arts.

[0225] In one embodiment, the antigen in a vaccine composition is a peptide, polypeptide, or immunogenic portion thereof. An "immunogenic portion," as used herein is a portion of a protein that is recognized (e.g., specifically bound) by a B cell and/or T cell surface antigen receptor. Such immunogenic portions generally comprise at least 5 amino acid residues, more preferably at least 10, and still more preferably at least 20 amino acid residues of an antigenic protein or a variant thereof.

[0226] Immunogenic portions of antigen polypeptides may generally be identified using well known techniques, such as those summarized in Paul, Fundamental Immunology, 3rd

ed., 243- 247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T cell lines or clones. As used herein, antisera and antibodies are “antigen-specific” if they specifically bind to an antigen (e.g., they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared using known techniques. An immunogenic portion of a protein is a portion that reacts with such antisera and/or T cells at a level that is not substantially less than the reactivity of the full length polypeptide (e.g., in an ELISA and/or T cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ¹²⁵I-labeled Protein A.

[0227] Peptide and polypeptide antigens may be prepared using any of a variety of well-known techniques. Recombinant polypeptides encoded by DNA sequences may be readily prepared from isolated DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, and higher eukaryotic cells, such as mammalian cells and plant cells. Preferably, the host cells employed are *E. coli*, yeast, or a mammalian cell line such as COS or CHO.

[0228] Portions and other variants of a protein antigen having less than about 100 amino acids, and generally less than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See, Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/ Applied BioSystems Division (Foster City, Calif.), and may be operated according to the manufacturer's instructions.

[0229] In certain embodiments, the nucleic acid encoding the antigen is DNA. Illustrative DNA-based vaccines of this type contain DNA encoding one or more polypeptide antigens, such that the antigen is generated in situ. Alternatively, the vaccine may be an RNA-based vaccine. In certain embodiments, the nucleic acid encoding the antigen is an mRNA. An mRNA may encode any polypeptide antigen of interest, including any naturally or non-naturally occurring or otherwise modified polypeptide. A polypeptide encoded by an mRNA may be of any size and may have any secondary structure or activity. In some embodiments, a polypeptide encoded by the mRNA may stimulate an immune response when expressed in a cell.

[0230] The vaccine compositions of the present disclosure may also contain other compounds, which may be biologically active or inactive. The vaccine or medicament may comprise an adjuvant or immunostimulant, or a polynucleotide encoding an adjuvant or immunostimulant (e.g., an adjuvative polypeptide). Adjuvants and immunostimulants are compounds or compositions that either directly or indirectly stimulate the immune system's response to a co-administered antigen. In some embodiments, the vaccines are not adjuvanted or are self-adjuvanting.

[0231] Suitable adjuvants are commercially available as, for example, Glucopyranosyl Lipid Adjuvant (GLA); Pam3CSK4; Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Mich.); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.); AS-2 (SmithKline Beecham); mineral salts (for example, aluminum, silica, kaolin, and carbon); aluminum salts such as aluminum hydroxide gel (alum), $\text{AlK}(\text{SO}_4)_2$, $\text{AlNa}(\text{SO}_4)_2$, $\text{AlNH}_4(\text{SO}_4)$, and $\text{Al}(\text{OH})_3$; salts of calcium (e.g., $\text{Ca}_3(\text{PO}_4)_2$), iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polynucleotides (for example, poly IC, poly AU acids, and CpG oligodeoxynucleotides (e.g., Class A or B)); polyphosphazenes; cyanoacrylates; polymerase-(DL-lactide-co- glycoside); bovine serum albumin; diphtheria toxoid; tetanus toxoid; edestin; keyhole-limpet hemocyanin; Pseudomonas Toxin A; cholera toxin; pertussis toxin; viral proteins; Quil A; aminoalkyl glucosamine phosphate compounds. In addition, adjuvants such as cytokines (e.g., GM-CSF or interleukin-2, -7, or -12), interferons, or tumor necrosis factor, may also be used as adjuvants. Protein and polypeptide adjuvants may be obtained from natural or recombinant sources according to methods well known to those skilled in the art. When obtained from recombinant sources, the adjuvant may comprise a protein fragment comprising at least the immunostimulatory portion of the molecule.

[0232] Other known immunostimulatory macromolecules which can be used include, but are not limited to, polysaccharides, tRNA, non-metabolizable synthetic polymers such as polyvinylamine, polymethacrylic acid, polyvinylpyrrolidone, mixed polycondensates (with relatively high molecular weight) of 4',4'-diaminodiphenylmethane-3,3'-dicarboxylic acid and 4-nitro-2-aminobenzoic acid (See, Sela, M., *Science* 166: 1365-1374 (1969)) or glycolipids, lipids or carbohydrates.

[0233] In some embodiments, the adjuvantive polypeptide comprises immune activator proteins, such as CD70, CD40 ligand, and constitutively active TLR4, or polycationic peptides (e.g., protamine). In some embodiments, the adjuvantive polypeptide is a flagellin polypeptide. Commercially available mRNA encoding adjuvantive polypeptides are available, for example, as TriMix (See Bonehill, A. et al. *Mol. Ther.* **16**, 1170–1180 (2008), incorporated herein by reference). In some embodiments, the vaccine may comprise at least two separate polynucleotides, one encoding anti-Müllerian hormone receptor II extracellular domain (AMHR2-ED), as described above, and the other encoding an adjuvantive polypeptide (e.g., a flagellin polypeptide or immune activator protein).

[0234] Vaccine preparation is a well-developed art and general guidance in the preparation and formulation of vaccines is readily available from any of a variety of sources. One such example is *New Trends and Developments in Vaccines*, edited by Volier et al. University Park Press, Baltimore, Md., U.S.A. 1978. Vaccine compositions may generally be used for prophylactic and therapeutic purposes.

[0235] The amount of antigen in each vaccine dose is generally selected as an amount which induces an immunoprotective response without significant adverse side effects in typical vaccines. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Of course, the dosage administered may be dependent upon the age, weight, kind of concurrent treatment, if any, and nature of the antigen administered.

[0236] The immunogenic activity of a given amount of a vaccine composition can be readily determined, for example by monitoring the increase in titer of antibody against the antigen used in the vaccine composition (Dalsgaard, K. *Acta Veterinaria Scandinavica* 69: 1-40 (1978)). Another common method involves injecting CD-1 mice intradermally with various amounts of a vaccine composition, later harvesting sera from the mice and testing for anti-immunogen antibody, e.g., by ELISA. These and other similar approaches will be apparent to the skilled artisan.

4. Methods of Use

[0237] The disclosure provides methods for inducing or modulating an immune or inflammatory response. As used herein, the term “modulating” generally refers to the ability to alter, by increasing or decreasing, e.g., directly or indirectly promoting/stimulating/up-regulating or interfering with/inhibiting/down-regulating a specific concentration, level, expression, function or behavior (e.g., of the immune or inflammatory response). In some embodiments, the modulating is an increase and/or decrease of a certain concentration, level, activity, or function relative to a control, or relative to the average level of activity that would generally be expected or relative to a control level of activity.

[0238] Thus, in some embodiments, modulating an immune or inflammatory response refers to the ability of the compounds of the present invention to alter or modulate one or more aspects of the immune or inflammatory response. In some embodiments, the methods polarize macrophages. In some embodiments, the methods induce an interferon response. In some embodiments, the methods activate transcription factors (e.g., STAT6, IRF3) of the innate immune response. In some embodiments, the methods reduce or eliminate regulatory B cells (B_{regs}). In some embodiments, the methods overcome STING resistance. In some embodiments, the methods inhibit PI3K γ . Accordingly, the disclosure also provides methods for reducing or eliminating regulatory B cells (B_{regs}) and methods for inhibiting PI3K γ (e.g., in a subject).

[0239] The disclosure further provides methods for treating a disease or disorder comprising administration of a compound or composition as disclosed herein, to a subject in need thereof. In some embodiments, the subject is a human.

[0240] The disease or disorder may comprise cancer, autoimmune diseases, inflammatory diseases, and infectious diseases.

[0241] In some embodiments, the disease or disorder is an inflammatory disease or disorder. Inflammatory diseases are characterized by activation of the immune system in a tissue or an organ to abnormal levels that may lead to abnormal function and/or disease in the tissue or organ. The inflammatory diseases and disorders that may be treated by the methods of the present invention include, but are not limited to, arthritis, rheumatoid arthritis, asthma, inflammatory bowel disease (Crohn's disease or ulcerative colitis), chronic obstructive pulmonary disease (COPD), allergic rhinitis, vasculitis (polyarteritis nodosa, temporal arteritis, Wegener's granulomatosis, Takayasu's arteritis, or Behcet's syndrome), inflammatory neuropathy, psoriasis, systemic lupus erythematosus (SLE), chronic thyroiditis,

Hashimoto's thyroiditis, Addison's disease, polymyalgia rheumatica, Sjogren's syndrome, or Churg-Strauss syndrome.

[0242] In some embodiments, the disease or disorder is an autoimmune disease or disorder. Autoimmune diseases and disorders refer to conditions in a subject characterized by cellular, tissue and/or organ injury caused by an immunologic reaction of the subject to its own cells, tissues and/or organs. Autoimmune diseases and disorders that may be treated by the methods of the present invention include, but are not limited to, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, glomerulonephritis, Graves' disease, Guillain-Barre, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), irritable bowel disease (IBD), IgA neuropathy, juvenile arthritis, lichen planus, lupus erythematosus, Meniere's disease, mixed connective tissue disease, multiple sclerosis, type 1 or immune-mediated diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatics, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's phenomenon, Reiter's syndrome, Rheumatoid arthritis, sarcoidosis, scleroderma, Sjogren's syndrome, stiff-man syndrome, systemic lupus erythematosus, lupus erythematosus, takayasu arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegener's granulomatosis.

[0243] Some autoimmune disorders are also associated with an inflammatory condition. Examples of inflammatory disorders which are also autoimmune disorders that can be prevented, treated or managed in accordance with the methods of the invention include, but are not limited to, asthma, encephalitis, inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), allergic disorders, pulmonary fibrosis, undifferentiated spondyloarthropathy, undifferentiated arthropathy, arthritis, inflammatory osteolysis, and chronic inflammation resulting from chronic viral or bacterial infections. Examples of the types of psoriasis which can be treated in accordance with the compositions and methods of

the invention include, but are not limited to, plaque psoriasis, pustular psoriasis, erythrodermic psoriasis, guttate psoriasis and inverse psoriasis. Some autoimmune disorders are also associated with immune dysregulatory diseases including, for example, sinopulmonary infections, opportunistic pneumonias, inflammatory bowel disease, autoimmune hepatitis and juvenile idiopathic arthritis, and myelofibrosis.

[0244] In some embodiments, the disease or disorder is cancer. In some embodiments, the cancer comprises a solid tumor. In some embodiments, the cancer is metastatic cancer. In some embodiments, the disclosed compounds, compositions, or methods result in suppression of elimination of metastasis. In some embodiments, the disclosed compounds, compositions, or methods result in decreased tumor growth. In some embodiments, the disclosed compounds, compositions, or methods prevent tumor recurrence.

[0245] The compounds and compositions herein may be useful to treat a wide variety of cancers including carcinoma, sarcoma, lymphoma, leukemia, melanoma, mesothelioma, multiple myeloma, or seminoma. The cancer may be a cancer of the bladder, blood, bone, brain, breast, cervix, colon/rectum, endometrium, head and neck, kidney, liver, lung, lymph nodes, muscle tissue, ovary, pancreas, prostate, skin, spleen, stomach, testicle, thyroid, or uterus. In some embodiments, the cancer is wherein the disease or disorder is breast cancer, pancreatic cancer, lung cancer, or lymphoma. In select embodiments, the cancer is pancreatic cancer.

[0246] In some embodiments, the cancer is invasive and/or metastatic cancer (e.g., stage II cancer, stage III cancer or stage IV cancer). In some embodiments, the cancer is an early stage cancer (e.g., stage 0 cancer, stage I cancer), and/or is not invasive and/or metastatic cancer.

[0247] In some embodiments, the disease or disorder is an infectious disease. Infectious diseases that can be treated or prevented by the methods of the present invention are caused by infectious agents including, but not limited to, viruses, bacteria, fungi, protozoa, helminths, and parasites. The invention is not limited to treating or preventing infectious diseases caused by intracellular or extracellular pathogens. The infectious disease may be derived from: bacteria, such as *Mycobacterium tuberculosis*, *Chlamydia*, *Francisella tularensis*; DNA viruses, such as *Herpesviridae* (herpes simplex virus-1, Kaposi's sarcoma-associated virus and Epstein-Barr virus), *Papillomaviridae* (human papilloma virus), *Adenovirus* and *Hepadnaviridae* (Hepatitis B virus), or RNA viruses, such as *Retroviridae* (human immunodeficiency virus) *Flaviviridae* (Dengue virus, Hepatitis C virus),

Orthomyxoviridae (influenza), and Coronaviridae (human coronavirus and SARS coronavirus).

[0248] The compounds and compositions disclosed herein may be administered to a subject by a variety of methods. In any of the uses or methods described herein, administration may be by various routes known to those skilled in the art, including without limitation oral, inhalation, intravenous, intramuscular, topical, subcutaneous, systemic, and/or intraperitoneal administration to a subject in need thereof.

[0249] The amount of the compounds of the present disclosure required for use in the disclosed methods will vary not only with the particular compound selected but also with the route of administration, the nature and/or symptoms of the disease and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician. The determination of effective dosage levels, that is the dosage levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine methods, for example, human clinical trials, in vivo studies, and in vitro studies. For example, useful dosages can be determined by comparing their in vitro activity, and in vivo activity in animal models.

[0250] Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the modulating effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from in vivo and/or in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, FIPLC assays or bioassays can be used to determine plasma concentrations. Dosage intervals can also be determined using MEC value. Compositions should be administered using a regimen, which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

[0251] It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity or organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the symptoms to be treated and the route of administration. Further, the dose, and perhaps dose frequency, will also vary according to the age, body weight, and response of the

individual patient. A program comparable to that discussed above may be used in veterinary medicine.

[0252] The compounds and compositions disclosed herein can be evaluated for efficacy and toxicity using known methods. For example, the toxicology of a particular compound or a subset of the compounds sharing certain chemical moieties, or a composition thereof, may be established by determining in vitro toxicity towards a cell line, such as a mammalian, and preferably human, cell line. The results of such studies are often predictive of toxicity in animals, such as mammals, or more specifically, humans. Alternatively, the toxicity of particular compounds in an animal model, such as mice, rats, rabbits, dogs, or monkeys, may be determined using known methods. Efficacy may be established using several recognized methods, such as in vitro methods, animal models, or human clinical trials. When selecting a model to determine efficacy, the skilled artisan can be guided by the state of the art to choose an appropriate model, dose, route of administration and/or regime.

[0253] A wide range of second therapies may be used in conjunction with the compounds of the present disclosure. The second therapy may be administration of an additional therapeutic agent or may be a second therapy not connected to administration of another agent. Such second therapies include, but are not limited to, surgery, immunotherapy, radiotherapy.

[0254] The second therapy may be administered at the same time as the initial therapy, either in the same composition or in a separate composition administered at substantially the same time as the first composition. In some embodiments, the second therapy may precede or follow the treatment of the first therapy by time intervals ranging from hours to months.

[0255] In some embodiments, a therapeutically effective amount of a compound disclosed herein, or compositions thereof, is administered alone or in combination with a therapeutically effective amount of at least one additional therapeutic agent. In some embodiments, effective combination therapy is achieved with a single composition or pharmacological formulation that includes both agents, or with two distinct compositions or formulations, administered at the same time or separated by a time interval, wherein one composition includes a compound of this invention, and the other includes the at least one additional therapeutic agent.

[0256] In some embodiments, the at least one additional therapeutic agent comprises an immune modulator, a chemotherapeutic agent, a nucleic acid (e.g., mRNA, aptamers, antisense oligonucleotides, ribozyme nucleic acids, interfering RNAs, antigene nucleic

acids), a decongestant, a steroid, an analgesic, an antimicrobial agent, an immunotherapy, or a combination thereof.

