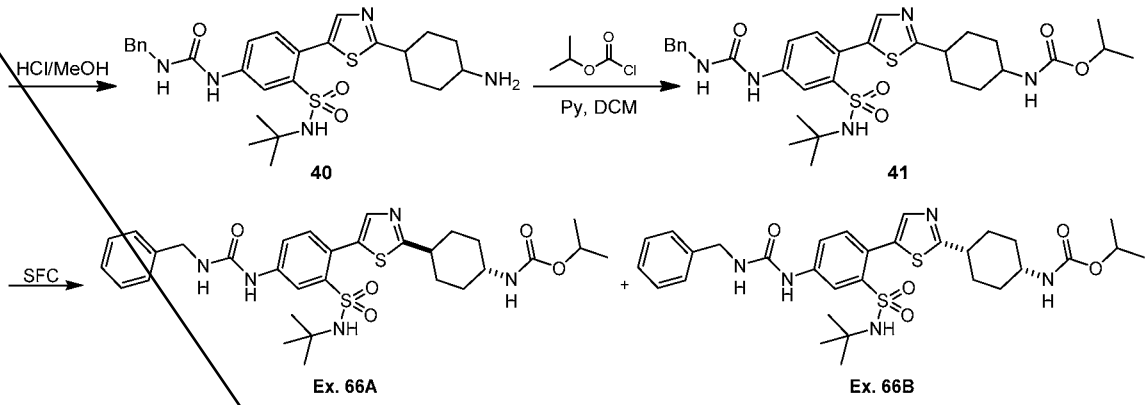
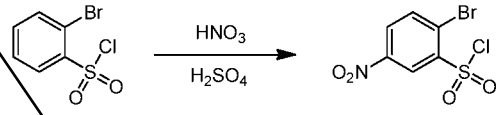
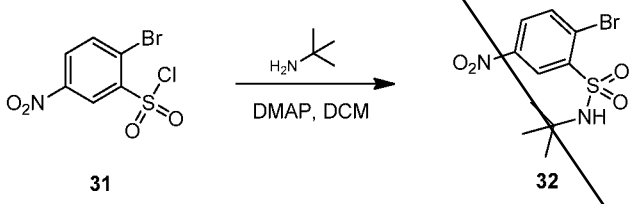


Preparation of compound 31.



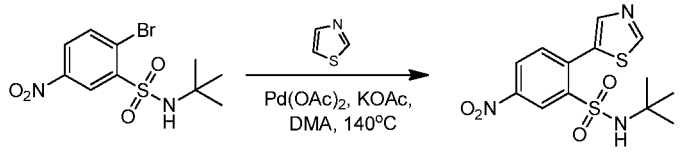
To a solution of ***2-bromobenzenesulfonyl chloride*** (100.00 g, 391.36 mmol, 1.00 *eq.*) in H_2SO_4 (1.0 L) was added a solution of HNO_3 (79.95 g, 1.21 mol, 57.11 mL, 95% purity, 3.08 *eq.*) in H_2SO_4 (0.50 L) drop-wise at 0°C. The mixture was stirred at 26°C for 2 hrs. TLC (Petroleum ether: EtOAc =10:1, R_f =0.40) showed the reaction was complete. The mixture was added slowly to ice water (5 L) with vigorous stirring and then filtered. The filter cake was washed with H_2O (1 L X 3) and dried to give ***2-bromo-5-nitro-benzenesulfonyl chloride*** (105.00 g, crude) as a yellow solid. ^{1}H NMR (400MHz, CHLOROFORM-d) δ = 8.98 (d, J=2.4 Hz, 1H), 8.37 (dd, J=2.5, 8.7 Hz, 1H), 8.09 (d, J=8.6 Hz, 1H).

Preparation of compound 32.



To a mixture of ***2-methylpropan-2-amine*** (200 g, 2.73 mol, 287.36 mL, 3.29 *eq.*) and DMAP (10 g, 81.85 mmol, 0.1 *eq.*) in DCM (2 L) was added ***2-bromo-5-nitro-benzenesulfonyl chloride*** (250 g, 831.91 mmol, 1 *eq.*) portionwise at 0°C. The mixture was warmed to 15°C and stirred for 1 hr. LCMS showed the reaction was complete. The mixture was washed with HCl (1 N, 2 L), sat.aq. $NaHCO_3$ (500 mL) and brine (500 mL), dried over Na_2SO_4, filtered and concentrated to give ***2-bromo-N-tert-butyl-5-nitro-benzenesulfonamide*** (240 g, crude) as a gray solid. ESI [M+H] = 336.9/338.9.

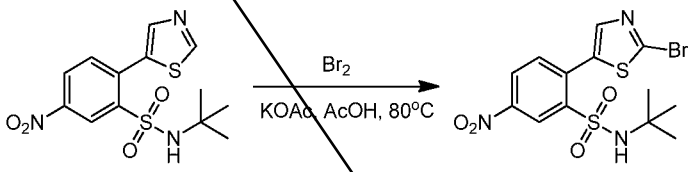
Preparation of compound 33.



32 33

To a solution of ***2-bromo-N-tert-butyl-5-nitro-benzenesulfonamide*** (35 g, 103.80 mmol, 1 *eq.*) in DMA (300 mL), were added thiazole (26.51 g, 311.40 mmol, 3 *eq.*), $Pd(OAc)_2$ (2.33 g, 10.38 mmol, 0.1 *eq.*) and KOAc (30.56 g, 311.40 mmol, 3 *eq.*). The mixture was stirred at 140°C for 16 hrs under N_2. LCMS showed the reaction was complete, the mixture was poured into water (3 L) and filtered. The filter cake was washed with water (200 mL x 3) and then dried to give ***N-tert-butyl-5-nitro-2-thiazol-5-yl-benzenesulfonamide*** (25.1 g, crude) as a black brown solid. ^{1}H NMR (400MHz, DMSO-d6) δ = 9.30 (s, 1H), 8.81 (d, J=2.4 Hz, 1H), 8.46 (dd, J=2.4, 8.3 Hz, 1H), 8.12 (s, 1H), 7.83 (d, J=8.3 Hz, 1H), 7.60 (s, 1H), 1.07 (s, 9H). ESI [M+H] = 342.0.

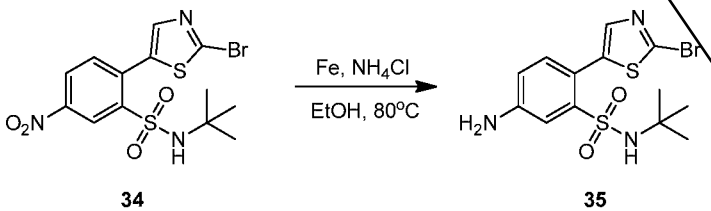
Preparation of compound 34.



33 34

To a solution of ***N-tert-butyl-5-nitro-2-thiazol-5-yl-benzenesulfonamide*** (15 g, 43.94 mmol, 1 *eq.*) in AcOH (200 mL), were added KOAc (21.56 g, 219.68 mmol, 5 *eq.*) and Br_2 (35.11 g, 219.68 mmol, 5 *eq.*). The mixture was stirred at 80°C for 3 hrs. LCMS showed the reaction was complete, the mixture was quenched by sat.aq.Na_2CO_3 (1 L) and extracted with EtOAc (300 mL x 3). The combined organic phase was dried over Na_2SO_4, filtered and concentrated to give ***2-(2-bromothiazol-5-yl)-N-tert-butyl-5-nitro-benzenesulfonamide*** (18 g, crude) as a green solid which was used without any purification.

Preparation of compound 35.