[0257] Exemplary immune modulators include: indoleamine 2,3-dioxygenase (IDO) inhibitors and analogs thereof, such as, epacadostat, BMS-986205, indoximod, PF-06840003, and analogs thereof; signal transducer and activator of transcription 3 (Stat3) inhibitors and analogs thereof, such as, SM-36 and its analogs; toll-like receptor (TLR) agonists and analogs thereof, such as, imiquimod, resiquimod, selgantolimod, gardiquimod, SM-360320, TMX-101, TMX-202, TMX-302, TMX-306, GSK2245035, CL097, 852A, AZD-8848, DSP-3025, GS-9620, RO7020531, RO6871765, ANA773, DSP-0509, NJH395, BNT411, TQ-A3334, JNJ-4964, LHC165, CV8102, VTX-1463, VTX-2337, IMO-8400, IMO-3100, IRS-954, and analogs thereof; and statins or other lipid-lowering medications and analogs thereof, such as, atorvastatin, pravastatin, fluvastatin, simvastatin, lovastatin, mevastatin, pitavastatin, rosuvastatin, and analogs thereof.

[0258] In some embodiments, the at least one additional therapeutic agent comprises at least one chemotherapeutic agent. As used herein, the term “chemotherapeutic” or “anti-cancer drug” includes any small molecule or other drug used in cancer treatment or prevention. Chemotherapeutics include, but are not limited to, cyclophosphamide, methotrexate, 5-fluorouracil, doxorubicin, docetaxel, daunorubicin, bleomycin, vinblastine, dacarbazine, cisplatin, paclitaxel, raloxifene hydrochloride, tamoxifen citrate, abemaciclib, afinitor (Everolimus), alpelisib, anastrozole, pamidronate, anastrozole, exemestane, capecitabine, epirubicin hydrochloride, eribulin mesylate, toremifene, fulvestrant, letrozole, gemcitabine, goserelin, ixabepilone, emtansine, lapatinib, olaparib, megestrol, neratinib, palbociclib, ribociclib, talazoparib, thiotepa, toremifene, methotrexate, and tucatinib. In select embodiments, the chemotherapeutic agent comprises paclitaxel.

[0259] In some embodiments of the methods disclosed herein, the compound or composition can be co-administered with an antimicrobial (e.g., antiviral or antibacterial) agent. In some embodiments, the additional antimicrobial agent is an antiviral agent, including but not limited to, abacavir, acyclovir, adefovir, amantadine, amprenavir, atazanavir, baloxavir marboxil, bictegravir, boceprevir, bulevirtide, cidofovir, cobicistat, daclatasvir, darunavir, delavirdine, didanosine, docosanol, dolutegravir, doravirine, edoxudine, efavirenz, elvitegravir, emtricitabine, enfuvirtide, entecavir, etravirine, famciclovir, fomivirsen, fosamprenavir, foscarnet, ganciclovir, ibacitabine, ibalizumab, idoxuridine, imiquimod, immunovir, indinavir, lamivudine, letermovir, lopinavir, loviride, maraviroc, methisazone, moroxydine, nelfinavir, nevirapine, nexavir, nitazoxanide,

oseltamivir, penciclovir, peramivir, penciclovir, peramivir, pleconaril, podophyllotoxin, raltegravir, remdesivir, ribavirin, rilpivirine, rilpivirine, rimantadine, rintatolimod, ritonavir, saquinavir, simeprevir, sofosbuvir, stavudine, taribavirin, telaprevir, telbivudine, tenofovir (e.g., tenofovir alafenamide or tenofovir disoproxil), tipranavir, trifluridine, tromantadine, umifenovir, valganciclovir, valganciclovir, vicriviroc, vidarabine, zalcitabine, zanamivir, and zidovudine, and any combination thereof.

[0260] In some embodiments, the additional antimicrobial agent is an antibacterial agent. Exemplary antibacterial agents include sulfonamides, amphenicols, spectinomycin, trimethoprim, glycylicyclines, macrolides (e.g., erythromycin, clarithromycin, azithromycin, roxithromycin), oxazolidinones (e.g., linezolid), tetracyclines (e.g., doxycycline, tetracycline, minocycline), β -lactams (e.g., penicillin, methicillin, cloxacillin), carbapenems (e.g., imipenem, meropenem, aztreonam), aminoglycosides (e.g., gentamicin, tobramycin, amikacin), quinolones and fluoroquinolones (e.g., levofloxacin, ciprofloxacin, moxifloxacin), glycopeptides (e.g., vancomycin), polymyxins (e.g., polymyxin, colistin).

[0261] In some embodiments, the second therapy includes immunotherapy. Immunotherapies include chimeric antigen receptor (CAR) T-cell or T-cell transfer therapies, cytokine therapy, immunomodulators, cancer vaccines, or administration of antibodies (e.g., monoclonal antibodies).

[0262] In some embodiments, the immunotherapy comprises administration of antibodies. The antibodies may target antigens either specifically expressed by tumor cells or antigens shared with normal cells. In some embodiments, the immunotherapy may comprise an antibody targeting, for example, CD20, CD33, CD52, CD30, HER (also referred to as erbB or EGFR), VEGF, CTLA-4 (also referred to as CD152), epithelial cell adhesion molecule (EpCAM, also referred to as CD326), and PD-1/PD-L1. Suitable antibodies include, but are not limited to, rituximab, blinatumomab, trastuzumab, gemtuzumab, alemtuzumab, ibritumomab, tositumomab, bevacizumab, cetuximab, panitumumab, ofatumumab, ipilimumab, brentuximab, pertuzumab, and the like). In some embodiments, the additional therapeutic agent may comprise anti-PD-1/PD-L1 antibodies, including, but not limited to, pembrolizumab, nivolumab, cemiplimab, atezolizumab, avelumab, durvalumab, and ipilimumab. The antibodies may also be linked to a chemotherapeutic agent. Thus, in some embodiments, the antibody is an antibody-drug conjugate.

[0263] The immunotherapy (e.g., administration of antibodies) may be administered to a subject by a variety of methods. In any of the uses or methods described herein, administration may be by various routes known to those skilled in the art, including without

limitation oral, inhalation, intravenous, intramuscular, topical, subcutaneous, systemic, and/or intraperitoneal administration to a subject in need thereof. The immunotherapy may be administered by parenteral administration (including, but not limited to, subcutaneous, intramuscular, intravenous, intraperitoneal, intracardiac and intraarticular injections). In some embodiments, the immunotherapy may be administered in the same or different manner than the disclosed compounds or compositions.

5. Kits

[0264] In another aspect, the disclosure provides kits comprising at least one disclosed compound or a pharmaceutically acceptable salt thereof, or a composition comprising the compound or a pharmaceutically acceptable salt thereof, and instructions for using the compound or composition.

[0265] The kits can also comprise other agents and/or products co-packaged, co-formulated, and/or co-delivered with other components. For example, a drug manufacturer, a drug reseller, a physician, a compounding shop, or a pharmacist can provide a kit comprising a disclosed compound and/or product and another agent for delivery to a patient.

[0266] The kits can also comprise instructions for using the components of the kit. The instructions are relevant materials or methodologies pertaining to the kit. The materials may include any combination of the following: background information, list of components, brief or detailed protocols for using the compositions, troubleshooting, references, technical support, and any other related documents. Instructions can be supplied with the kit or as a separate member component, either as a paper form or an electronic form which may be supplied on computer readable memory device or downloaded from an internet website, or as recorded presentation.

[0267] It is understood that the disclosed kits can be employed in connection with the disclosed methods. The kit may further contain containers or devices for use with the methods or compositions disclosed herein. The kits optionally may provide additional components such as buffers and disposable single-use equipment (e.g., pipettes, cell culture plates or flasks).

[0268] The kits provided herein are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging, and the like. Individual member components of the kits may be physically packaged together or separately.

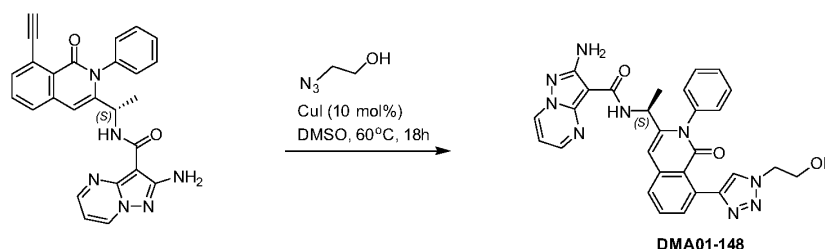
6. Examples

[0269] Abbreviations used in the schemes and examples that follow are: DCM is dichloromethane; DIPEA is N,N-diisopropylethylamine; DMAP is 4-dimethylaminopyridine; DMF is dimethylformamide; DMSO is dimethyl sulfoxide; EtOAc is ethyl acetate; HATU is hexafluorophosphate azabenzotriazole tetramethyl uronium; MeOH is methanol; RT or r.t. is room temperature; TBAF is tetrabutyl ammonium fluoride; THF is tetrahydrofuran; and TBSCl is tert-butyldimethylsilyl chloride.

Example 1

Syntheses of Starting Materials

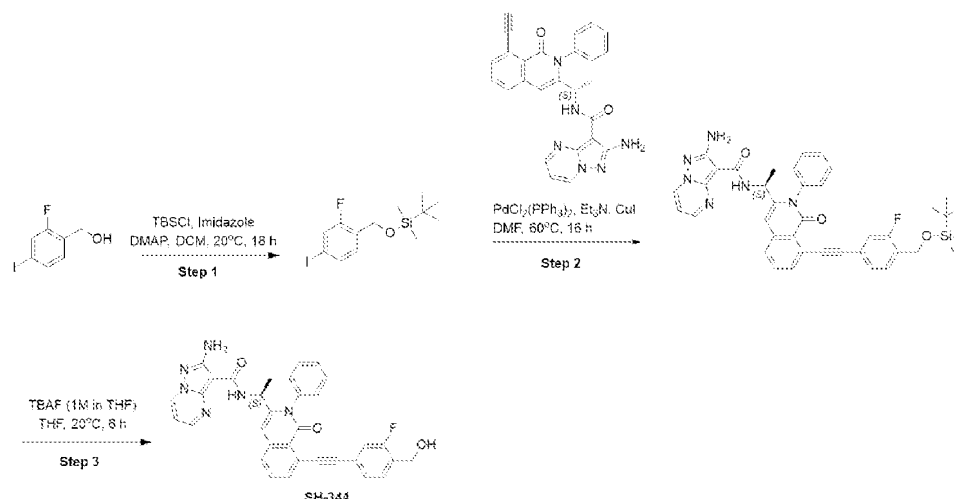
(S)-2-amino-N-(1-(8-(1-(2-hydroxyethyl)-1H-1,2,3-triazol-4-yl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (DMA01-148)



[0270] A screw cap vial was charged (S)-2-amino-N-(1-(8-ethynyl-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (purchased from WuXi AppTec) (2 g, 4.46 mmol, 1.0 equiv.), 2-azidoethanol (3.1 g, 2.72 mL, 35.68 mmol, 8 eq) and CuI (85 mg, 0.45 mmol, 0.1 eq). DMSO (22 mL, 0.2 M) was added and the suspension was purged with Nitrogen for 5 min in sonic bath. The mixture was heated at 60°C for 18 hours. The reactions was cooled down to rt, diluted with brine (300 mL) and extracted with DCM (3 x 200 mL). The organics were combined, dried (Na2SO4), concentrated on celite in vacuo and purified by chromatography on silica gel (80 g cartridge, 0-8% MeOH/DCM) to afford (S)-2-amino-N-(1-(8-(1-(2-hydroxyethyl)-1H-1,2,3-triazol-4-yl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (1.845 g, 73% yield) as an light yellow solid. The product was analyzed by analytical HPLC (Sunfire® C18, 5 µm, 4.6 x 150 mm column, elution conditions 0.1% TFA in water/Methanol 70%, run time 15 min), retention time 2.56 min, purity 97% @254 nm. ¹H NMR (599 MHz, DMSO) δ 8.93 (dd, *J* = 6.7, 1.7 Hz, 1H), 8.56 (dd, *J* = 4.5, 1.6 Hz, 1H), 8.13 (s, 1H), 8.01 (d, *J* = 6.7 Hz, 1H), 7.75 – 7.69 (m, 2H), 7.63 (dd, *J* = 6.6, 2.2 Hz, 1H), 7.56 – 7.51 (m, 1H), 7.50 – 7.43 (m, 2H), 7.42 – 7.39 (m, 1H), 7.37 – 7.33 (m, 1H), 7.02 (dd,

$J = 6.7, 4.5$ Hz, 1H), 6.81 (s, 1H), 6.43 (s, 2H), 4.99 (t, $J = 5.3$ Hz, 1H), 4.56 (p, $J = 6.8$ Hz, 1H), 4.38 (t, $J = 5.5$ Hz, 2H), 3.77 (q, $J = 5.5$ Hz, 2H), 1.36 (d, $J = 6.8$ Hz, 3H). Contained 4.5wt% of DCM.

(S)-2-amino-N-(1-(8-((3-fluoro-4-(hydroxymethyl)phenyl)ethynyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (SH-344)



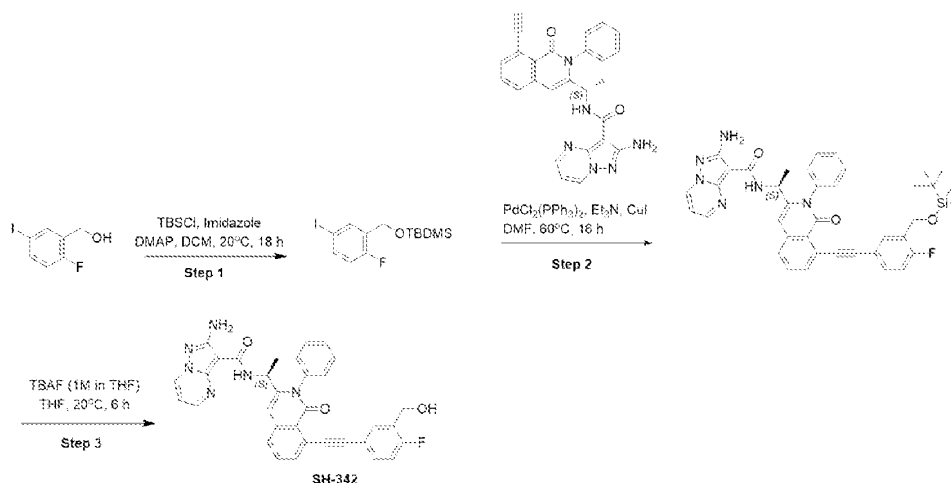
[0271] Step 1: A solution of (2-fluoro-4-iodophenyl)methanol (3 g, 11.9 mmol, 1 eq), TBSCl (2.33 g, 15.47 mmol, 1.3 eq), imidazole (1.62 g, 23.81 mmol, 2 eq), DMAP (145 mg, 1.19 mmol, 0.1 eq) in DCM (70 mL, 0.2 M) was stirred at 20°C for 18 hours. The reaction was diluted with sat. NH_4Cl (150 mL) and extracted with DCM (2 x 100 mL). The combined organic phases were dried (Na_2SO_4), concentrated in vacuo on celite, and purified by chromatography on silica gel (80 g cartridge, 0-20% EtOAc/n-Hexane) to afford tert-butyl((2-fluoro-4-iodobenzyl)oxy)dimethylsilane (4.17 g, 98% yield) as a colorless oil. ^1H NMR (599 MHz, DMSO) δ 7.62 – 7.57 (m, 2H), 7.25 – 7.19 (m, 1H), 4.69 (s, 2H), 0.88 (s, 9H), 0.08 (s, 6H).

[0272] Step 2: A screw cap vial was charged with (S)-2-amino-N-(1-(8-ethynyl-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (purchased from WuXi AppTec) (2 g, 4.46 mmol, 1.0 equiv.), Bis(triphenylphosphine)palladium(II) dichloride (157 mg, 0.22 mmol, 0.05 eq), Copper iodide (85 mg, 0.45 mmol, 0.1 eq) triethylamine (3.61 g, 5 mL, 35.69 mmol, 8 eq), and tert-butyl((2-fluoro-4-iodobenzyl)oxy)dimethylsilane (1.63 g, 1.22 mL, 4.46 mmol, 1 eq). DMF (25 mL, 0.2 M) was added, the mixture was purged with Nitrogen for 5 min in sonic bath,

and then heated at 60°C for 16 hours. The reaction was cooled down to rt. The orange solution was slowly added to an iced cold aqueous solution of sat. NH_4Cl (400 mL). The yellow solid was filtered off, dissolved in DCM/MeOH (1:1, 150 mL), concentrated on celite in vacuo and purified by chromatography on silica gel (80 g cartridge, 0-4% MeOH/DCM) to afford (S)-2-amino-N-(1-(8-(((4-(((tert-butyl)dimethylsilyl)oxy)methyl)-3-fluorophenyl)ethynyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (1.96 g, 61% yield) as a light-yellow solid. ^1H NMR (599 MHz, DMSO) δ 8.93 (dd, J = 6.8, 1.6 Hz, 1H), 8.55 (dd, J = 4.5, 1.7 Hz, 1H), 8.01 (d, J = 6.7 Hz, 1H), 7.74 – 7.66 (m, 3H), 7.61 – 7.55 (m, 1H), 7.55 – 7.42 (m, 4H), 7.42 – 7.37 (m, 1H), 7.34 (d, J = 1.6 Hz, 1H), 7.28 (d, J = 1.6 Hz, 1H), 7.02 (dd, J = 6.7, 4.6 Hz, 1H), 6.78 (s, 1H), 6.43 (s, br, 2H), 4.75 (s, 2H), 4.56 (p, J = 6.8 Hz, 1H), 1.36 (d, J = 6.8 Hz, 3H), 0.88 (s, 9H), 0.08 (s, 6H). The residue contained 3.4wt% of DMF.

[0273] Step 3: To a solution of (S)-2-amino-N-(1-(8-(((4-(((tert-butyl)dimethylsilyl)oxy)methyl)-3-fluorophenyl)ethynyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (1.95 g, 2.69 mmol, 1.0 eq) in THF (110 mL, 0.03M) was added TBAF (1 M in THF, 4 mL, 4.04 mmol, 1.5 eq). The mixture was stirred at 20°C for 6 hours. The reaction was diluted with saturated NaHCO_3 (150 mL) and extracted with DCM (2 x 150 mL). The organics were combined, dried (Na_2SO_4), concentrated in vacuo and purified by chromatography on silica gel (40 g cartridge, 0-8% MeOH/DCM) to afford (S)-2-amino-N-(1-(8-((3-fluoro-4-(hydroxymethyl)phenyl)ethynyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (1.2 g, 69% yield) as a light-yellow solid. The product was analyzed by analytical HPLC (Sunfire® C18, 5 μm , 4.6 x 150 mm column, elution conditions 0.1% TFA in water/Methanol 80%, run time 15 min), retention time 3.22 min, purity 100% @254 nm. ^1H NMR (599 MHz, DMSO) δ 8.93 (dd, J = 6.7, 1.6 Hz, 1H), 8.55 (dd, J = 4.5, 1.6 Hz, 1H), 8.01 (d, J = 6.7 Hz, 1H), 7.74 – 7.66 (m, 3H), 7.61 – 7.55 (m, 1H), 7.55 – 7.45 (m, 4H), 7.42 – 7.37 (m, 1H), 7.32 (dd, J = 7.8, 1.5 Hz, 1H), 7.25 (dd, J = 10.6, 1.5 Hz, 1H), 7.06 – 6.98 (m, 1H), 6.78 (s, 1H), 6.43 (s, br, 2H), 4.55 (d, J = 7.2 Hz, 3H), 1.36 (d, J = 6.8 Hz, 3H). The residue contained DCM 11wt%.

(S)-2-amino-N-(1-(8-((4-fluoro-3-(hydroxymethyl)phenyl)ethynyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (SH-342)



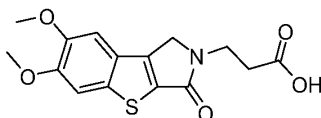
[0274] Step 1: A solution of (2-fluoro-5-iodophenyl)methanol (3 g, 11.90 mmol, 1 eq), TBSCl (2.33 g, 15.47 mmol, 1.3 eq), imidazole (1.62 g, 23.81 mmol, 2 eq), DMAP (145 mg, 1.19 mmol, 0.1 eq) in DCM (70 mL, 0.2 M) was stirred at 20°C for 18 hours. The reaction was diluted with sat. NH_4Cl (150 mL) and extracted with DCM (2 x 100 mL). The combined organic phases were dried (Na_2SO_4), concentrated in vacuo on celite, and purified by chromatography on silica gel (80 g cartridge, 0-20% EtOAc/n-Hexane) to afford tert-butyl((2-fluoro-5-iodobenzyl)oxy)dimethylsilane (3.93 g, 88% yield) as a light pink oil. ^1H NMR (599 MHz, DMSO) δ 7.77 – 7.72 (m, 1H), 7.69 – 7.61 (m, 1H), 7.03 (app t, 1H), 4.71 (s, 2H), 0.89 (s, 9H), 0.09 (s, 6H).

[0275] Step 2: A screw cap vial was charged with (S)-2-amino-N-(1-(8-ethynyl-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (purchased from WuXi AppTec) (2 g, 4.46 mmol, 1.0 equiv.), Bis(triphenylphosphine)palladium(II) dichloride (157 mg, 0.22 mmol, 0.05 eq), Copper iodide (85 mg, 0.45 mmol, 0.1 eq) triethylamine (3.61 g, 5 mL, 35.69 mmol, 8 eq), and tert-butyl((2-fluoro-5-iodobenzyl)oxy)dimethylsilane (1.63 g, 1.22 mL, 4.46 mmol, 1 eq). DMF (25 mL, 0.2 M) was added, the mixture was purged with Nitrogen for 5 min in sonic bath, and then heated at 60°C for 16 hours. The reaction was cooled down to rt. The orange solution was slowly added to an iced cold aqueous solution of sat. NH_4Cl (400 mL). The yellow solid was filtered off, dissolved in DCM/MeOH (1:1, 150 mL), concentrated on celite in vacuo and purified by chromatography on silica gel (80 g cartridge, 0-4% MeOH/DCM) to afford (S)-2-amino-N-(1-(8-(((tert-butyl)dimethylsilyl)oxy)methyl)-4-fluorophenyl)ethynyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-

a]pyrimidine-3-carboxamide (1.44 g, 45% yield) as a light-yellow solid. ¹H NMR (599 MHz, DMSO) δ 8.87 (dd, *J* = 6.8, 1.6 Hz, 1H), 8.49 (dd, *J* = 4.5, 1.6 Hz, 1H), 7.95 (d, *J* = 6.7 Hz, 1H), 7.65 – 7.61 (m, 2H), 7.61 – 7.58 (m, 1H), 7.53 – 7.49 (m, 2H), 7.47 – 7.37 (m, 4H), 7.35 – 7.31 (m, 1H), 7.18 – 7.10 (m, 1H), 6.98 – 6.93 (m, 1H), 6.71 (s, 1H), 6.37 (s, br, 2H), 4.67 (s, 2H), 4.50 (p, *J* = 6.8 Hz, 1H), 1.29 (d, *J* = 6.8 Hz, 3H), 0.80 (s, 9H), 0.00 (s, 6H). The residue contained 2.1wt% of DMF.

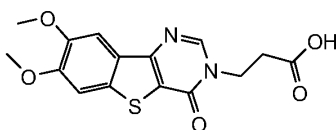
[0276] Step 3: To a solution of (S)-2-amino-N-(1-(8-(((tert-butyl)dimethylsilyl)oxy)methyl)-4-fluorophenyl)ethynyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (1.35 g, 1.86 mmol, 1.0 eq) in THF (37 mL, 0.05M) was added TBAF (1 M in THF, 2.8 mL, 2.8 mmol, 1.5 eq). The mixture was stirred at 20°C for 6 hours. The reaction was diluted with saturated NaHCO₃ (150 mL) and extracted with DCM (2 x 150 mL). The organics were combined, dried (Na₂SO₄), concentrated in vacuo and purified by chromatography on silica gel (40 g cartridge, 0-8% MeOH/DCM) to afford (S)-2-amino-N-(1-(8-((4-fluoro-3-(hydroxymethyl)phenyl)ethynyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (0.99 g, 90% yield) as a light-yellow solid. The product was analyzed by analytical HPLC (Sunfire® C18, 5 μm, 4.6 x 150 mm column, elution conditions 0.1% TFA in water/Methanol 80%, run time 15 min), retention time 3.18 min, purity 100% @254 nm. ¹H NMR (599 MHz, DMSO) δ 8.93 (dd, *J* = 6.8, 1.7 Hz, 1H), 8.55 (dd, *J* = 4.5, 1.6 Hz, 1H), 8.01 (d, *J* = 6.7 Hz, 1H), 7.71 – 7.63 (m, 3H), 7.62 – 7.55 (m, 2H), 7.55 – 7.46 (m, 3H), 7.46 – 7.37 (m, 2H), 7.21 – 7.15 (m, 1H), 7.04 – 6.98 (m, 1H), 6.77 (s, 1H), 6.43 (s, br, 2H), 4.56 (p, *J* = 6.8 Hz, 1H), 4.52 (s, 2H), 1.36 (d, *J* = 6.8 Hz, 3H).

3-(6,7-dimethoxy-3-oxo-1,3-dihydro-2H-benzo[4,5]thieno[2,3-c]pyrrol-2-yl)propanoic acid



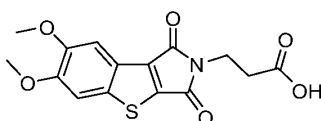
[0277] This compound was prepared as described in International Patent Application No. PCT/US2024/053620 (see compound 12d therein), which is incorporated herein by reference.

3-(7,8-dimethoxy-4-oxobenzo[4,5]thieno[3,2-d]pyrimidin-3(4H)-yl)propanoic acid (SH-250)



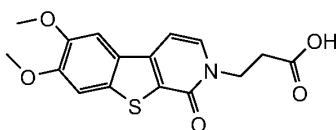
[0278] Compound SH-250 was prepared as described in International Patent Application No. PCT/US2024/053620 (see compound 28c therein).

3-(6,7-dimethoxy-1,3-dioxo-1,3-dihydro-2H-benzo[4,5]thieno[2,3-c]pyrrol-2-yl)propanoic acid (ZSA-51)



[0279] Compound ZSA-51 was prepared as described in International Patent Application No. PCT/US2024/053620 (see compound 23 therein).

3-(6,7-dimethoxy-1-oxobenzo[4,5]thieno[2,3-c]pyridin-2(1H)-yl)propanoic acid

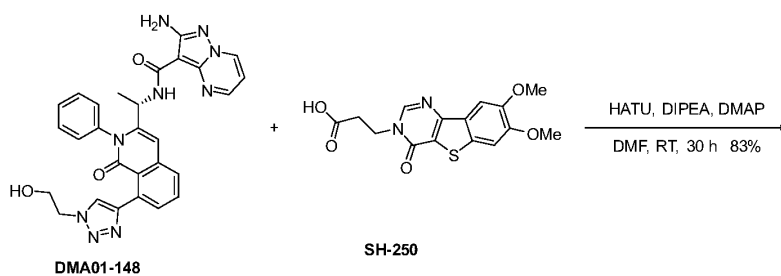


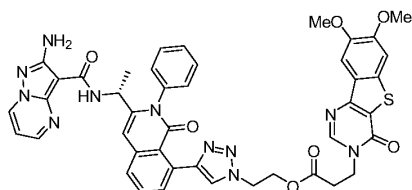
[0280] This compound was prepared as described in International Patent Application No. PCT/US2024/053620 (see compound 39 therein).

Example 2

Compound Syntheses

Synthesis of SH-269

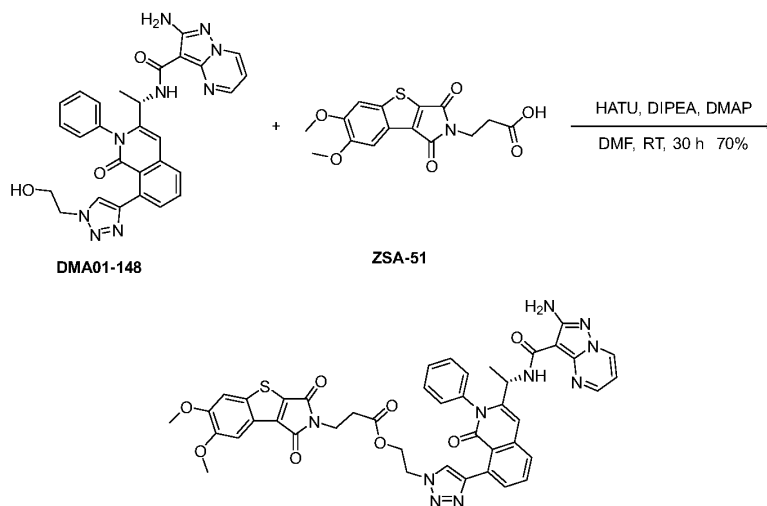




SH-269

[0281] A solution of **DMA01-148** (176 mg, 0.33 mmol), **SH-250** (115 mg, 0.34 mmol), HATU (188 mg, 0.495 mmol), DIPEA (63.86 mg, 0.495 mmol), and DMAP (4 mg, 0.033 mmol) in DMF (1.5 mL) was stirred at room temperature for 38 h. Upon completion of the reaction, the mixture was concentrated to yield a gray powder, which was purified using column chromatography (20:1 ethyl acetate/methanol) to provide the title compound **SH-269** (233 mg, 83% yield) as a white powder. ^1H NMR (500 MHz, CDCl_3) δ 8.38 (dd, $J = 6.7, 1.8$ Hz, 1H), 8.35 (dd, $J = 4.5, 1.7$ Hz, 1H), 8.09 (s, 1H), 7.97 (s, 1H), 7.87 (d, $J = 7.0$ Hz, 1H), 7.80 (dd, $J = 7.5, 1.3$ Hz, 1H), 7.62 – 7.52 (m, 2H), 7.45 (dd, $J = 8.0, 1.2$ Hz, 1H), 7.35 (ddd, $J = 10.1, 6.4, 2.1$ Hz, 2H), 7.32 – 7.25 (m, 2H), 7.23 (s, 1H), 6.74 (dd, $J = 6.8, 4.4$ Hz, 1H), 6.63 (s, 1H), 5.51 (s, 2H), 4.70 (t, $J = 6.9$ Hz, 1H), 4.52 (q, $J = 4.3, 3.9$ Hz, 2H), 4.41 (td, $J = 5.2, 1.7$ Hz, 2H), 3.99 – 3.88 (s, 6H), 2.73 – 2.68 (m, 2H), 1.33 (d, $J = 6.8$ Hz, 3H).

Synthesis of SH-273

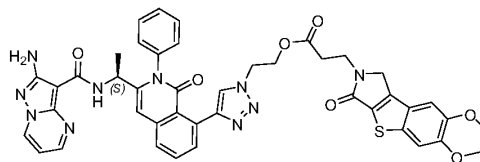


SH-273

[0282] A solution of **DMA01-148** (66.9 mg, 0.125 mmol), **ZSA-52** (44 mg, 0.13 mmol), HATU (71.3 mg, 0.187 mmol), DIPEA (24.2 mg, 0.187 mmol), and DMAP (15 mg, 0.125

mmol) in DMF (1 mL) was stirred at room temperature for 38 h. Upon completion of the reaction, the mixture was concentrated to yield a dark yellow powder, which was purified using column chromatography (20:1 ethyl acetate/methanol) to provide the title compound **SH-273** (74.55 mg, 70% yield). as a yellow powder. ^1H NMR (599 MHz, CDCl_3) δ 8.40 (dd, $J = 6.7, 1.7$ Hz, 1H), 8.36 (dd, $J = 4.5, 1.6$ Hz, 1H), 8.12 (s, 1H), 7.95 (s, 1H), 7.88 (d, $J = 7.0$ Hz, 1H), 7.78 (dd, $J = 7.6, 1.4$ Hz, 1H), 7.55 (t, $J = 7.7$ Hz, 1H), 7.43 (d, $J = 8.9$ Hz, 3H), 7.39 – 7.33 (m, 3H), 7.23 (s, 2H), 6.75 (dd, $J = 6.8, 4.5$ Hz, 1H), 6.62 (s, 1H), 5.53 (s, 2H), 4.72 (t, $J = 6.9$ Hz, 1H), 4.57 – 4.49 (m, 2H), 4.43 (t, $J = 5.2$ Hz, 2H), 3.92 (s, 6H), 3.73 – 3.67 (m, 1H), 3.68 – 3.60 (m, 2H), 3.11 (dd, $J = 7.5, 4.3$ Hz, 1H), 2.73 (s, 3H), 2.48 (t, $J = 6.5$ Hz, 2H), 1.40 (dd, $J = 6.9, 5.0$ Hz, 4H), 1.35 (dd, $J = 9.4, 6.7$ Hz, 4H).