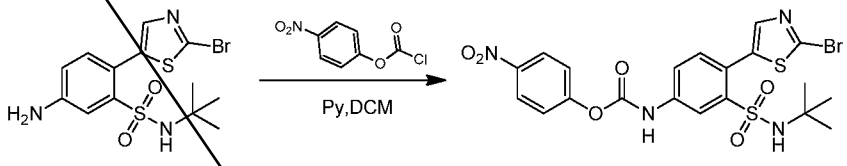


34 35

To a solution of ***2-(2-bromothiazol-5-yl)-N-tert-butyl-5-nitro-benzenesulfonamide*** (8.5 g, 20.22 mmol, 1 *eq.*) in EtOH (70 mL), THF (40 mL) and H_2O (20 mL), were added Fe (3.39 g, 60.67 mmol, 3 *eq.*) and NH_4Cl (3.25 g, 60.67 mmol, 2.12 mL, 3 *eq.*). The mixture was

stirred at 90°C for 1 hr. LCMS showed the reaction was complete. The mixture was filtered, the filtrate was concentrated to remove organic solvent and extracted with DCM (200 mL x 2). The organic phase was dried over Na_2SO_4, filtered and concentrated to afford ***5-amino-2-(2-bromothiazol-5-yl)-N-tert-butyl- benzenesulfonamide*** (6 g, crude) as a yellow solid. ^{1}HNMR showed the structure was correct. 1H NMR (400MHz, DMSO-d6) δ = 7.58 - 7.52 (m, 1H), 7.30 (d, J=2.4 Hz, 1H), 7.11 (d, J=8.3 Hz, 1H), 6.99 (s, 1H), 6.73 (dd, J=2.4, 8.3 Hz, 1H), 5.94 (s, 2H), 1.07 (s, 9H). ESI [M+H] = 390.0/392.0.

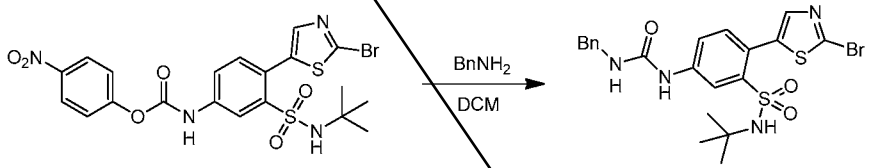
Preparation of compound 36.



35 36

General method D, ***(4-nitrophenyl) N-[4-(2-bromothiazol-5-yl)-3-(tert-butylsulfamoyl)phenyl]carbamate*** as DCM solution ESI [M+H] = 555.0/557.0

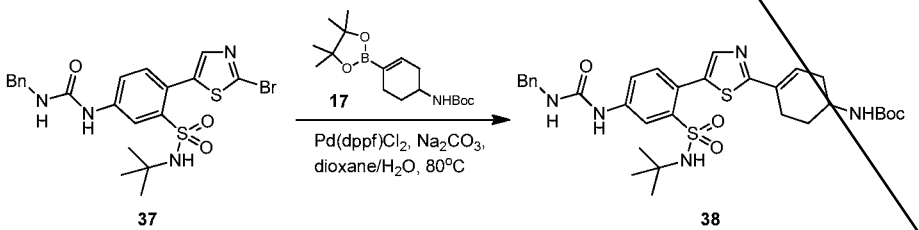
Preparation of compound 37.



36 37

General method H, ***1-benzyl-3-[4-(2-bromothiazol-5-yl)-3-(tert-butylsulfamoyl) phenyl]urea***. ESI [M+H] = 523.0/525.0.

Preparation of compound 38.

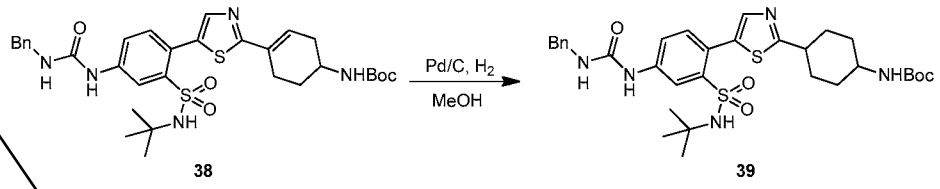


37 38

General method B (*Suzuki reaction)*, ***tert-butyl-N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl]thiazol-2-yl]cyclohex-3-en-1-yl]carbamate (Compound 38)***.

To a solution of ***Compound 17*** (1 *eq.*) in dioxane and H_2O, were added $Pd(dppf)Cl_2$ (0.1 *eq.*), ***Compound 37*** (0.9 *eq.*) and Na_2CO_3 (3 *eq.*). The mixture was stirred at 80°C for 12 hrs under N_2. LCMS showed the reaction was complete. The mixture was concentrated and the residue was purified by prep-TLC (PE/EtOAc=1:1) to yield product **38**. ESI [M+H] = 640.5

Preparation of compound 39.

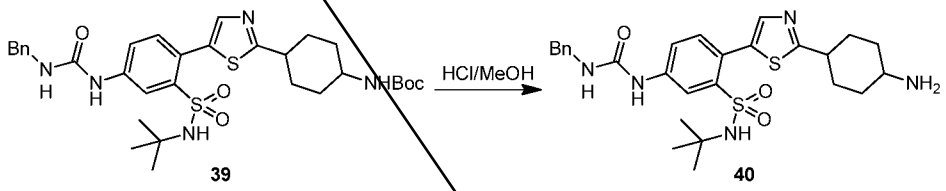


38 39

tert-butyl N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert- butylsulfamoyl)phenyl]thiazol-2-yl]cyclohexyl]carbamate.

To a solution of ***Compound 38*** (1 *eq.*) and AcOH (0.1 *eq.*) in EtOAc was added Pd/C (10% purity, 1.00 *eq.*). The mixture was stirred under H_2 (15 psi) at 40°C for 1 hr. LCMS showed the reaction was complete and then the mixture was filtered and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate =10/1 to 1:1) to give ***Compound 39.*** ESI [M+H] = 642.5

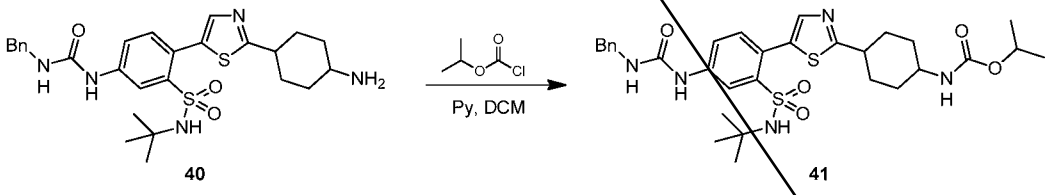
Preparation of compound 40.



39 40

General method F, ***1-[4-[2-(4-aminocyclohexyl)thiazol-5-yl]-3-(tert-butylsulfamoyl) phenyl]-3-benzyl-urea***. ESI [M+H] = 542.5.

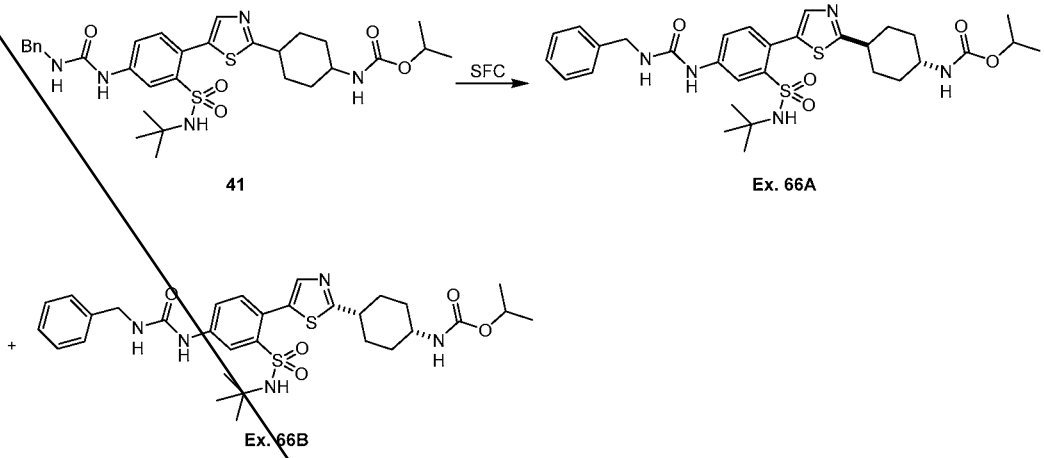
Preparation of compound 41



40 41

General method D, ***isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl]thiazol-2-yl]cyclohexyl]carbamate***, ESI [M+H] = 628.3.

Preparation of Compound Ex. 66A and Ex. 66B.



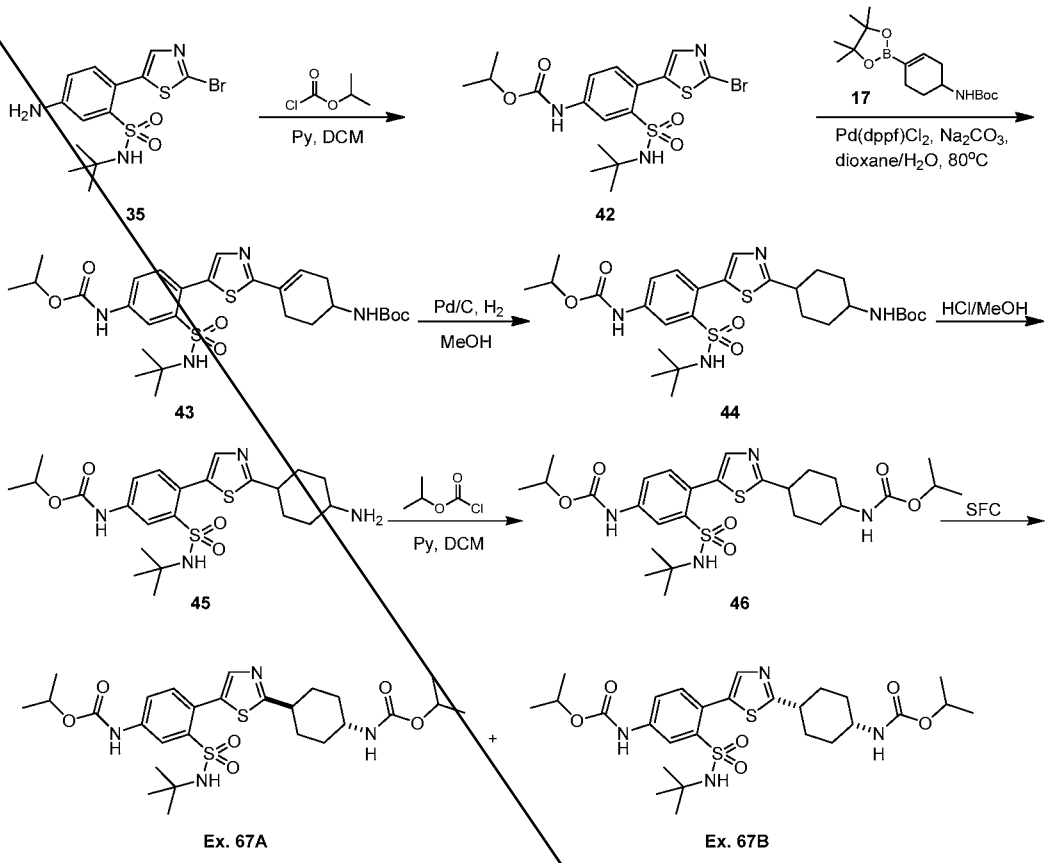
Compound 41 was separated by SFC (Instrument: Thar SFC80 preparative SFC; Column: Chiralpak AD-H 250*30mm i.d. 5u; Mobile phase: A for CO_2 and B for IPA(0.1%NH_3H_2O); Gradient: B%=42%; Flow rate:70 g/min; Wavelength:220 nm; Column temperature: 40℃; System back pressure: 100 bar; Cycle time:20 min; Injection amount: 4 mg per injection), and then purified by prep-HPLC (Column: Luna C18 100*30 5u; mobile phase: [water(0.1%TFA)-ACN];B%: 35%-75%,5min). ***trans-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl]thiazol-2-yl]cyclohexyl]carbamate Ex. 66A*** (11.54 mg, 18.38 umol, 5.53% yield, 100% purity) and ***cis-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl] thiazol-2-yl]cyclohexyl]carbamate Ex. 66B*** (9.38 mg, 14.94 umol, 4.50% yield, 100% purity) were obtained.

Trans-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl] thiazol-2-yl]cyclohexyl]carbamate, ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.24 (d, J=2.2 Hz, 1H), 7.72 (s, 1H), 7.69 (dd, J=2.6, 8.3 Hz, 1H), 7.37 - 7.30 (m, 5H), 7.27 - 7.22 (m, 1H), 4.85 - 4.78 (m, 1H), 4.41 (s, 2H), 3.50 - 3.39 (m, 1H), 3.06 - 2.96 (m, 1H), 2.22 (br d, J=11.8 Hz, 2H), 2.07 (br d, J=10.1 Hz, 2H), 1.76 - 1.62 (m, 2H), 1.41 (dq, J=3.1, 12.7 Hz, 2H), 1.22 (br d, J=6.1 Hz, 6H), 1.11 (s, 9H). ESI [M+H] = 628.2.

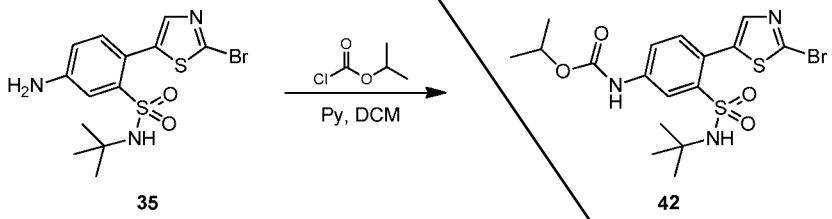
Cis-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl]thiazol-2-yl]cyclohexyl]carbamate. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.25 (d, J=2.2 Hz, 1H), 7.74 (s, 1H), 7.70 (dd, J=2.2, 8.3 Hz, 1H), 7.38 - 7.30 (m, 5H), 7.28 - 7.22 (m, 1H), 4.82 (br d, J=6.1 Hz, 1H), 4.41 (s, 2H), 3.74 (br s, 1H), 3.20 - 3.13 (m, 1H), 2.03 - 1.95 (m, 4H), 1.86 - 1.72 (m, 4H), 1.23 (d, J=6.1 Hz, 6H), 1.12 (s, 9H). ESI [M+H] = 628.2.

Examples 67A and 67B

Scheme 26:

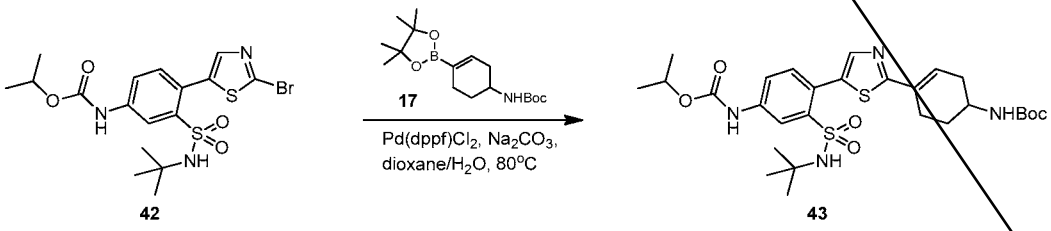


Preparation of compound 42.



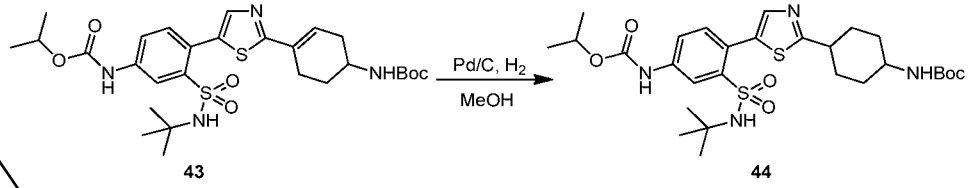
General method D, ***isopropyl N-[4-(2-bromothiazol-5-yl) -3-(tert-butylsulfamoyl) phenyl]carbamate.*** ESI [M+H] =476.0/478.0.