Synthesis of DMA02-187



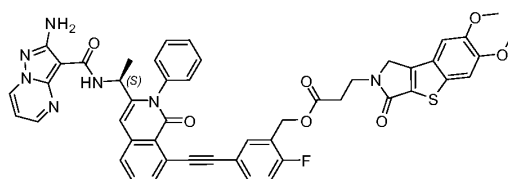
DMA02-187

[0283] A mixture of (S)-2-amino-N-(1-(8-(1-(2-hydroxyethyl)-1H-1,2,3-triazol-4-yl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (DMA01-148) (100 mg, 0.19 mmol, 1 eq), 3-(6,7-dimethoxy-3-oxo-1,3-dihydro-2H-benzo[4,5]thieno[2,3-c]pyrrol-2-yl)propanoic acid (65 mg, 0.16 mmol, 0.87 eq), EDC.HCl (38 mg, 0.2 mmol, 1.07 eq), DMAP (5 mg, 0.04 mmol, 0.2 eq) in DCM (5 mL, 0.04 M) was stirred at 50°C for 14 hours. The reaction was cooled down to rt, diluted with sat. NH_4Cl (75 mL) and extracted with DCM (3 x 75 mL). The organics were dried (Na_2SO_4), concentrated on celite under reduced pressure, and purified by chromatography on silica gel (12 g cartridge, 0-8% MeOH/DCM). The fractions containing the product were combined, concentrated on celite, and purified by reverse phase (40 g column, RediSep Gold® C18, 5-60% ACN/0.1% formic acid in water) to afford (S)-2-(4-(3-(1-(2-aminopyrazolo[1,5-a]pyrimidine-3-carboxamido)ethyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-8-yl)-1H-1,2,3-triazol-1-yl)ethyl 3-(6,7-dimethoxy-3-oxo-1,3-dihydro-2H-benzo[4,5]thieno[2,3-c]pyrrol-2-yl)propanoate (50 mg, 35% yield) as a light yellow solid. The product was analyzed by analytical HPLC (Sunfire® C18, 5 μm , 4.6 x 150 mm column, elution conditions 0.1% TFA in water/Methanol 80%, run time 15 min), retention time 2.51 min, purity 100% @254 nm. ^1H NMR (599 MHz, DMSO) δ 8.92 (dd, $J = 6.8, 1.6$ Hz, 1H), 8.55 (dd, $J = 4.5, 1.6$ Hz, 1H),

8.19 (s, 1H), 8.00 (d, $J = 6.7$ Hz, 1H), 7.72 – 7.64 (m, 3H), 7.59 (dd, $J = 6.2, 2.6$ Hz, 1H), 7.51 – 7.46 (m, 1H), 7.46 – 7.35 (m, 4H), 7.35 – 7.30 (m, 1H), 7.03 – 6.98 (m, 1H), 6.79 (s, 1H), 6.42 (s, 2H), 4.67 – 4.60 (m, 2H), 4.56 (p, $J = 6.8$ Hz, 1H), 4.49 – 4.35 (m, 4H), 3.84 (s, 3H), 3.78 (s, 3H), 3.59 (t, $J = 6.7$ Hz, 2H), 2.53 (t, $J = 6.6$ Hz, 2H), 1.34 (d, $J = 6.8$ Hz, 3H).

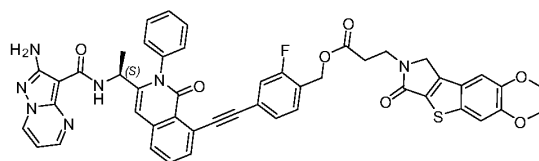
The residue contained 1wt% of DCM.

Synthesis of DMA02-188



DMA02-188

[0284] A mixture of (S)-2-amino-N-(1-(8-((4-fluoro-3-(hydroxymethyl)phenyl)ethynyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (500 mg, 0.87 mmol, 1 eq), 3-(6,7-dimethoxy-3-oxo-1,3-dihydro-2H-benzo[4,5]thieno[2,3-c]pyrrol-2-yl)propanoic acid (305 mg, 0.76 mmol, 0.87 eq), EDC.HCl (179 mg, 0.93 mmol, 1.07 eq), DMAP (21 mg, 0.17 mmol, 0.2 eq) in DCM (22 mL, 0.04 M) was stirred at 50°C for 6 hours. The reaction was cooled down to rt, diluted with sat. NH₄Cl (150 mL) and extracted with DCM (3 x 150 mL). The organics were dried (Na₂SO₄), concentrated on celite under reduced pressure, and purified by chromatography on silica gel (40 g cartridge, 0-8% MeOH/DCM). The fractions containing the product were combined, concentrated on celite, and purified by reverse phase (100 g column, RediSep Gold® C18, 5-60% ACN/0.1% formic acid in water) to afford (S)-5-((3-(1-(2-aminopyrazolo[1,5-a]pyrimidine-3-carboxamido)ethyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-8-yl)ethynyl)-2-fluorobenzyl 3-(6,7-dimethoxy-3-oxo-1,3-dihydro-2H-benzo[4,5]thieno[2,3-c]pyrrol-2-yl)propanoate (255 mg, 34% yield) as an off-white solid. ¹H NMR (599 MHz, DMSO) δ 8.93 (dd, $J = 6.8, 1.7$ Hz, 1H), 8.55 (dd, $J = 4.5, 1.7$ Hz, 1H), 8.00 (d, $J = 6.7$ Hz, 1H), 7.71 – 7.62 (m, 4H), 7.59 (dd, $J = 7.2, 2.2$ Hz, 1H), 7.58 – 7.53 (m, 1H), 7.51 – 7.45 (m, 4H), 7.42 (s, 1H), 7.39 – 7.36 (m, 1H), 7.23 – 7.16 (m, 1H), 7.04 – 6.99 (m, 1H), 6.77 (s, 1H), 6.42 (s, 2H), 5.14 (s, 2H), 4.61 – 4.49 (m, 3H), 3.82 (d, $J = 11.8$ Hz, 6H), 3.74 (t, $J = 6.8$ Hz, 2H), 2.73 (t, $J = 6.7$ Hz, 2H), 1.35 (d, $J = 6.8$ Hz, 3H). The residue contained 1.7wt% of MeCN, 2.1wt% of n-hexane and some baseline minor impurities.

Synthesis of DMA02-189**DMA02-189**

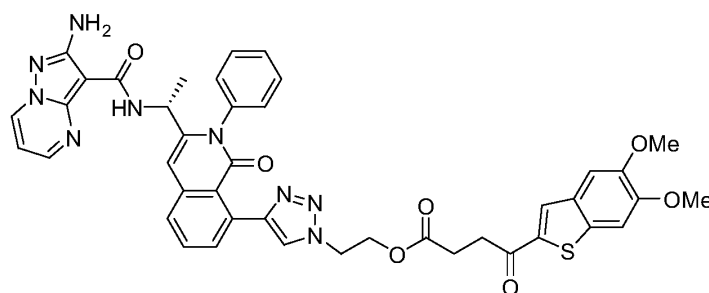
[0285] A mixture of (S)-2-amino-N-(1-(8-((3-fluoro-4-(hydroxymethyl)phenyl)ethynyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (SH-344) (500 mg, 0.87 mmol, 1 eq), 3-(6,7-dimethoxy-3-oxo-1,3-dihydro-2H-benzo[4,5]thieno[2,3-c]pyrrol-2-yl)propanoic acid (305 mg, 0.76 mmol, 0.87 eq), EDC.HCl (179 mg, 0.93 mmol, 1.07 eq), DMAP (21 mg, 0.17 mmol, 0.2 eq) in DCM (22 mL, 0.04 M) was stirred at 50°C for 6 hours. The reaction was cooled down to rt, diluted with sat. NH₄Cl (150 mL) and extracted with DCM (3 x 150 mL). The organics were dried (Na₂SO₄), concentrated on celite under reduced pressure, and purified by chromatography on silica gel (40 g cartridge, 0-8% MeOH/DCM). The fractions containing the product were combined, concentrated on celite, and purified by reverse phase (100 g column, RediSep Gold® C18, 5-60% ACN/0.1% formic acid in water) to afford (S)-4-((3-(1-(2-aminopyrazolo[1,5-a]pyrimidine-3-carboxamido)ethyl)-1-oxo-2-phenyl-1,2-dihydroisoquinolin-8-yl)ethynyl)-2-fluorobenzyl 3-(6,7-dimethoxy-3-oxo-1,3-dihydro-2H-benzo[4,5]thieno[2,3-c]pyrrol-2-yl)propanoate (268 mg, 36% yield) as an off-white solid. ¹H NMR (599 MHz, DMSO) δ 8.93 (dd, *J* = 6.8, 1.7 Hz, 1H), 8.55 (dd, *J* = 4.5, 1.7 Hz, 1H), 8.01 (d, *J* = 6.7 Hz, 1H), 7.74 – 7.65 (m, 4H), 7.57 (td, *J* = 7.4, 2.3 Hz, 1H), 7.55 – 7.46 (m, 3H), 7.46 – 7.38 (m, 3H), 7.31 – 7.22 (m, 2H), 7.01 (dd, *J* = 6.7, 4.5 Hz, 1H), 6.79 (s, 1H), 6.43 (s, 2H), 5.15 (s, 2H), 4.56 (d, *J* = 3.3 Hz, 3H), 3.83 (d, *J* = 1.3 Hz, 6H), 3.76 (t, *J* = 6.8 Hz, 2H), 2.76 (t, *J* = 6.8 Hz, 2H), 1.36 (d, *J* = 6.8 Hz, 3H).

[0286] Additional compounds can be prepared by analogous methods using appropriate starting materials.

Example 3**Biological Efficacy**

[0287] THP-1 ISG Blue cell analysis is a standard assay to test STING activation in vitro. THP1-Blue™ ISG Cells, which were specifically designed for monitoring the interferon signaling pathway, were obtained from InvivoGen. The assay was developed for the monitoring of Human interferon regulatory factor (IRF) activation by determining the activity

of SEAP reporter construct, which can be readily assessed with QUANTI-Blue™, a SEAP detection reagent. Briefly, 100 μ L of different concentrations of the compounds were incubated with 100 μ L of THP1-Blue™ ISG cell suspension (10^6 cells/mL) for 24 hrs at 37 °C. The SEAP levels were measured using Synergy 2 microplate reader (Biotek) at 620 nm in absorbance model. The results indicated that SH-269 showed similar bioactivities compared with MSA-2 in THP1-Blue™ ISG cells and SH-273 showed better bioactivity (FIG. 1). Bioactivity of DMA01-166 (D166) is also shown.



DMA01-166

[0288] The in vivo efficacy of the compounds was tested using a pancreatic KPC inoculated mice model. Data shows that D166, SH269 and SH-273 have significantly enhanced anticancer efficacy with anti-PD-1 antibody (FIG. 2).

Example 4

Dual Targeting of STING and PI3K γ for Pancreatic Cancer

[0289] Pancreatic cancer is the deadliest type of cancer with a 5-year survival at 11%, but is lacks effective treatment options. The only two available therapies for pancreatic cancer, FOLFIRINOX (leucovorin, fluorouracil, irinotecan, Oxaliplatin) and Abraxane (albumin nanoparticle of paclitaxel)/gemcitabine, have very limited efficacy. Additionally, some pancreatic cancer patients do not respond to the breakthrough immunotherapy using anti-PD-1/PD-L1 due to the immunosuppressive tumor microenvironment (TME). The TME in pancreatic cancer is predominately regulated by myeloid-derived immune cells, such as tumor associated macrophages (TAM), dendritic cells (DCs), myeloid-derived suppressor cells (MDSC), regulatory B cells (B_{regs}). However, effort to remodel the TME using immuno-modulators have not been successful.

[0290] STING agonist has emerged as the most effective immune modulator to remodel the immunosuppressive TME to enhance the efficacy of immunotherapy. However, three challenges remain for STING agonist especially in pancreatic cancer. STING agonists also

strongly induce B_{regs} in pancreatic cancer to elicit strong immunosuppressive TME, leading to an intrinsic resistance to STING agonist. Most STING agonists are used by local intra-tumor injection in clinical trials, which generate local immunity to shrink the local tumors, but fail to stimulate systemic immunity to inhibit distal tumors or metastasis. Intra-tumor injections are not feasible in pancreatic cancer patients. Systemic use of STING agonists may have liver toxicity.

[0291] *STING agonists expand Breg cells in PDAC Mice*

[0292] Recent studies suggest that immunosuppression in PDAC is mediated by both immune suppressive regulatory B cells and myeloid cells. To validate the presence of Breg cells in Pancreatic ductal adenocarcinoma (PDAC) mouse model, their frequency was analyzed in tumors and lymph nodes of KPC (KrasG12D, P53R172H, Pdx1-Cre) transgenic mice, which spontaneously develop pancreatic adenocarcinoma in comparison with normal C57BL/6 mice. The results revealed a significant increase in both IL35⁺ and IL10⁺ Breg cell frequencies in the tumor, lymph nodes of KPC mice compared to normal mice. Specifically, Breg cell frequencies were 4.6 and 36.2 times higher in KPC mouse tumors (10.2% and 19.9%) than in pancreatic tissues of normal mice (2.2% and 0.55%), 32.4 and 310 times higher in KPC mouse lymph nodes (22% and 18.6%) than in normal lymph nodes (0.68% and 0.06%). (FIGS. 3A-3B). Additionally, a high level of M2 macrophages was also observed in the tumor and lymph nodes of PDAC mice (FIGS. 3C-3D).

[0293] Recent studies also suggest that STING agonists further expand Breg cells, contributing to intrinsic STING resistance in PDAC. To confirm this, the in vivo effects of two STING agonists, diABZi and cGAMP, were evaluated in C57BL/6 mice implanted with PDAC KPC 6422 cells (FIG. 10A). Following five doses of diABZi administered either intratumorally (it) or intravenously (iv), with or without anti-PD1 antibody, minimal inhibition of tumor growth (FIG. 10A) was observed, and similar results were observed after cGAMP treatment. Both intratumorally and intravenously administrations of STING agonists further expanded IL35⁺ and IL10⁺ Breg cells in tumor tissues and lymph nodes (FIGS. 10B-10C and 15). This persistence of high-level Breg cells may explain the limited efficacy of STING agonists with or without combined with anti-PD1 in KPC mice, which underscores the importance of eliminating Breg cells to overcome STING resistance in PDAC.

[0294] *STING agonist activated PI3K signaling in B cells, but not in myeloid cells.*

[0295] RNA-seq was used to study differential gene expressions and pathway enrichments with or without STING agonist (MSA2) treatment, in B cells and bone marrow-derived dendritic cells (BMDCs) isolated from transgenic KPC mice with pancreatic cancer (LSL-

KrasG12D, LSL-Trp53R172H/+, Pdx1cre/+) (FIG. 11A and 16A). Interestingly, MSA2 significantly activated PI3K pathway in B cells, in addition to STING signaling that are similar to viral infection. In contrast, MSA2 did not significantly activate PI3K pathways in BMDCs (FIG. 11A and 16A).