Preparation of compound 43



General method B, ***isopropyl N-[4-[2-[4-(tert -butoxycarbonylamino)cyclohexen-1-yl] thiazol-5-yl]-3-(tert-butylsulfamoyl)phenyl]carbamate***. ESI [M+H] =593.3.

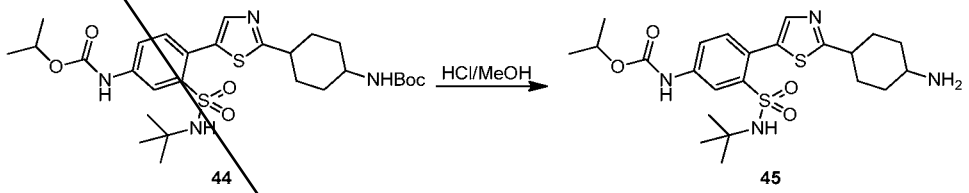
Preparation of compound 44.



43 44

General method I, ***isopropyl N-[4-[2-[4-(tert-butoxycarbonylamino)cyclohexyl] thiazol-5-yl]-3-(tert-butylsulfamoyl)phenyl]carbamate***. ESI [M+H] =595.3.

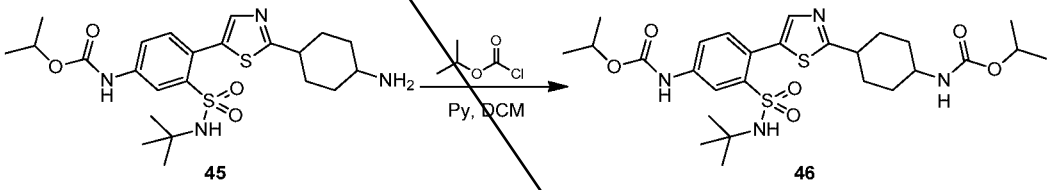
Preparation of compound 45.



44 45

General method F, ***isopropyl N-[4-[2-(4-aminocyclohexyl) thiazol-5-yl]-3-(tert-butylsulfamoyl)phenyl]carbamate***. ESI [M+H] =495.2.

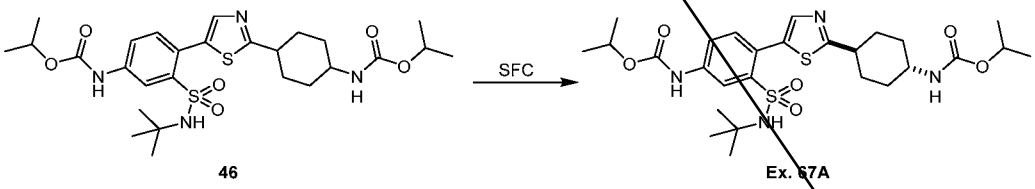
Preparation of compound 46.



45 46

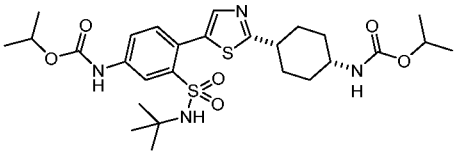
General method D, ***isopropyl N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino) cyclohexyl]thiazol-5-yl]phenyl]carbamate***. ESI [M+H] =581.2.

Preparation of Ex. 67A and Ex. 67B.



46 Ex. 67A

+



Ex. 67B

Compound 46 was separated by SFC (Instrument: Thar SFC80 preparative SFC; Column: ChiralpakAD-H 250*30mm i.d. 5u; Mobile phase: A for CO_2 and B for IPA(0.1%NH_3H_2O); Gradient: B%=30%; Flow rate:70 g/min; Wavelength:220 nm; Column temperature: 40°C; System back pressure: 100 bar; Cycle time:8 min; Injection amount: 3 mg per injection); and then purified by prep-HPLC (Column: Agela Durashell C18 150*25 5u; mobile phase:

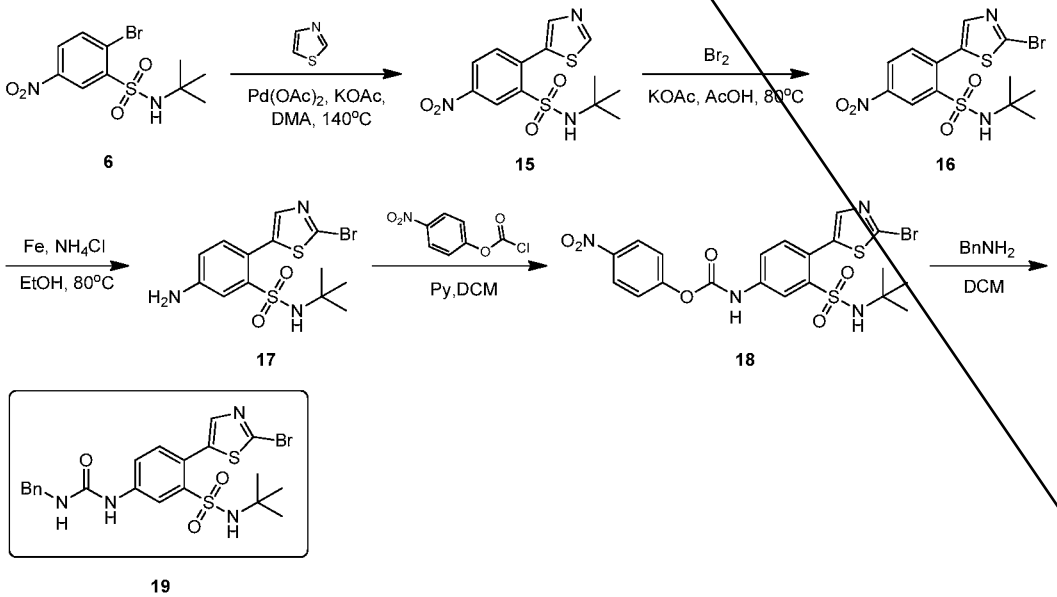
[water(0.1%TFA)-ACN];B%: 55%-85%,12min), ***trans-isopropylN-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate Ex. 67A*** (5.76 mg, 100% purity) and ***cis-isopropylN-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino) cyclohexyl]thiazol-5-yl]phenyl]carbamate Ex. 67B*** (3.95 mg , 100% purity) ***were obtained as a pale yellow solid.***

Trans-isopropylN-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl] thiazol-5-yl]phenyl]carbamate (Compound S12). ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.37 (d, J=2.3 Hz, 1H), 7.75 (s, 1H), 7.69 (dd, J=2.2, 8.4 Hz, 1H), 7.40 (d, J=8.3 Hz, 1H), 5.01 (td, J=6.3, 12.5 Hz, 1H), 4.86 - 4.82 (m, 1H), 3.52 - 3.42 (m, 1H), 3.04 (tt, J=3.5, 12.0 Hz, 1H), 2.35 - 2.19 (m, 2H), 2.15 - 1.99 (m, 2H), 1.72 (dq, J=3.0, 12.9 Hz, 2H), 1.43 (dq, J=3.3, 12.6 Hz, 2H), 1.34 (d, J=6.2 Hz, 6H), 1.25 (br d, J=6.1 Hz, 6H), 1.14 (s, 9H). ESI [M+H] = 581.2.

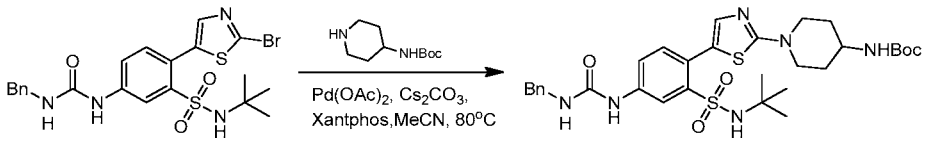
Cis-isopropylN-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate (Compound S13). ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.37 (d, J=2.2 Hz, 1H), 7.76 (s, 1H), 7.70 (dd, J=2.2, 8.3 Hz, 1H), 7.40 (d, J=8.3 Hz, 1H), 5.01 (td, J=6.3, 12.5 Hz, 1H), 4.84 (br s, 1H), 3.77 (br s, 1H), 3.23 - 3.15 (m, 1H), 2.05 - 1.98 (m, 4H), 1.89 - 1.72 (m, 4H), 1.34 (d, J=6.2 Hz, 6H), 1.25 (d, J=6.1 Hz, 6H), 1.16 (s, 9H). ESI [M+H] = 581.2

Example 68. Synthesis of *isopropyl N-[1-[5-[4-(benzylcarbamoylamino)-2-(tert-butyl sulfamoyl)phenyl]thiazol-2-yl]-4-piperidyl]carbamate.*

Scheme 27:



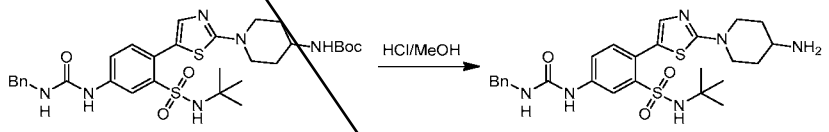
Preparation of compound 24.



19 24

A mixture of ***1-benzyl-3-[4-(2-bromothiazol-5-yl)-3-(tert-butylsulfamoyl)phenyl]urea*** (0.06 g, 114.62 umol, 1.0 eq.), tert-butyl N-(4-piperidyl)carbamate (40 mg, 199.72 umol, 1.74 eq.), Xantphos (6.63 mg, 11.46 umol, 0.1 eq.), $Pd(OAc)_2$ (2.57 mg, 11.46 umol, 0.1 eq.) and Cs_2CO_3 (112.04 mg, 343.86 umol, 3.0 eq.) in ACN (3 mL) was heated to 80°C for 12 hrs under N_2. LCMS showed the reaction was complete and the mixture was concentrated. The residue was purified by prep-TLC (Petroleum ether: Ethyl acetate = 1:1) to give ***tert-butyl N-[1-[5-[4-(benzylcarbamoylamino) -2-(tert-butylsulfamoyl)phenyl]thiazol-2-yl]-4-piperidyl]carbamate*** (0.03 g, crude) as a yellow solid. ESI [M+H] = 643.1.

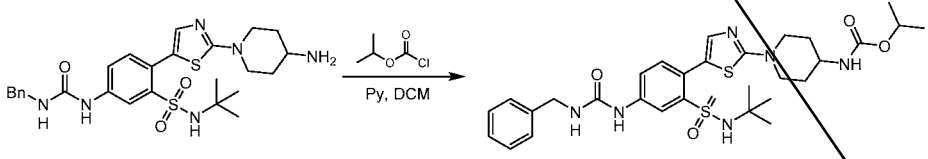
Preparation of compound 25.



24 25

Tert-butyl N-[1-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl] thiazol-2-yl]-4-piperidyl]carbamate (30 mg, 46.67 umol, 1.0 eq.) was dissolved into HCl/MeOH (4M, 1 mL) and the mixture was stirred at 20°C for 0.5 hr. LCMS showed the reaction was complete and the mixture was concentrated to give ***1-[4-[2-(4-amino-1-piperidyl)thiazol-5-yl]-3-(tert-butylsulfamoyl)phenyl]-3-benzyl-urea*** (27 mg, crude, HCl salt) as a yellow solid. ESI [M+H] = 543.2.

Preparation of Ex. 68.

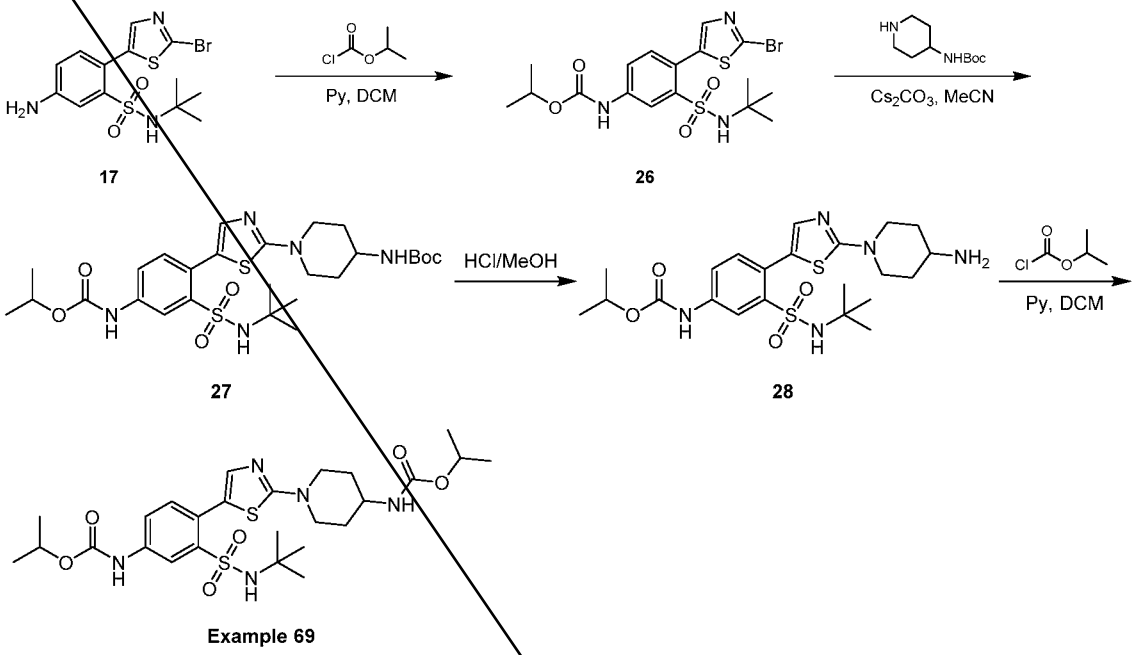


25 Ex. 68

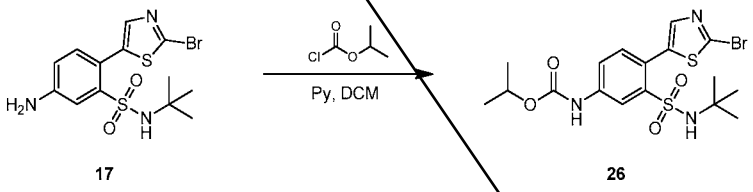
General method D, ***isopropyl N-[1-[5-[4-(benzylcarbamoylamino)-2-(tert-butyl sulfamoyl)phenyl]thiazol-2-yl]-4-piperidyl]carbamate***. ^1H NMR (400MHz, METHANOL-d4) δ = 8.24 (d, J=2.2 Hz, 1H), 7.69 (dd, J=2.4, 8.4 Hz, 1H), 7.39 (d, J=8.4 Hz, 1H), 7.36 - 7.19 (m, 6H), 4.82 - 4.73 (m, 1H), 4.40 (s, 2H), 3.92 (br d, J=13.7 Hz, 2H), 3.75 - 3.68 (m, 1H), 3.43 (br t, J=11.0 Hz, 2H), 2.10 - 2.02 (m, 2H), 1.71 - 1.60 (m, 2H), 1.22 (br d, J=6.2 Hz, 6H), 1.18 (s, 9H). ESI [M+H] = 629.2.