[0296] Since PI3K isoforms (PI3K γ and PI3K δ) are predominantly expressed in immune cells¹⁶, the differential gene expression levels of subunits of PI3K γ and PI3K δ was analyzed with or without STING agonist (MSA-2) treatment. The data showed that MSA-2 increased expression of regulatory subunit of PI3K γ (Pik3r5-p101), but not catalytic subunit of PI3K γ (Pik3cg-p110 γ) in B cells (FIG. 16B). In contrast, MSA2 did not significantly alter expression levels of subunits of PI3K γ in BMDCs (FIG. 16C). This result is further confirmed by western blot analysis (FIG. 16D). Moreover, MSA-2 also slightly increased the gene expression of catalytic subunit of PI3K δ (Pik3cd-p110 δ) and regulatory subunits of PI3K δ (Pik3cd-p85 α) in B cells (FIG. 16E), but MSA2 did not significantly change the expression of subunits of PI3K δ in BMDCs (FIG. 16F). To confirm the expressions of STING and PI3K γ in different tissues and immune cells, western blot was used to detect their protein levels. The data showed that STING is present in tissues including tumor and lymph nodes of KPC mice, normal pancreas and normal lymph nodes in C57BL/6 mice, as well as different immune cells including B cells, bone marrow derived macrophages (BMDM), bone marrow derived dendritic cells (BMDC) in both KPC mice and normal mice (FIG. 17). In comparison, PI3K γ and PI3K δ is also expressed in whole tumor tissues (including tumor infiltrated immune cells) and lymph node tissues, as well as different immune cells. (FIG. 17

[0297] *Blocking PI3K γ abolished STING-induced IRF3 phosphorylation in B cells, while PI3K γ inhibition sustained STING-induced IRF3 phosphorylation in myeloid cells.*

[0298] STING activation triggers IRF3 phosphorylation in myeloid cells, enhancing type I interferon expression, while in B cells, IRF3 phosphorylation boosts IL-10 and IL-35 expression, expands B regulatory (Breg) cells. To investigate effect of PI3K γ inhibition on STING-induced IRF3 phosphorylation across cell types, western blot was used to analyze IRF3 phosphorylation in the presence/absence of PI3K γ inhibitor (IPI-549) and STING agonist (MSA-2) in B cells, Bone Marrow derived dendritic cell (BMDC), bone marrow derived macrophage (BMDM), CD4 T cells, CD8 T cells from KPC transgenic mice from KPC mice, as well as THP-1 hSTING^{HAQ} monocyte cell line (FIG. 4A and 18A). STING activation by MSA-2 shows a time-dependent increase of IRF3 phosphorylation in B cells, BMDC and BMDM (FIG. 4A and 19), peaking around 2 to 4 hours. PI3K γ inhibition by IPI-549 completely abolished STING-induced IRF3 phosphorylation in B cells in a concentration

dependent manner (0.5 - 10 μ M). However, PI3K γ inhibition by IPI-549 sustained STING-induced IRF3 phosphorylation in CD4 T, CD8 T, NK, and myeloid cells, including BMDC, BMDM from KPC mice, as well as THP-1 hSTING^{HAQ} monocyte cell line (FIG. 11C, 18B-18C, and 19). In addition, PI3K γ inhibitor IPI-549 did not alter STING-induced IRF3 phosphorylation in human CD4 T, CD8 T and NK cells (FIG. 18C).

[0299] To further study if inhibitors of different PI3K isoform had distinct function to regulate STING-induced IRF3 phosphorylation in B cells, human B cells were treated with PI3K α inhibitor Alpelisib, PI3K β inhibitor TGX-211, PI3K δ inhibitor TGR-1202, PI3K γ inhibitor IPI-549. The IRF3 phosphorylation under combination of STING agonist MSA2 and these PI3K inhibitors were monitored using western blotting (FIG. 11C). Data showed that PI3K γ inhibitor IPI-549 dramatically reduced STING-induced IRF3 phosphorylation in B cells, while PI3K δ inhibitor TGR-1202 also slightly decreased STING-induced IRF3 phosphorylation in B cells. In contrast, PI3K α inhibitor Alpelisib and PI3K β inhibitor TGX-211 did not change STING-induced IRF3 phosphorylation in B cells.

[0300] Inhibitors of these pathway were also tested to examine IRF3 phosphorylation in B cells. Inhibitors targeting MAPK signaling (Raf inhibitor SB 590885, MEK inhibitor Binimetinib, ERK inhibitor Temuterkib) and Calcium signaling (PP2B inhibitor Cyclosporin A, IP3R inhibitor Xestospongine C) did not alter STING-induced IRF3 phosphorylation in B cells (FIG. 11B). Taken together, the data suggest that only PI3K γ inhibitor could significantly abolish STING-induced IRF3 phosphorylation in B cells, and PI3K δ inhibitor has less effect on STING-induced IRF3 phosphorylation in B cells, but inhibitors targeting MAPK pathway and calcium signaling did not alter STING-induced IRF3 phosphorylation in B cells.

[0301] *Blocking PI3K γ eliminated STING-induced Breg cells expansion, while PI3K γ inhibition preserved STING induced myeloid cell activation.*

[0302] To further analyze how did PI3K γ inhibition alter Breg cell populations, flow cytometry was used to monitor IL-35⁺ and IL-10⁺ Breg cells in B cells from PBMC of PDAC patients or healthy people, as well as splenocytes isolated from KPC and STING knockout mice (Tmem173^{-/-}), treated with or without treatment by the STING agonist MSA-2 and the PI3K γ inhibitor IPI-549 (FIGS. 11D-11E, 20 and 21). The data showed that STING agonist (MSA-2) significantly increased IL-35⁺ and IL-10⁺ Breg cells by 2.0 and 2.8 - fold, but PI3K γ inhibitor (IPI-549) eliminated the Breg cells induced by MSA-2 ($p < 0.0001$) in B cells from both PBMCs of PDAC patients (FIGS. 11D-11E), human healthy donor (FIG. 20B), and KPC mice (FIG. 20C). Similar results were observed in standard B cell activation

condition where B cells were pretreated with or without anti-IgM&anti-CD40 (FIG. 21A). To confirm this effect is STING-dependent, IL-35+ and IL-10+ Breg cells were also monitored in the splenocytes from STING knockout mice (Tmem173^{-/-} mice) under treatment of STING agonist (MSA-2) and PI3K γ inhibitor (IPI-549). The data showed that neither MSA-2 nor IPI-549 has any effect on IL-35+ and IL-10+ Breg cells with STING knockout (FIG. 20D and 21B), strongly suggesting this effect is STING dependent. Furthermore, no significant induction of IL-35 and IL-10 was found in BMDCs, BMDMs and THP-1 cells under similar treatment conditions (FIGS. 22-23), suggesting that STING-induced IL-35 and IL-10 expressions are exclusively in B cells.

[0303] To investigate whether PI3K γ inhibition would impact STING activation in myeloid cells, STING induced type I interferon expression was measured in BMDC (FIG. 11F), monocyte THP1 hSTING^{R232} (FIG. 11G), and THP-1-BlueTM ISG (hSTING^{HAQ}) (FIG. 11H), and THP-1 hSTING^{KO} (FIG. 11I) cells. STING agonist MSA-2 stimulated IFN-beta secretion in these STING positive cells but not in STING knockout cells, while PI3K γ inhibition (IPI-549) sustained STING activation in these STING positive cells.

[0304] To study whether PI3K γ inhibition would influence STING induced activation of myeloid cells, dendritic cell activation and M1-macrophage polarization were measured in BMDCs and BMDMs from KPC mice, as well as macrophage cell line RAW264.7 cells. STING agonist (MSA-2), with/without PI3K γ inhibitor IPI-549, activated BMDCs (FIGS. 5G, 11J, and 24A) and BMDMs (FIGS. 5G, 11K and 24B) as measured by CD86+ populations, stimulated M1-macrophage polarization (FIG. 5J and 11L) and increased TNF-alpha (FIG. 11M) secretion in macrophage RAW264.7. PI3K γ inhibition preserved the STING induced activation in these myeloid cells (FIGS. 5J, 11F-11M, and 24).

[0305] In summary, PI3K γ inhibition abolished STING-induced IRF3 phosphorylation in B cells, thereby eliminating Breg cell expansion. However, PI3K γ inhibition sustained STING-induced IRF3 phosphorylation in myeloid cells, promoting type I interferon production and facilitating the activation of myeloid cells.

[0306] *Dual targeting compound SH-273 and its albumin nanoformulation inhibit PI3K γ to eliminates Breg cells and stimulate STING function to activate myeloid cells.*

[0307] To simultaneously activate STING and inhibit PI3K γ , a dual targeting compound (SH-273) was developed and encapsulated into albumin nanoformulation (Nano-273) for intravenous administration to achieve systemic immunity (FIG. 7A). SH-273 was designed with two active moieties, one to inhibit PI3K γ and one to activate STING. Nano-273 helped to deliver SH-273 inside cells. This design of SH-273 and its albumin nanoformulation

(Nano-273) improved STING agonist activity, where Nano-273's IC₅₀ is 0.03 μ M, SH-273's IC₅₀ is 1.67 μ M, and ZSA2's IC₅₀ is 2 μ M (FIG. 26A) in stimulating STING activity in THP-1 Blue ISG cells (STING reporter) (FIG. 12A); SH-273 inhibited the PI3K γ and PI3K δ -induced downstream AKT (473) phosphorylation in BMDC and BMDM at 400 nM (FIG. 12B, and 25A-25B), but did not alter PI3K α and PI3K β -induced AKT phosphorylation (FIG. 25C). The IC₅₀ of DMA01-148 (active moiety of SH-273) to inhibit four isoforms of PI3K α , β , δ , γ are 1920, 2012, 40, and 20 nM, respectively (IC₅₀) (Table 1)

Table 1. Binding IC₅₀ of PI3K isoforms for IPI-549, TGR1202, SH-273, and DMA01-148 (released active moiety of SH-273 to inhibit PI3K).

Compounds	IPI-549	TGR-1202	DH-273	DMA01-148
PI3K γ IC ₅₀ (nM)	5.468	3395	1083	20.24
PI3K δ IC ₅₀ (nM)	968.8	28.4	855.4	39.59
PI3K β IC ₅₀ (nM)	1566	>10000	>10000	2012
PI3K α IC ₅₀ (nM)	1248	>10000	>10000	1920

[0308] SH-273 abolished the STING-induced IRF3 phosphorylation in B cells from splenocytes of KPC PDAC mice (FIG. 12C and 27A). SH-273 eliminated STING-induced expansion of IL35⁺ and IL10⁺ Breg cells in B cells of PBMC from human PDAC and healthy donor (FIGS. 12D-12E and 27B-27C) and the B cells from splenocytes of KPC transgenic mice (FIG. 12F). As a control, SH-273 did not show such effect in the splenic B cells from STING knockout mice (Tmem173^{-/-}), suggesting SH-273 effect is indeed STING dependent (FIG. 12G). In contrast, SH-273 sustained activity to stimulate IRF3 phosphorylation in myeloid cells, and thus SH-273 preserved STING function to activate myeloid cells (FIG. 26B).

[0309] An albumin nanoformulation of SH-273 (Nano-273) was prepared for intravenous administration and effective delivery to tumor and lymph nodes. Nano-273 showed a uniform morphology with an average hydrodynamic diameter of 135 nm, a polydispersity index (PDI) of 0.095, maintaining stable even after 1000-fold dilution (FIG. 6A and 7B). Nano-273 showed effective delivery to pancreatic tumors and lymph nodes compared to SH-273 in transgenic KPC PDAC mice (FIGS. 6A and 28). The active moieties of DMA01-148 and ZSA-2 from Nano-273 in the tumor and lymph nodes were also significantly higher than that from SH273 (FIGS. 6A and 28). 2D and 3D confocal imaging showed that albumin nanoformulation of fluorescent dye delivered more and penetrate deeper into tumor tissue and tumor organoids from KPC PDAC mice than free fluorescent dye (FIG. 29). 2D confocal imaging revealed albumin nanoformulation of fluorescent dye delivered more and deeper into

lymph nodes of KPC PDAC mice than free fluorescent dye (FIG. 30). 3D confocal imaging showed albumin nanoformulation of fluorescent dye penetrated deep into the lymph nodes with accumulation in the macrophages and B cells.

[0310] *Nano-273 extended median survival to 201 days in KPC PDAC mice through activating systemic immunity without exhibiting toxicity.*

[0311] To assess the in vivo efficacy of Nano-273, Nano-273 was tested in combination with anti-PD1 antibody in transgenic KPC PDAC mice with spontaneous pancreatic tumors. The data showed that the combination of Nano-273 and anti-PD1 extended median survival to 201 days compared to control group (median survival at 120 days). MSA-2 combined with anti-PD1 slightly extended median survival to 146 days. Neither the anti-PD1 antibody alone nor combined with the albumin nanoformulation of paclitaxel (Nano-P) significantly improved median survival of KPC transgenic mice. (FIG. 7C and 13A). Nano-273 combined with anti-PD1 showed superior efficacy in KPC transgenic mice compared to other treatment groups (FIG. 31), which includes combination of IPI549, MSA-2 plus anti-PD1 and Nano-P and IPI549 plus anti-PD1. At the endpoint of the study, the metastasis in the lung, liver, spleen, and kidney tissues was also examined. Notably, Nano-273 substantially decreased tumor metastasis and local invasion to lung (FIG. 13B).

[0312] In addition, the efficacy of Nano-273 was also tested in xenograft model using KPC 6422 cells in C57BL/6 mice. Nano-273 significantly delayed tumor growth compared to the MSA-2, with or without combination with anti-PD1 antibody (FIG. 13C). Nano-273 demonstrated superior efficacy over free SH-273, in combination with anti-PD1 antibody (FIG. 6D). SH-273, in combination with anti-PD1 antibody increased DC activation in BMDC, M1 macrophage polarization, as well as serum concentration of IFN β and IFN α in KPC transgenic mice (FIGS. 13D-13F). MSA-2 significantly enhanced the IL-35 and IL-10 serum level, while SH-273 eliminated the IL-35 and IL-10 in serum (FIG. 13G). The serum cytokine results were also confirmed in KPC xenograft mice mode after treatment from FIG. 13C. Nano-273 plus anti-PD1 largely decreased serum IL35 and IL10 cytokines, but maintained a similar level of IFN- β and TNF- α level compared to MSA-2 plus anti-PD1 (FIGS. 31C-31D). SH-273 can preserve STING activation function but overcome the STING resistance due to IL-35 and IL-10 secretion.

[0313] In order to evaluate the systemic anticancer immunity of Nano-273 through intravenous administration, the anticancer efficacy of Nano-273 to inhibit bilaterally inoculated KPC 6422 cells in C57BL/6 mice was compared for intravenous (iv), subcutaneous (sc), or intratumorally (it) injections, (FIG. 13H). Intratumorally injections of

Nano-273 only suppressed local tumor growth, but had minimal inhibition to the tumor at the distal site, highlighting the importance of using STING agonists systematically to induce systemic immunity. Indeed, both intravenous and subcutaneous injections of Nano-273 elicited robust systemic immune responses, significantly reducing tumor burden at both local and distal sites (FIG. 9B and 33).

[0314] To investigate how systemic delivery of Nano-273 achieves systemic immunity in comparison with intratumoral injection, flow cytometry was used to analyze the M1-macrophage polarization in local, distal tumors and lymph nodes. The data showed that Nano-273 (iv and sc) significant increased M1 macrophages at distal tumors and lymph nodes, while intra-tumoral injection of Nano-273 did not increase M1-macrophage in either distal tumors or lymph nodes (FIG. 13I). To confirm if systemic efficacy of Nano-273 indeed depends on the systemic immune cell trafficking, fingolimod (FTY-720) was used to inhibit lymphocyte egress from lymph nodes, which reversed effect of Nano-273 after iv administration (FIGS. 34A-34B). Fingolimod also decreased immune infiltration in distal tumor and reduced M1 macrophage in the lymph nodes and distal tumor sites (FIGS. 34C-34E). These data suggested that systemic delivery of Nano-273 achieve systemic immunity for its superior systemic anticancer efficacy in KPC PDAC mice.