Example 69. Synthesis of *isopropyl N-[1-[5-[2-(tert-butylsulfamoyl)-4-(isopropoxy carbonylamino)phenyl]thiazol-2-yl]-4-piperidyl]carbamate.*

Scheme 28:

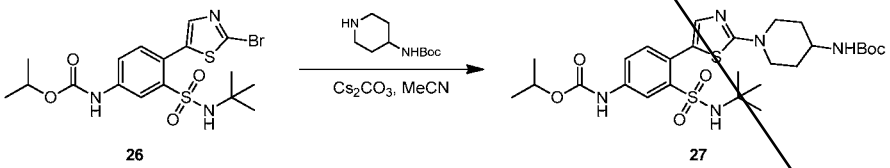


Preparation of compound 26.



General method D, ***isopropyl N-[4-(2-bromothiazol-5-yl)-3-(tert-butylsulfamoyl) phenyl]carbamate***. ESI [M+H] = 478.0/476.0.

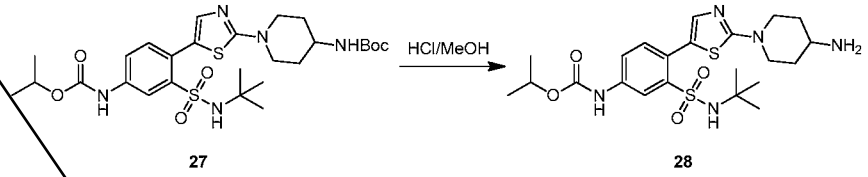
Preparation of compound 27.



To a solution of ***isopropyl N-[4-(2-bromothiazol-5-yl)-3-(tert-butylsulfamoyl) phenyl]carbamate*** (0.05 g, 104.95 umol, 1.0 eq.) in MeCN (2 mL) were added Cs_2CO_3 (102.59 mg, 314.86 umol, 3.0 eq.), KI (17.42 mg, 104.95 umol, 1.0 eq.) and tert-butyl N-(4-piperidyl)carbamate (105.10 mg, 524.76 umol, 5.0 eq.). The mixture was stirred at 95°C for 12 hrs and then concentrated. The residue was purified by prep-TLC (SiO_2, Petroleum ether: Ethyl acetate = 4:3) to give ***N-[4-[2-[4-(tert-butoxycarbonylamino)-1-piperidyl]thiazol-5-yl]-***

3-(tert-butylsulfamoyl) phenyl]carbamate (0.025 g, crude) as a white solid. ESI [M+H] = 596.2.

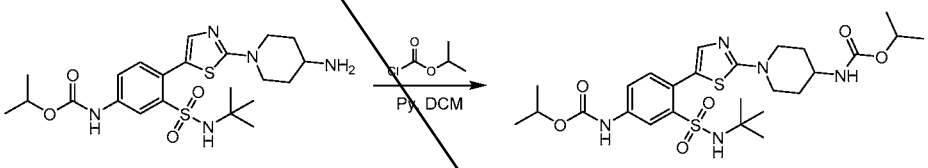
Preparation of compound 28.



27 28

Isopropyl N-[4-[2-[4-(tert-butoxycarbonylamino)-1-piperidyl]thiazol-5-yl]-3-(tert-butylsulfamoyl)phenyl]carbamate (0.025 g, 41.96 umol, 1.0 eq.) was dissolved into HCl/MeOH (4M, 1.5 mL) and the mixture was stirred at 20°C for 0.5 hr. LCMS showed the reaction was complete and the mixture was concentrated to give the ***N-[4-[2-(4-amino-1-piperidyl)thiazol-5-yl]-3-(tert-butylsulfamoyl)phenyl] carbamate*** (0.02 g, crude, HCl salt) as a white solid. ESI [M+H] = 496.2.

Preparation of Ex. 69.

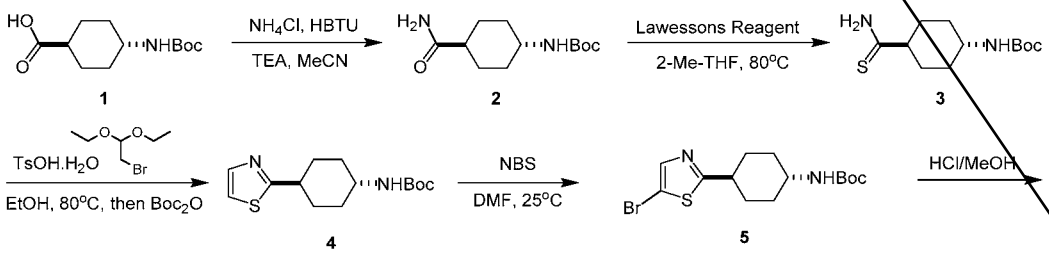


28 Ex. 69

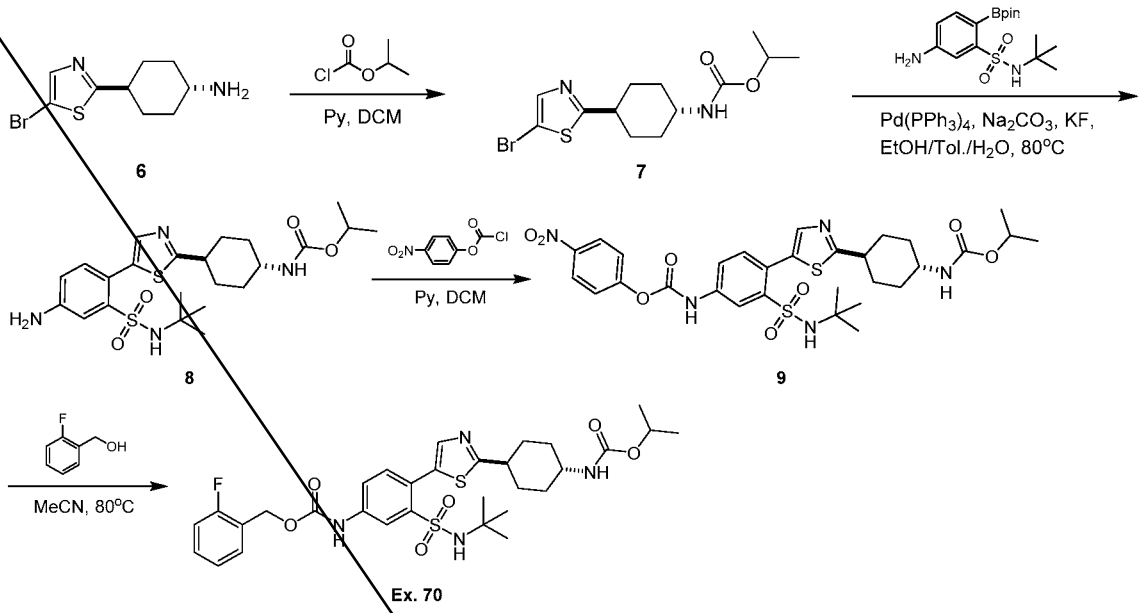
General method D, ***isopropyl N-[1-[5-[2-(tert-butylsulfamoyl)-4-(isopropoxy carbonylamino)phenyl]thiazol-2-yl]-4-piperidyl]carbamate***. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.35 (s, 1H), 7.66 (br d, J=8.4 Hz, 1H), 7.42 (d, J=8.4 Hz, 1H), 7.31 (s, 1H), 4.98 (td, J=6.2, 12.5 Hz, 1H), 4.84 - 4.77 (m, 1H), 3.93 (br d, J=13.5 Hz, 2H), 3.77 - 3.67 (m, 1H), 3.44 (br t, J=12.3 Hz, 2H), 2.06 (br dd, J=3.1, 13.2 Hz, 2H), 1.71 - 1.61 (m, 2H), 1.31 (d, J=6.4 Hz, 6H), 1.23 (br d, J=6.2 Hz, 6H), 1.19 (s, 9H). ESI [M+H] = 582.2.

Example 70. Synthesis of trans-isopropyl N-[4-[5-[2-(tert-butylsulfamoyl)-4-[(2-fluorophenyl)methoxy carbonylamino]phenyl]thiazol-2-yl]cyclohexyl]carbamate.

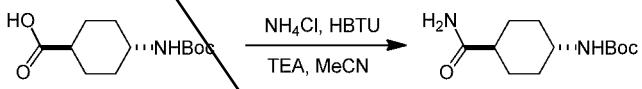
Scheme 29:



1 2 3 4 5

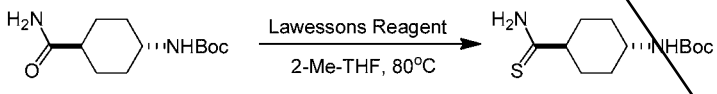


Preparation of compound 2.



To a mixture of ***trans-4-(tert-butoxycarbonylamino)cyclohexanecarboxylic acid*** (65.0 g, 267.2 mmol, 1.0 *eq.*), NH_4Cl (21.4 g, 400.7 mmol, 1.5 *eq.*) and TEA (801.5 mmol, 111.6 mL, 3 *eq.*) in MeCN (1.3 L) was added HBTU (111.5 g, 293.9 mmol, 1.1 *eq.*) and the mixture was stirred at 25°C for 3 hrs. The mixture was filtered and then the filter cake was washed with petroleum ether (200 mL) and dried to give ***trans-tert-butyl N-(4-carbamoylcyclohexyl)carbamate*** (140 g, crude，2 batches) as a white solid. ^1H NMR (METHANOL-d4, 400MHz) δ = 3.25-3.34 (m, 1H), 2.14 (tt, J=12.3, 3.5 Hz, 1H), 1.83-1.99 (m, 4H), 1.52 (qd, J=13.1, 2.9 Hz, 2H), 1.42 (s, 9H), 1.21 (qd, J=12.7, 3.5 Hz, 2H)

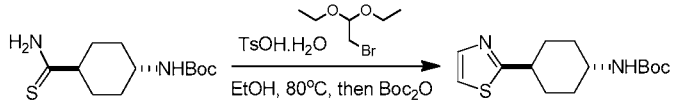
Preparation of compound 3.



A mixture of ***trans-tert-butyl N-(4-carbamoylcyclohexyl)carbamate*** (90.0 g, 371.4 mmol, 1.0 *eq.*), Na_2CO_3 (39.4 g, 371.4 mmol, 1.0 *eq.*) and Lawesson's reagent (82.6 g, 204.3 mmol, 0.55 *eq.*) in 2-Me-THF (600 mL) was stirred at 80°C for 2 hrs and then the reaction mixture was poured into H_2O (200 mL). The aqueous phase was extracted with EtOAc (500 mL x 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to give ***trans-tert-butyl N-(4-carbamothioylcyclohexyl)carbamate*** (180 g, crude，2 batches) as a

white solid. ^{1}H NMR (METHANOL-d4, 400MHz) δ = 3.35-3.46 (m, 1H), 2.87-3.00 (m, 1H), 2.09-2.20 (m, 2H), 1.99-2.09 (m, 2H), 1.54-1.68 (m, 2H), 1.26-1.45 (m, 2H), 1.14-1.25 (m, 9H)

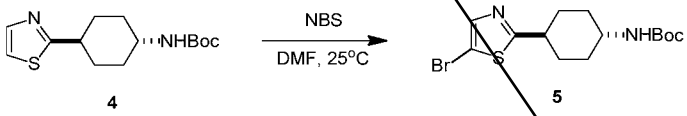
Preparation of compound 4.



3 4

A mixture of ***trans-tert-butyl N-(4-carbamothioylcyclohexyl)carbamate*** (180.0 g, 696.7 mmol, 1.0 *eq.*), 2-bromo-1,1-diethoxy-ethane (137.3 g, 696.7 mmol, 1.0 *eq.*) and TsOH.H_2O (265 g, 1.4 mol, 2 *eq.*) in EtOH (2.0 L) was stirred at 80°C for 6 hrs. Then the mixture was cooled to RT and adjusted to PH=9 with aq.sat.Na_2CO_3 and Boc_2O (152 g, 696.7 mmol, 1 *eq.*) was added. The mixture was stirred at 30°C for 3 hrs, then concentrated and diluted with H_2O (2 L). The mixture was extracted with EtOAc (800 mL x 3) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was triturated with petroleum ether (1.5 L) to give ***trans-tert-butyl N-(4-thiazol-2-ylcyclohexyl) carbamate*** (70 g, 247.87 mmol, 35.58% yield) as a white solid. ^{1}H NMR (METHANOL-d4, 400MHz) δ = 7.67 (d, J=3.1 Hz, 1H), 7.44 (d, J=3.5 Hz, 1H), 3.33-3.44 (m, 1H), 2.93-3.06 (m, 1H), 2.12-2.21 (m, 2H), 2.00-2.09 (m, 2H), 1.57-1.71 (m, 2H), 1.41-1.48 (m, 9H), 1.29-1.38 (m, 1H), 1.13-1.28 (m, 1H).

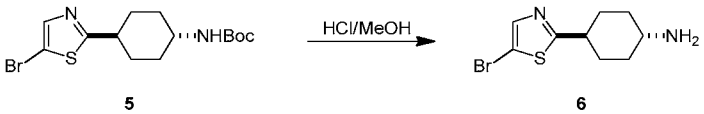
Preparation of compound 5.



4 5

A mixture of ***trans-tert-butyl N-(4-thiazol-2-ylcyclohexyl)carbamate*** (68 g, 240.8 mmol, 1 *eq.*) and NBS (47.1 g, 264.9 mmol, 1.1 *eq.*) in DMF (500 mL) was stirred at 25°C for 10 hrs and then poured into H_2O (2 L) and extracted with EtOAc (500 mL x 3). The combined organic layers were washed with brine (300 mL x 5), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate = 20:1 to 10:1) to give ***trans-tert-butyl N-[4-(5-bromothiazol-2-yl)cyclohexyl]carbamate*** (78 g, crude) as a yellow solid. ESI [M+H] = 363.0/361.0

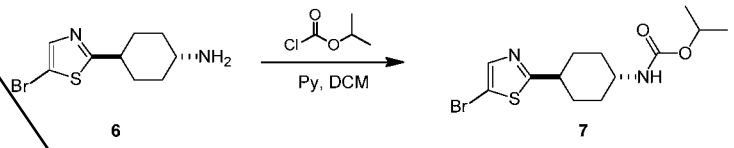
Preparation of compound 6.



5 6

A mixture of ***trans-tert-butyl N-[4-(5-bromothiazol-2-yl)cyclohexyl]carbamate*** (50 g, 138.4 mmol, 1 *eq.*) in HCl/MeOH (4 M, 700 mL) was stirred at 25°C for 0.5 hr and then concentrated to give ***trans-4-(5-bromothiazol-2-yl) cyclohexanamine*** (45 g, crude, HCl salt) as a yellow solid. ESI [M+H] = 263.0/261.0

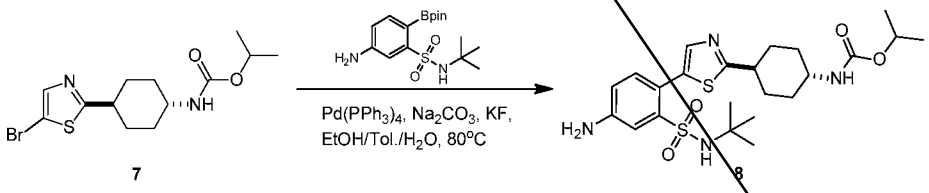
Preparation of compound 7.