[0315] To confirm if systemic delivery of Nano-273 will not cause toxicity in major organs, extensive single dose acute toxicity and multiple dose chronic 28-day toxicity studies were performed. At the therapeutic dose regimen, Nano-273 and SH273 (iv injection five doses of Nano-273 and SH273 at 15 mg/kg) did not show any toxicity in the liver, kidney, and blood cells as measured by liver enzymes (AST, GST), kidney function (BUN, creatinine) and complete blood cell counts (FIG. 35). Pathological staining of major organs (liver, kidney, spleen, lung, and heart) also did not show toxicity as indicated healthy tissue structures (FIG. 36). To perform single dose acute toxicity in C57BL/6 mice, a high single intravenous dose of Nano-273 (200 mg/kg) was administered to 3 mice, with body weight monitored daily for 10 days. No acute toxicity was observed. All mice maintained their body weight throughout the study (FIG. 37A). Toxicity studies were performed in Es1^{-/-} mice (B6.Cg-Ces1c^{tm1.1Loc}/J) that better mimic human condition using Nano-273 with single dose of 100, 150, or 200 mg/kg and multiple dose 15, 50, and 100 mg/kg every three days for 28 days in Es1^{-/-} mice. No signs of toxicity were observed at doses ranging from 15 – 150 mg/kg, as evidenced by stable body weights (FIGS. 37B-37E), normal complete blood counts (CBCs) (white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells, platelets, hematocrit) (FIG. 38), normal blood chemistry (alanine

aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, hemoglobin, creatine, blood urine nitrogen, albumin, glucose, calcium (FIG. 39), clinical observations, and pathology examinations (FIG. 40) of major organs in brain, heart, lung, liver, kidney, spleen.

[0316] *Nano-273 eliminated STING-induced Breg cells expansion and remodeled immune microenvironment in tumor and lymph nodes for systemic anticancer immunity.*

[0317] Flow cytometry was used to examine immune cell infiltration following the treatments of MSA-2, SH-273, or Nano-273, in combination with anti-PD1 antibody. Both Nano-273 and SH-273 significantly increased immune cell infiltration to tumors compared to STING agonist (MSA-2) alone (FIG. 14A). Subsequently, single-cell RNA-sequencing (RNA-Seq) was employed to analyze all immune cells in the tumor tissues post-treatments. Immune cell phenotypes were identified based on RNA expression levels and corroborated by TotalSeq™-C antibody surface staining (FIG. 6E and 14B), which showed increased B and CD8 T cell populations. The data showed that both Nano-273 and SH-273 significantly decreased IL35⁺ and IL10⁺ Breg cells, while increased ICOSL⁺ B cell populations in tumors (FIG. 14C-14D). In contrast, STING agonist (MSA-2) significantly increased IL35⁺ and IL10⁺ Breg cells in tumors (FIG. 14C-14D).

[0318] To verify these findings in single cell RNA-seq, flow cytometry was used to assess Breg cell frequencies in tumors, lymph nodes, and spleens post-treatment in xenograft model inoculated with KPC cells. The Nano-273 plus anti-PD1 significantly decreased Breg cell frequencies by 4.2 to 8.9 - folds in tumors (IL35⁺ Breg cell: 4.6% vs 40.0% and 27.9%, IL10⁺ Breg cell 7.3% vs 43.4% and 30.3%), 2.4 to 5.4 - folds in lymph nodes (IL35⁺ Breg cell: 2.3% vs 12.2% and 5.4%; IL10⁺ Breg cell: 2.1% vs 11.0% and 4.9%) in comparison with MSA-2 plus anti-PD1 and anti-PD1 groups in tumors (FIG. 14E). The results were also confirmed in KPC transgenic mice, Nano-273 plus anti-PD1 decreased IL35⁺ and IL10⁺ Breg cell in lymph node compared to MSA-2 plus anti-PD1 (FIG. 41A).

[0319] To investigate if Nano-273 also showed efficacy in other cancer models, efficacy experiments were conducted in C57BL/6 mice inoculated with LL/2 lung tumor or MC38 colon cancer. Nano-273 demonstrated superior antitumor efficacy to MSA-2 in lung cancer model (FIG. 14F and 41B). Immune profiling in the LL/2 lung cancer model revealed high Bregs percentages in both lymph nodes and tumors, and MSA-2 treatment further increasing Bregs populations. In contrast, Nano-273 significantly reduced Bregs in lymph nodes (Nano-273 vs. MSA-2 and Control, IL35⁺: 4.5% vs. 11.2% and 8.6%; IL10⁺: 2.8% vs. 9.3% and 6.9%) and in tumors (Nano-273 vs. MSA-2 and Control, IL35⁺: 6.8% vs. 40.4% and 36.7%;

IL10⁺: 3.5% vs. 40.8% and 38.9%) (FIG. 14G and 41C). Furthermore, the anticancer efficacy of Nano-273 was evaluated in C57BL/6 mice inoculated with MC 38 tumor (FIG. 42A), which is sensitive to STING agonist treatment. Both MSA-2 and Nano-273 showed potent tumor growth inhibition. Low levels of Bregs populations were observed in lymph nodes and in tumors for both MSA-2 and Nano-273 groups (FIGS. 42B-42C), indicating that STING agonist did not trigger Bregs in MC-38 colon cancer cells that are sensitive to STING agonist. to cause resistance.

[0320] *Efficacy of Nano-273 is dependent on NK cells and cytotoxic CD8 T cells.*

[0321] To understand whether the anticancer efficacy of Nano-273 is due to the enhanced NK function through elimination Bregs, experiments were performed to investigate the anticancer efficacy of SH-273 in in a xenograft pancreatic cancer model with KPC6422 cells after depletion of NK cell (FIG. 14H). The antitumor efficacy of Nano-273 + α PD1 was significantly reduced after depletion of NK cells. In addition, the NK cell populations in the lymph nodes were monitored after treatment of STING agonist MSA2, IPI-549 and Nano-273 in KPC transgenic mice (FIG. 14I). Nano-273 dramatically increased the percentage of NK cells expressing Granzyme B. How CD 8 T cells attributed to the anticancer efficacy of Nano-273 was examined in a xenograft pancreatic cancer model with KPC6422 cells after depletion of CD8 T cell (FIG. 42D). CD 8 T cell depletion also reduced antitumor efficacy of Nano-273, but with a less effect compared to NK cell depletion. Nano-273 effectively eliminated STING-induced Breg cells expansion and enhanced immune cell infiltration, DC cell activation, macrophage polarization and NK cell activation.

Example 5

Methods

[0322] *Animal experiments* All animal experiments were conducted according to protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA). Animals were maintained under pathogen-free conditions, in temperature- and humidity-controlled housing, with free access to food and water, under a 12-hour light-dark rhythm at the unit for laboratory animal medicine (ULAM), part of the University of Michigan medical school office of research. CD1 and C57BL/6 mice were obtained from The Jackson Laboratories. The KPC (LSL-KrasG12D/+;LSL-Trp53R172H/+;Pdx-1-Cre) transgenic mice were bred, genotyped and maintained by ULAM. Tumor experiments by xenograft implanting were performed using 6 - week - old wild-type C57BL/6 mice. Endpoints for anti-tumor efficacy studies were determined using the End-Stage Illness Scoring System,

mice receiving an End-Stage Illness Score greater than 6 were euthanized by CO₂ asphyxiation.

[0323] *Cells* All cells were maintained at 37 °C in a 5% CO₂/95% air atmosphere and 85% relative humidity. Primary B-cells, CD4 T cells, CD8 T cells and splenocytes were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum, 2-Mercaptoethanol (50 µM) and 1% pen/strep. THP-1-Blue™ ISG, THP-1 hSTING^{KO} and THP-1 hSTING^{R232} cells were cultured in RPMI 1640 supplemented with 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum, 100 µg/mL normocin and 1% pen/strep. Bone marrow-derived dendritic cells (BMDCs) and bone marrow-derived macrophages (BMDMs) were isolated from mouse bone marrow cells and cultured following previously reported protocols. RAW264.7 macrophages were cultured in complete RPMI-1640 media supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% MEM nonessential amino acid solution, 1% sodium pyruvate and 1% pen/strep. KPC 6422 cells obtained from kerafast were cultured in DMEM supplemented with 10% fetal bovine serum, Glutamax and 1% pen/strep. SK-OV-3 cells were cultured in ATCC-formulated McCoy's 5a Medium Modified supplemented with 10% fetal bovine serum, and 1% pen/strep. 786-O cells were cultured in ATCC-formulated RPMI-1640 Medium supplemented with 10% fetal bovine serum, and 1% pen/strep. MC-38 cell line was cultured in Dulbecco's modified MEM with 10% fetal bovine serum, 2mM glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 10 mM Hepes, 50ug/ml gentamycin sulfate and 1% pen/strep. LL/2 cell line was cultured in ATCC-formulated Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, and 1% pen/strep.

[0324] *Analyze Breg cells and macrophages in various tissues and assess the effectiveness of STING agonist in pancreatic cancer mouse models* To analyze Breg cells and macrophage proportions, lymph node, tumor, and spleen tissues from KPC (LSL-Kras G12D, LSL-P53R172H, Pdx1-cre) transgenic mice were harvested and prepared for single-cell suspension. CD45, CD3, CD19, IL35, IL10 and IL27 were used to identify regulatory B cells by flow cytometry. Similarly, CD45, CD11b, F4/80, CD80, and CD206 were used to identify M1 and M2 macrophages. To evaluate the in vivo efficacy of the STING agonists, 6-week-old C57BL/6 male mice were subcutaneously inoculated at the right flank with 5*10⁵ KPC 6422 cells/mouse. diABZi (20 µg/mouse for intratumorally injection and 1.5 mg/kg for intravenous injection) and 2'3'-cGAMP (10 µg/mouse for intratumorally injection) were administrated 5 days post tumor inoculation, every 3 days for a total of 5 times. Tumor volumes were calculated as volume = (width)² × length/2.

[0325] *Immunoblotting* Lymph node, pancreas and tumor were harvested from C57BL/6 mice or transgenic KPC mice. B cells were isolated from PBMCs of healthy donor or splenocytes of C57BL/6 mice or transgenic KPC mice using the negative B cell isolation kit (Miltenyi Biotech or STEMCELL Technologies). Bone marrow-derived dendritic cells (BMDCs) and bone marrow-derived macrophages (BMDMs) were isolated from bone marrow cells of transgenic KPC mice and cultured following previously reported protocols. SK-OV-3 cells and 786-O cells were obtained from ATCC. Cells were sonicated and lysed with RIPA lysis extraction buffer (Thermo Fisher), supplemented with protease and phosphatase inhibitor cocktails (Thermo Fisher), on ice for 30 minutes. The lysates were centrifuged at 16,000g for 15 minutes at 4 °C. Clarified supernatants were loaded onto Mini-PROTEAN® TGX™ Precast Protein Gels (4–15%, Bio-Rad), transferred to PVDF membranes (Bio-Rad), and blocked for 1 hour with 3% Blotting-Grade Blocker (Bio-Rad) in TBST buffer. Membranes were incubated overnight at 4 °C with primary antibodies in blocking buffer. After five washes, membranes were incubated with secondary antibodies for 1 hour at room temperature. Proteins were detected using Clarity™ Western ECL Substrate (Bio-Rad).

[0326] *Study the role of PI3Kγ in STING activation in different immune cells* B and CD4+ T cells were isolated from the spleen using the STEMCELL EasySep™ mouse B cell isolation kit and EasySep™ mouse CD4+ T cell isolation kit, respectively. To investigate the phosphorylation of IRF3, B cells (pre-treated with 5 µg/mL anti-IgM& anti-CD40), CD4+ T cells, BMDCs, BMDMs, and THP-1-Blue™ ISG cells were seeded in 12-well plates at a density of 3×10^6 cells/well and incubated with or without MSA-2 (5 µg/mL) and with or without IPI-549 (17 µM). Proteins were analyzed by western blot analysis after cell collection and lysis. IL35+ or IL10+ cells were analyzed by flow cytometry after cell collection and staining with anti-EBI3, anti-IL10 and anti-IL27p28 antibodies. Mouse Splenic B cells (pre-treated with or without 5 µg/mL anti-IgM and 5 µg/mL anti-CD40 or 10 µg/mL lipopolysaccharide and 5 µg/mL anti-CD40) from KPC transgenic mice or STING knock out mice (C57BL/6J-Sting^{fl}/J) were then incubated with or without MSA-2 (5 µg/mL) or IPI-549 (5 µM or 2 µM) or SH-273 (5 µM or 2 µM) for 7 hours. Cells were then stained with CD45, CD3, CD19, Ebi3, IL10 and IL27p28 and processed for flow cytometry. Mouse Splenic B cells were also incubated with or without MSA-2 (5 µg/mL) or Raf inhibitors (SB 590885, 1µM), MEK inhibitors (Binimetinib, 1µM), ERK inhibitors (Temuterkib, 1µM), PP2B inhibitors (Cyclosporin A, 1µM), IP3R inhibitors (Xestospongic C, 1µM) or PI3Kγ inhibitor (IPI-549, 1µM) for 1.5 hours, and then proceed to collect protein for western

blotting. For STING activation in BMDCs, BMDMs, THP-1 cell lines, and RAW264.7 cells, the cells were treated with or without 10 μ M of MSA-2 and with or without 10 μ M of IPI-549. Supernatants were collected for cytokine measurement via ELISA. BMDCs were collected and stained with CD80 and CD86 for activation analysis by flow cytometry. BMDMs and RAW264.7 cells were collected and stained with CD80 or CD86 to access M1 polarization ratios by flow cytometry.

[0327] *Human Samples* The study was carried out in accordance with The University of Michigan Medical School guidelines and was approved by Institutional Review Board ethics committees. The study was conducted in accordance with ethical standards as in the Declaration of Helsinki. Informed consent was obtained from the pancreatic ductal adenocarcinoma (PDAC) patients and healthy donors. The Sex and gender information of participants were determined on self-report. The patients' information was deidentified. Peripheral Blood Mononuclear Cells (PBMC) were isolated from 4 PDAC patients from the University of Michigan and healthy donors that were purchased from commercial vendors (New York Blood center, HumanCells Biosciences and IQ Biosciences). No sex or gender were identified in the pooled blood samples from the commercial vendor. No sex and gender-based analysis was performed, nor did sex or gender factor into the study design since blood samples were only used from 4 PDAC patients, and no influence of sex or gender were reported for the responses to STING activation and PI3K inhibition. Human PBMCs from deidentified PDAC patients or healthy donors were harvested by using density gradient centrifugation with Lymphoprep™ and SepMate™ Tubes (STEMCELL Technologies). PBMCs (pre-treated with 5 μ g/mL anti-IgM and 5 μ g/mL anti-CD40) were then incubated with or without MSA-2 (5 μ g/mL) or IPI-549 (5 μ M or 2 μ M) or SH-273 (5 μ M or 2 μ M) for 7 hours before analysis of Breg induction by flow cytometry. Human CD19⁺ B cells isolated from healthy donor by negative selection (Miltenyi Biotec) were incubated with or without MSA-2 (10 μ g/mL) and with or without inhibitors of different PI3K isoforms (α , Alpelisib, 2 μ M; β , TGX-211, 2 μ M; δ , TGR-1202, 5 μ M; γ , IPI-549, 10 μ M) for 1.5 hours. Cells were then collected for lysis, sonication, and centrifugation for western blot analysis.

[0328] *Preparation of albumin nanoformulation of SH-273* Albumin nano formulation of SH-273 (Nano-273) was then prepared following previously established protocols. Briefly, SH-273 (10 - 12 mg) was dissolved in 1mL of chloroform and then mixed with mouse albumin solution (100 mg/20 mL) to generate a milky emulsion using a rotor-stator homogenizer. This emulsion was processed through five to six cycles at 26,000 psi in a high-pressure homogenizer (Nano DeBEE) at 4°C. Subsequently, the organic solvent was removed

using a rotary evaporator at 25°C. The Nano-273 suspension was then filtered (0.22 µm), lyophilized, and stored at -20°C. Size distribution and morphology were assessed using dynamic light scattering and transmission electron microscopy (TEM).

[0329] *SH-273 cellular STING potency and class-I PI3K isoforms biochemical assay and cellular assay.* The cellular IC₅₀ of SH-273 was measured using THP-1-Blue™ ISG cells. THP-1-Blue™ ISG cells were seeded in a 96-well plate with a density of 1*10⁵ cells/well and incubated with different concentrations of MSA-2 and Nano-273 for 24 hrs at 37 °C. Cell media were then collected, mixed, and incubated with QUANTI-Blue™ solution. SEAP levels were subsequently measured using Synergy 2 microplate reader (Biotek) at 620 nm in absorbance mode.

[0330] Class-I PI3K isoforms binding affinity was evaluated using with PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ assay kits (BPS Bioscience) from BPS Bioscience, following the provided protocols. To investigate the inhibitions of SH-273 in PI3K isoforms at cellular level, SK-OV-3 (PI3Kα) cells, 786-O (PI3Kβ) cells, BMDCs (PI3Kδ and PI3Kγ) and BMDMs from KPC transgenic mice were cultured and pretreated with 200 ng/mL SDF-1α for 1 hour. Alpelisib (PI3Kα inhibitor), 1µM; TGX-211 (PI3Kβ), 1µM; TGR-1202 (PI3Kδ inhibitor), 1 µM or 150 nM; IPI-549 (PI3Kγ), 1µM, 150 nM or 20 nM and SH-273, 2µM, 400 nM or 40 nM were then incubated with those cell lines for 1.5 hours. Cells were then collected for lysis, sonication, and centrifugation for western blot analysis of phosphorylation of Akt (473).

[0331] *Pharmacokinetics and toxicity of SH-273 and Nano-273 in mice* To investigate the pharmacokinetics of SH-273 and Nano-273, KPC transgenic mice (approximately 100 days old with spontaneous tumors) were divided into two groups (n = 9) and treated with SH-273 (i.v. 17.6 µmol/kg) or Nano-273 (i.v. 17.6 µmol/kg). Tissue samples (plasma, liver, tumor, lymph nodes) were collected at 0.5, 2, and 7 hours post-administration, processed, and analyzed. SH-273 concentrations were measured by LC-MS using An AB SCIEX QTRAP 5500 mass spectrometer with a Turbo V electrospray ionization source (Applied Biosystems) coupled to a Shimadzu LC-20AD HPLC system, was used for the quantification. HPLC separation was performed on a waters XBridge® C18 3.5 µm, 5 cm x 2.1 mm column (Waters Corporation). Mobile phase A (water containing 0.1% formic acid) was first kept at 95% for 0.8 min, then decreased to 1% during 1.2 min, and maintained for 1.5 min, returned to 5% B (acetonitrile containing 0.1% formic acid) and maintained for 2 min. The flow rate was 400 µL/min. Positive ion MS/MS was conducted to detect SH273. The mass spectrometric conditions were set as follows: source temperature, 500°C; curtain gas (CUR),

30 psi; ion spray voltage, 5500 V; ion source gas 1 (GS1), 60 psi; ion source gas 2 (GS2), 40 psi; collision gas (CAD), high; entrance potential (EP), 10 eV; collision energy (CE), 45 eV. The MS/MS transition $853.2 > 648.1$ was used to detect SH273, and a dwell time of 50 ms was used for the transition. And the second transition $473 > 431.1$ was used for the internal standard Clofazimine (CFZ). Data acquisition and quantitation were performed using Analyst software (Applied Biosystems). To determine the SH273 in mice plasma, 140 μ L of internal standard solution (CFZ 50 ng/mL in Acetonitrile) and 30 μ L of acetonitrile were added into 30 μ L of plasma samples. The mixture was vortexed for 10 min and centrifuged at 3500 rpm for 10 min. Then, the supernatant was transferred to the autosampler vials for LC–MS/MS analysis. tissue samples were homogenized (Precellys tissue homogenizer, Bertin Technologies) with the addition of 20% Acetonitrile-water solution with a ratio of 5:1 volume (mL) to weight of tissue (g). Then, 140 μ L of internal standard solution and 30 μ L of acetonitrile were added into 30 μ L of tissue homogenization samples for protein precipitation. The mixture was vortexed for 10 min and centrifuged at 3500 rpm for 10 min. The supernatant was transferred to the autosampler vials for LC–MS/MS analysis. Blank plasma and tissues, the samples from un-treated control groups, were used to exclude contamination or interference. The SH273 analytical curves were constructed with 12 standards spiked with blank plasma or tissue, by plotting the peak area ratio of SH273 to the internal standard versus the sample concentration. The concentration range evaluated was from 1 to 5000 ng/mL in plasma and tissues. All samples for calibration curve were made as the method mentioned above.

[0332] To study the in vivo toxicity, CD1 mice (6 weeks) were randomly assigned to two groups ($n = 4$) and administered with SH-273 (15 mg/kg) or Nano-273 (15 mg/kg) every three days for 5 times. Blood was collected on day 0, day 3, and day 15 following the initial dose for whole blood cell count. At day 15, plasma was collected for test of liver enzymes and kidney functions. At day 15, organs (heart, liver, spleen, lung and kidney) were harvest and collected for pathological staining. For single dose acute toxicity in in C57BL/6 mice, a high single intravenous dose of Nano-273 (200 mg/kg) was administered to 3 mice, with body weight monitored daily for 10 days. For high single dose acute toxicity in Es1-/- mice (B6.Cg-Ces1c^{tm1.1Loc}/J), Nano-273 was administered intravenously at doses of 100, 150, or 200 mg/kg to 3 mice per group. For multiple dose chronic (28 days) toxicity in Es1-/- mice (B6.Cg-Ces1c^{tm1.1Loc}/J), Nano-273 was administered intravenously at doses of 15, 50, and 100 mg/kg every three days for 28 days. The mice were assessed via body weights, complete blood counts (CBCs), blood chemistry, clinical observations, and pathology examination of

major organs. All blood cell count, liver enzymes, kidney functions and tissues pathological staining were conducted by ULAM.

[0333] *In vivo anti-tumor efficacy* KPC transgenic mice were randomly assigned to various treatment groups (n = 10). Treatments started at 8 weeks of age, with doses administered weekly for a total of five times. The survival dates of the mice were documented either at the time of death or upon reaching the endpoint as defined by the End-Stage Illness Scoring System. Lung tissues were collected and underwent pathological staining to analyze metastasis in KPC mice that reached the endpoint in each treatment group by ULAM. C57BL/6 mice at 6 weeks of age were subcutaneously inoculated with 5×10^5 KPC 6422 cells at the right flank or both flanks. MSA-2 (i.v. 34.0 $\mu\text{mol/kg}$) plus anti-PD1 antibody (100 μg), IPI-549 (i.v. 18.9 $\mu\text{mol/kg}$) plus anti-PD1 antibody (100 μg), SH-273 (i.v. 17.6 $\mu\text{mol/kg}$) plus anti-PD1 antibody (100 μg) and Nano-273 (i.v. 17.6 $\mu\text{mol/kg}$) plus anti-PD1 antibody (100 μg) were then administered to the mice 5 days post-tumor inoculation and repeated every 3 days for a total of five times. For antitumor efficacy in immunodeficient mice, NSG (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ) mice at 6 weeks of age were subcutaneously inoculated with 5×10^5 KPC 6422 cells at the right flank. Nano-273 (i.v. 17.6 $\mu\text{mol/kg}$) plus anti-PD1 antibody (100 μg) were then administered to the mice 5 days post-tumor inoculation and repeated every 3 days for a total of five times. For antitumor efficacy in CD8T cells or NK cells depletion mice, C57BL/6 mice at 6 weeks of age were received 200 μg of anti-CD8 (clone YTS 169.4, BioXcell) or anti-NK (clone PK136, BioXcell) depleting antibody or IgG isotype control every 3 days, 7 days prior tumor (5×10^5 KPC 6422 cells) inoculation. Nano-273 (i.v. 17.6 $\mu\text{mol/kg}$) plus anti-PD1 antibody (100 μg) were then administered to the mice 5 days post-tumor inoculation and repeated every 3 days for a total of five times. For LL/2 and MC-38 antitumor efficacy, C57BL/6 mice at 6 weeks of age were subcutaneously inoculated with 5×10^5 LL/2 or MC-38 cells at the right flank. MSA-2 (i.v. 34.0 $\mu\text{mol/kg}$) plus anti-PD1 antibody (100 μg), Nano-273 (i.v. 17.6 $\mu\text{mol/kg}$) and Nano-273 (i.v. 17.6 $\mu\text{mol/kg}$) plus anti-PD1 antibody (100 μg) were then administered to the mice 5 days post-tumor inoculation and repeated every 3 days for a total of five times. Tumor volumes were calculated as volume = (width)² × length/2.

[0334] *Immune profiling of lymph nodes and tumors after treatments in vivo by flow cytometry* For in vivo M1 macrophage ratio measurements, lymph nodes and distal tumors were harvested and processed into single-cell suspensions 10 days following the final dosage. Markers CD45, CD11b, F4/80, CD80, and CD206 were used to identify M1 macrophages by flow cytometry. For in vivo Breg cell measurements, lymph nodes and tumors were harvested

and processed into single-cell suspensions 10 days after the final dosage. CD45, CD4, CD19, IL35, and IL10 markers were used to identify regulatory B cells by flow cytometry.

[0335] *RNA Sequencing* Total RNA was extracted from splenic B cells and BMDCs from KPC transgenic mice with or without treatment of MSA-2 using the RNeasy plus mini kit (QIAGEN). RNA was assessed by Qubit RNA broad-range assay (Thermofisher Q10211) and TapeStation (Agilent 5067-5576). Samples with RINs (RNA Integrity Numbers) of 8 or greater were prepared using the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB E7490), NEBNext UltraExpress RNALibrary Prep Kit for Illumina (NEB E3330L), and NEBNext Multiplex Oligos for Illumina Unique dual (NEB E6440L). Where 130ng of total RNA was converted to mRNA using a polyA purification. The mRNA is then fragmented and copied into first strand cDNA using reverse transcriptase and random primers. The 3 prime ends of the cDNA are then adenylated and adapters are ligated. The products are purified and enriched by PCR to create the final cDNA library. The products are purified and enriched by PCR to create the final cDNA library. Final libraries were checked for quantity and quality by Qubit hsDNA (Thermofisher Q33231) and LabChip (Perkin Elmer CLS1444006). The samples were pooled and sequenced on the Illumina NovaSeqX 10B Paired-end 150bp (Illumina 20085594), according to manufacturer's recommended protocols. Bcl2fastq2 Conversion Software (Illumina) is used to generate de-multiplexed Fastq files. The count matrix for all samples was used to perform differential expression analysis using the DESeq2 R package, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for DEGs was conducted with the clusterProfiler R package.

[0336] *Single-cell RNA sequencing for immune fingerprints of tumors after treatments in vivo* Tumor samples were harvested and dissociated into single-cell suspensions 9 days after the last dose. Dead cells were excluded using a dead cell removal kit (Miltenyi Biotec). The single-cell suspensions were then stained with TotalSeq™-C Mouse antibody. These suspensions were subjected to final cell counting on a Countess II Automated Cell Counter (Thermo Fisher) and diluted to a concentration of 700-1000 nuclei/μL. 3' single-nucleus libraries were constructed using the 10x Genomics Chromium Controller and followed the manufacturer's protocol for 3' V3.1 chemistry with NextGEM Chip G reagents (10x Genomics). The final library quality was assessed using a TapeStation 4200 (Agilent), and the libraries were quantified by Kapa qPCR (Roche). Pooled libraries were subjected to 150 bp paired-end sequencing according to the manufacturer's protocol (Illumina NovaSeq 6000). Bcl2fastq2 Conversion Software (Illumina) was used to generate demultiplexed Fastq files,

and a CellRanger Pipeline (10x Genomics) was used to align reads and generate count matrices.

[0337] *Statistical analyses* Data were analysed using Prism version 10.3.1 software (GraphPad) and are presented as mean values $\pm$ s.e.m. Unpaired two-tailed Student's t-tests, one-way analysis of variance with turkey comparisons test, two-way analysis of variance with turkey comparisons test and log-rank (Mantel–Cox) test were used with P value less than 0.05 as statistically significant.

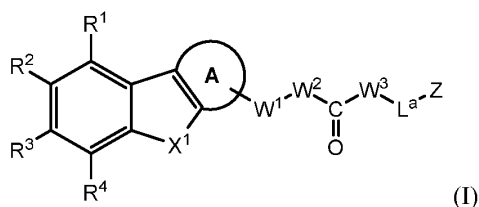
[0338] *Data Availability* All raw and processed RNA-Seq and scRNA-seq data are deposited into GEO repository under accession numbers GSE275780 (RNA-seq) and GSE275785 (scRNA-Seq).

[0339] It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the disclosure, which is defined solely by the appended claims and their equivalents.

[0340] Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art and may be made without departing from the spirit and scope thereof.

CLAIMS

1. A compound of formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

R^1 , R^2 , R^3 , and R^4 are each independently selected from hydrogen, C_1 - C_6 alkoxy, halo, and C_1 - C_6 alkyl;

X^1 is selected from S and NH;

A is selected from a five- or six-membered cycloalkyl, heterocyclyl, heteroaryl, or aryl, each of which is optionally substituted with 1 or 2 oxo groups;

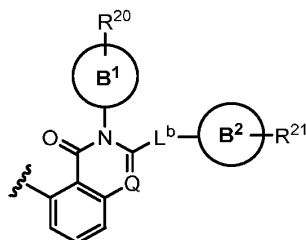
W^1 is a bond or O;

W^2 is selected from C_1 - C_6 alkylene, C_3 - C_6 cycloalkylene, C_3 - C_6 cycloalkylene- C_1 - C_6 alkylene, arylene, and hydroxy- C_1 - C_6 -alkylene;

W^3 is O or NH;

L^a is a linker; and

Z is



wherein:

Q is CH or N;

B^1 is aryl or a 5- or 6-membered monocyclic heteroaryl having 1, 2, 3, or 4 heteroatoms independently selected from N, O, S, and P;

R^{20} is selected from hydrogen, halo, C_1 - C_4 alkyl, C_3 - C_6 cycloalkyl, C_1 - C_4 haloalkyl, $-OR^{a1}$, $-N(R^{a2})(R^{a3})$, $-SO_2R^{a4}$, $-SO_2N(R^{a5})(R^{a6})$, and $-NHSO_2R^{a7}$,

wherein R^{a1} , R^{a2} , R^{a3} , R^{a4} , R^{a5} , R^{a6} , and R^{a7} are each independently selected from hydrogen, C₁-C₄ alkyl, and C₁-C₄ haloalkyl;

R^{21} is selected from hydrogen and a group $-L^{21}-E$, wherein:

L^{21} is a bond, C₁-C₂ alkylene, $-\text{CH}=\text{CH}-$, $-\text{C}\equiv\text{C}-$, $-\text{C}(\text{O})-$, $-\text{O}-$, $-\text{NH}-$, $-\text{S}-$, $-\text{C}(\text{O})\text{O}-$, $-\text{C}(\text{O})\text{NH}-$, $-\text{C}(\text{O})\text{S}-$, arylene, cycloalkylene, heteroarylene, or heterocyclylene, or wherein L^{21} comprises a combination of any two of such groups;

E is a bicyclic heterocyclyl or bicyclic heteroaryl, each of which is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from halo, C₁-C₄ alkyl, C₃-C₆ cycloalkyl, C₃-C₆-cycloalkyl-C₁₋₄-alkyl, C₁-C₄ haloalkyl, oxo, $-\text{OR}^{b1}$, $-\text{N}(\text{R}^{b2})(\text{R}^{b3})$, $-\text{SO}_2\text{R}^{b4}$, $-\text{SO}_2\text{N}(\text{R}^{b5})(\text{R}^{b6})$, and $-\text{NHSO}_2\text{R}^{b7}$, wherein R^{b1} , R^{b2} , R^{b3} , R^{b4} , R^{b5} , R^{b6} , and R^{b7} are each independently selected from hydrogen, C₁-C₄ alkyl, and C₁-C₄ haloalkyl;

L^b is $-(\text{CR}^{c1}\text{R}^{c2})_m-\text{G}^b-$, wherein:

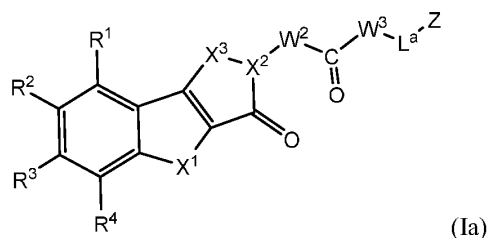
R^{c1} and R^{c2} are independently selected from hydrogen and C₁-C₄ alkyl;

m is 0, 1, or 2; and

G^b is a bond, $-\text{NHC}(\text{O})-$, $-\text{NH}-$, $-\text{O}-$, or $-\text{S}-$; and

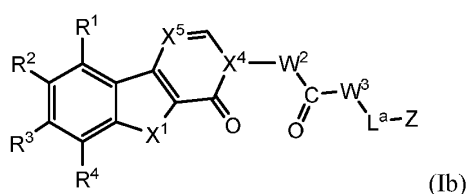
B^2 is a bicyclic heteroaryl or bicyclic heterocyclyl, each of which is optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from halo, C₁-C₄ alkyl, C₃-C₆ cycloalkyl, C₁-C₄ haloalkyl, optionally substituted aryl, $-\text{OR}^{d1}$, $-\text{N}(\text{R}^{d2})(\text{R}^{d3})$, $-\text{SO}_2\text{R}^{d4}$, $-\text{SO}_2\text{N}(\text{R}^{d5})(\text{R}^{d6})$, and $-\text{NHSO}_2\text{R}^{d7}$, wherein R^{d1} , R^{d2} , R^{d3} , R^{d4} , R^{d5} , R^{d6} , and R^{d7} are each independently selected from hydrogen, C₁-C₄ alkyl, and C₁-C₄ haloalkyl.

2. The compound of claim 1, wherein the compound is a compound of formula (Ia):



or a pharmaceutically acceptable salt thereof, wherein X^2 is CH or N, and X^3 is CH_2 or CO.

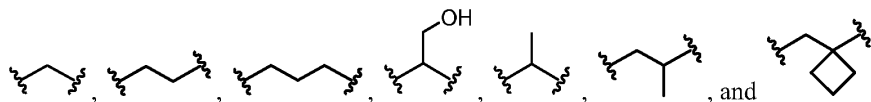
3. The compound of claim 2, or a pharmaceutically acceptable salt thereof, wherein X^2 is CH.
4. The compound of claim 2, or a pharmaceutically acceptable salt thereof, wherein X^2 is N.
5. The compound of any of claims 2-4, or a pharmaceutically acceptable salt thereof, wherein X^3 is CH_2 .
6. The compound of any of claims 2-4, or a pharmaceutically acceptable salt thereof, wherein X^3 is CO.
7. The compound of claim 1, wherein the compound is a compound of formula (Ib):



or a pharmaceutically acceptable salt thereof, wherein X^4 and X^5 are independently selected from N and CH.

8. The compound of claim 7, or a pharmaceutically acceptable salt thereof, wherein X^4 is N.
9. The compound of claim 7 or 8, or a pharmaceutically acceptable salt thereof, wherein X^5 is N.
10. The compound of claim 7 or 8, or a pharmaceutically acceptable salt thereof, wherein X^5 is CH.
11. The compound of any of claims 1-10, or a pharmaceutically acceptable salt thereof, wherein X^1 is S.
12. The compound of any of claims 1-10, or a pharmaceutically acceptable salt thereof, wherein X^1 is NH.
13. The compound of any of claims 1-12, or a pharmaceutically acceptable salt thereof, wherein W^2 is selected from $\text{C}_1\text{-C}_4$ alkylene, $\text{C}_4\text{-C}_5$ cycloalkylene, $\text{C}_4\text{-C}_5$ cycloalkylene- $\text{C}_1\text{-C}_2$ alkylene, phenylene, and hydroxy- $\text{C}_1\text{-C}_3$ -alkylene.

14. The compound of any of claims 1-13, or a pharmaceutically acceptable salt thereof, wherein W^2 is selected from:



15. The compound of any of claims 1-14, or a pharmaceutically acceptable salt thereof, wherein W^3 is O.

16. The compound of any of claims 1-14, or a pharmaceutically acceptable salt thereof, wherein W^3 is NH.

17. The compound of any of claims 1-16, or a pharmaceutically acceptable salt thereof, wherein R^2 and R^3 are each independently C_1 - C_6 alkoxy.

18. The compound of claim 17, or a pharmaceutically acceptable salt thereof, wherein R^2 and R^3 are each methoxy.

19. The compound of any of claims 1-18, or a pharmaceutically acceptable salt thereof, wherein R^1 and R^4 are each independently halo or hydrogen.

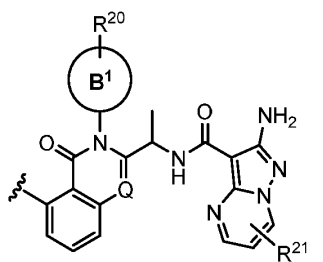
20. The compound of claim 19, or a pharmaceutically acceptable salt thereof, wherein R^1 is halo and R^4 is hydrogen.

21. The compound of any of claims 1-20, or a pharmaceutically acceptable salt thereof, wherein R^1 is halo, R^2 and R^3 are each independently C_1 - C_6 alkoxy, and R^4 is hydrogen.

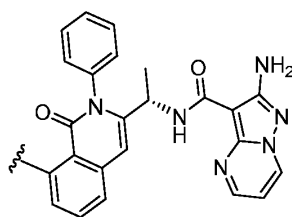
22. The compound of claim 21, or a pharmaceutically acceptable salt thereof, wherein R^1 is chloro or bromo, and R^2 and R^3 are methoxy.

23. The compound of any of claims 1-22, or a pharmaceutically acceptable salt thereof, wherein R^1 and R^4 are hydrogen, and R^2 and R^3 are methoxy.

24. The compound of any one of claims 1-23, or a pharmaceutically acceptable salt thereof, wherein Z is:



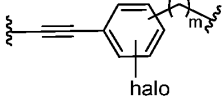
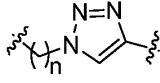
25. The compound of claim 24, or a pharmaceutically acceptable salt thereof, wherein B¹ is phenyl.
26. The compound of claim 24 or 25, or a pharmaceutically acceptable salt thereof, wherein R²⁰ is hydrogen.
27. The compound of any one of claims 24-26, or a pharmaceutically acceptable salt thereof, wherein Q is CH.
28. The compound of any one of claims 24-27, or a pharmaceutically acceptable salt thereof, wherein Z is:



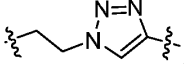
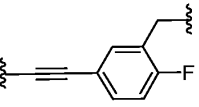
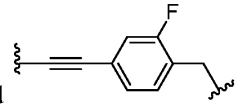
29. The compound of any one of claims 1-28, or a pharmaceutically acceptable salt thereof, wherein L^a comprises one or more groups independently selected from -C(R')₂-, -CH=CH-, -C≡C-, -O-, -NR'-, -BR'-, -S-, -C(O)-, -C(NR')-, -S(O)-, -S(O)₂-, arylene, heteroarylene, cycloalkylene, and heterocyclylene, wherein each R' is independently selected from hydrogen, C₁-C₈₀ alkyl, C₂-C₈₀ alkenyl, C₂-C₈₀ alkynyl, aryl, arylalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, and heteroarylalkyl, and wherein each alkyl, arylene, heteroarylene, cycloalkylene, and heterocyclylene is independently unsubstituted or substituted with 1, 2, or 3 substituents.
30. The compound of claim 29, or a pharmaceutically acceptable salt thereof, wherein L^a comprises one or more groups independently selected from -CH₂-, -C≡C-, arylene, and heteroarylene, wherein the arylene is unsubstituted or substituted with 1 or 2 halo groups.

31. The compound of claim 29, or a pharmaceutically acceptable salt thereof, wherein L^a comprises one or more groups independently selected from $-CH_2-$ and heteroarylene.

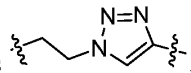
32. The compound of claim 29 or 30, or a pharmaceutically acceptable salt thereof,

wherein L^a is  or , wherein m and n are independently 1, 2, 3, 4, or 5.

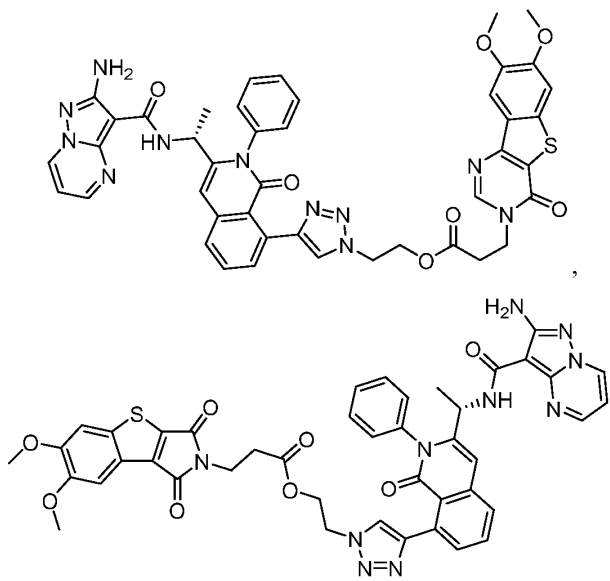
33. The compound of claim 32, or a pharmaceutically acceptable salt thereof, wherein L^a

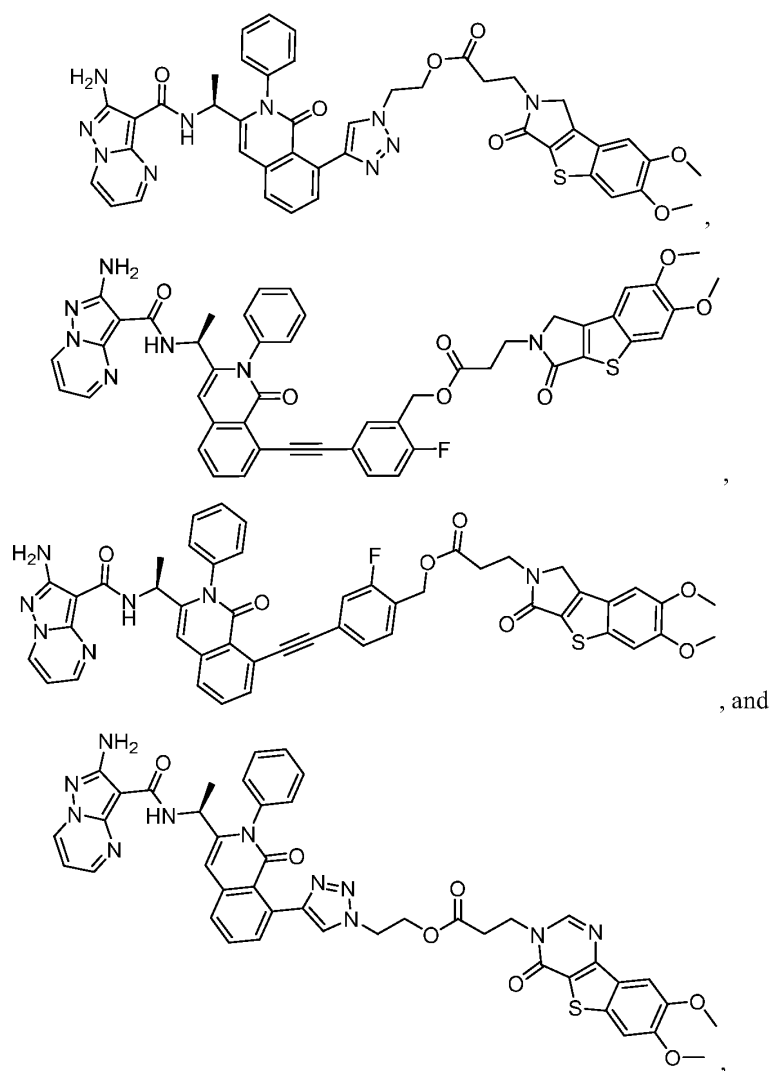
is selected from , , and .

34. The compound of claim 33, or a pharmaceutically acceptable salt thereof, wherein L^a

is .

35. The compound of claim 1, wherein the compound is selected from:





and pharmaceutically acceptable salts thereof.

36. A pharmaceutical composition comprising an effective amount of a compound of any one of claims 1-35, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

37. The pharmaceutical composition of claim 36, wherein the composition comprises albumin nanoparticles, liposomes, micelles, or lipid nanoparticles.

38. The pharmaceutical composition of claim 36 or 37, wherein the composition further comprises at least one additional therapeutic agent.

39. The pharmaceutical composition of claim 38, wherein the at least one additional therapeutic agent comprises an immune modulator, a chemotherapeutic agent, a nucleic acid, a decongestant, a steroid, an analgesic, an antimicrobial agent, or a combination thereof.
40. The pharmaceutical composition of claim 38 or 39, wherein the at least one additional therapeutic agent comprises an RNA selected from the group consisting of a small interfering RNA (siRNA), an asymmetrical interfering RNA (aiRNA), a microRNA (miRNA), a Dicer-substrate RNA (dsRNA), a small hairpin RNA (shRNA), a messenger RNA (mRNA), and mixtures thereof.
41. The pharmaceutical composition of claim 38-40, wherein the at least one additional therapeutic agent is selected from a chemotherapeutic agent, an IDO inhibitor, a Stat3 inhibitor, a TLR agonist, and a PI3K inhibitor.
42. The pharmaceutical composition of any of claims 36-41, further comprising one or more cell targeting epitopes.
43. The pharmaceutical composition of claim 42, wherein the one or more cell targeting epitopes are covalently attached or directly conjugated to an albumin.
44. The pharmaceutical composition of claim 42 or 43, wherein the cell targeting epitopes comprise an immune cell epitope.
45. The pharmaceutical composition of claim 42-44, further comprising one or more epitopes from a microbiological agent.
46. A vaccine comprising an effective amount of:
a compound of any one of claims 1-35, or a pharmaceutically acceptable salt thereof,
or a composition of any of one of claims 36-45; and
an antigen or a nucleic acid encoding thereof.
47. The vaccine of claim 46, the antigen is a tumor antigen, a self-antigen, or an infectious disease derived antigen.
48. The vaccine of claim 46 or 47, wherein the nucleic acid is messenger RNA (mRNA).
49. A method of treating or preventing a disease or disorder comprising administering an effective amount of a compound of any one of claims 1-35, or a pharmaceutically acceptable salt thereof, a composition of any of claims 36-45, or a vaccine of any of claims 46-48, to a subject in need thereof.

50. The method of claim 49, wherein the disease or disorder comprises cancer, an autoimmune disease, an inflammatory disease, or an infectious disease.
51. The method of claim 49 or 50, wherein the disease or disorder is cancer.
52. The method of claim 51, wherein the subject has cancer, has had cancer, is predisposed to cancer, or has a family history of cancer.
53. The method of any of claims 49-52, wherein the cancer comprises a solid tumor.
54. The method of any of claims 49-53, wherein the cancer is metastatic cancer.
55. The method of any of claims 49-54, wherein the method suppresses or eliminates cancer metastasis, decreases tumor growth, prevents tumor recurrences, or any combination thereof.
56. The method of any of claims 49-55, wherein the cancer is pancreatic cancer.
57. The method of any of claims 49-56, wherein the administering comprises an initial administration and at least one subsequent administration.
58. A method of inducing or modulating an immune or inflammatory response in a subject comprising administering the composition of a compound of any one of claims 1-65, or a pharmaceutically acceptable salt thereof, a composition of any of claims 36-45, or the vaccine of any of claims 46-48, to a subject in need thereof.
59. A method of reducing or eliminating regulatory B cells (B_{regs}) in a subject comprising administering the composition of a compound of any one of claims 1-35, or a pharmaceutically acceptable salt thereof, a composition of any of claims 35-45, or the vaccine of any of claims 46-48, to a subject in need thereof.
60. A method of inhibiting PI3K γ in a subject comprising administering the composition of a compound of any one of claims 1-35, or a pharmaceutically acceptable salt thereof, a composition of any of claims 36-45, or the vaccine of any of claims 46-48, to a subject in need thereof.
61. The method of any of claims 49-60, wherein the subject is human.
62. The method of any of claims 49-61, further comprising administering at least one additional therapeutic agent.

63. The method of claim 62, wherein the at least one additional therapeutic agent comprises an immune modulator, a chemotherapeutic agent, a nucleic acid, a decongestant, a steroid, an analgesic, an antimicrobial agent, or a combination thereof.
64. The method of claim 49-63, wherein the method overcomes STING resistance.
65. Use of a compound of any one of claims 1-35, or a pharmaceutically acceptable salt thereof, or a composition of any of claims 36-45 in the manufacture of a medicament for the treatment or prevention of a disease or disorder.
66. The use of claim 65, wherein the disease or disorder comprises cancer, an autoimmune disease, an inflammatory disease, or an infectious disease.