6 7

To a solution of ***trans-4-(5-bromothiazol-2-yl)cyclohexanamine*** (45 g, HCl salt, 172.3 mmol, 1 *eq.*), Pyridine (61.5 mmol, 69.5 mL, 5 *eq.*) and DMAP (4.2 g, 34.5 mmol, 0.2 *eq.*) in DCM (300 mL) was added isopropyl carbonochloridate (258.5 mmol, 35.9 mL, 1.5 *eq.*) dropwise at 0°C. The mixture was stirred at 25°C for 0.5 hr and then washed with HCl (1N, 1 L) and sat.aq.Na_2CO_3 (1 L). The organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate =15:1 to 10:1) to give ***trans-isopropyl N-[4-(5-bromothiazol-2-yl)cyclohexyl]carbamate*** (37 g, 106.55 mmol, 61.84% yield) as a yellow solid. ^1H NMR (METHANOL-d4, 400MHz) δ = 7.60 (s, 1H), 4.81 (dt, J=12.2, 6.2 Hz, 1H), 3.41 (tt, J=11.6, 4.0 Hz, 1H), 2.88-3.00 (m, 1H), 2.10-2.20 (m, 2H), 1.98-2.07 (m, 2H), 1.55-1.68 (m, 2H), 1.30-1.43 (m, 2H), 1.21 (br d, J=6.2 Hz, 6H).

Preparation of compound 8.

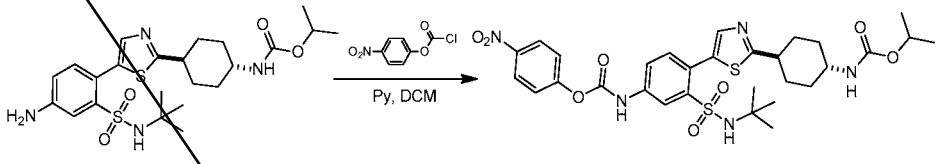


7 8

A mixture of ***trans-isopropyl N-[4-(5-bromothiazol-2-yl)cyclohexyl]carbamate*** (16.2 g, 46.7 mmol, 1 *eq.*), ***5-amino-N-tert-butyl-2-(4,4,5,5-tetramethyl-1,3,2- dioxaborolan-2-yl)benzenesulfonamide*** (19.9 g, 56.1 mmol, 1.2 *eq.*), KF (4.1 g, 70.1 mmol, 1.5 *eq.*), Na_2CO_3 (14.9 g, 140.2 mmol, 3 *eq.*) and $Pd(PPh_3)_4$ (1.6 g, 1.4 mmol, 0.03 *eq.*) in toluene (150 mL), EtOH (150 mL) and H_2O (50 mL) stirred at 80°C for 6 hrs under N_2 atmosphere. The reaction mixture was concentrated and the residue was diluted with H_2O (100 mL) and extracted with EtOAc (100 mL x 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate= 20:1 to 1:1) to give ***trans-isopropyl N-[4-[5-[4-amino-2-(tert-butylsulfamoyl) phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (13 g, 26.3 mmol, 56.2% yield) as a white solid.

^{1}H NMR (400MHz, METHANOL-d4) δ = 7.65 - 7.60 (m, 1H), 7.43 (d, J=2.2 Hz, 1H), 7.13 (d, J=8.3 Hz, 1H), 6.83 (dd, J=2.6, 8.3 Hz, 1H), 4.81 (br s, 1H), 3.76 - 3.71 (m, 1H), 3.05 - 2.86 (m, 1H), 2.20 (br d, J=12.3 Hz, 2H), 2.06 (br d, J=10.5 Hz, 2H), 1.75 - 1.60 (m, 2H), 1.47 - 1.34 (m, 2H), 1.22 (br d, J=6.1 Hz, 6H), 1.09 (s, 9H).

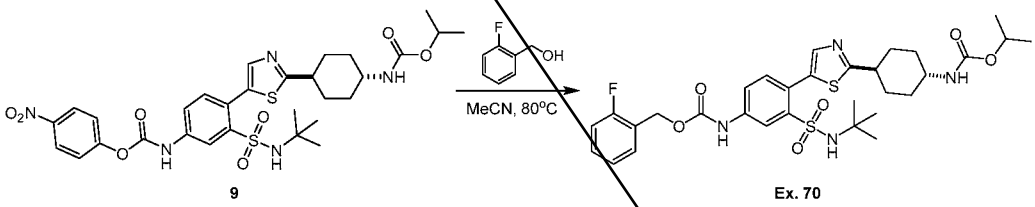
Preparation of compound 9.



8 9

To a solution of ***trans-isopropyl N-[4-[5-[4-amino-2-(tert-butylsulfamoyl)phenyl] thiazol-2-yl]cyclohexyl]carbamate*** (250 mg, 505.4 umol, 1 *eq.*) in DCM (4 mL) were added DMAP (6.2 mg, 50.5 umol, 0.1 *eq.*), Pyridine (120 mg, 1.5 mmol, 3 *eq.*) and (4-nitrophenyl) carbonochloridate (153 mg, 758 umol, 1.5 *eq.*). The mixture was stirred at 25°C for 0.5 hr and used directly for the next step. ESI [M+H] = 660.2

Preparation of compound Ex. 70.

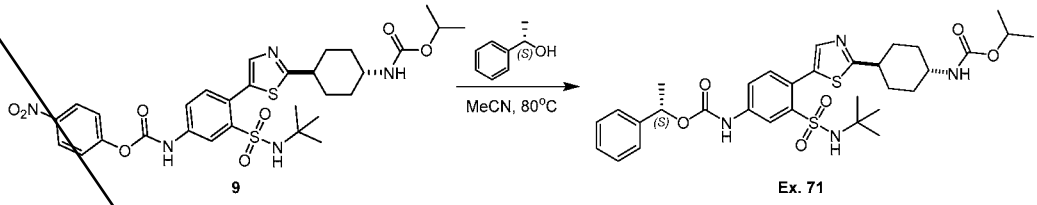


9 Ex. 70

To a solution of (2-fluorophenyl)methanol (45.9 mg, 363.8 umol, 3 *eq.*) and DIEA (47 mg, 363.8 umol, 3 *eq.*) in MeCN (1 mL) was added the above solution (1 mL). The mixture was stirred at 80°C for 1 hr, then concentrated and the residue was purified by prep-HPLC (Column: Waters Xbridge 150*25 5u; Mobile phase: [water (10 mM NH_4HCO_3)-ACN]; B%: 42%-72%, 12 min) to give ***trans-isopropyl N-[4-[5-[2-(tert-butylsulfamoyl)-4-[(2-fluorophenyl)methoxy carbonylamino]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (16.34 mg, 25.26 umol, 20.83% yield, 100% purity) as a yellow solid. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.36 (s, 1H), 7.77 - 7.63 (m, 2H), 7.52 (t, J=7.0 Hz, 1H), 7.44 - 7.31 (m, 2H), 7.23 - 7.08 (m, 2H), 5.29 (s, 2H), 4.83 (br s, 1H), 3.46 (br d, J=11.8 Hz, 1H), 3.00 (br t, J=11.8 Hz, 1H), 2.22 (br d, J=12.7 Hz, 2H), 2.07 (br d, J=11.4 Hz, 2H), 1.76 - 1.62 (m, 2H), 1.47 - 1.35 (m, 2H), 1.22 (br d, J=6.1 Hz, 6H), 1.12 (s, 9H). ESI [M+H] = 647.2

Example 71. Synthesis of trans-[(1S)-1-phenylethyl] N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate.

Scheme 30:

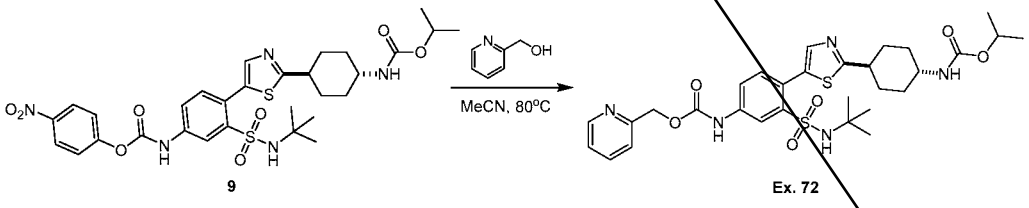


Preparation of Ex. 71.

To a solution of (1S)-1-phenylethanol (29.6 mg, 242.5 umol, 2 *eq.*) and DIEA (47 mg, 363.8 umol, 3 *eq.*) in MeCN (2 mL) was added a solution of ***trans-(4-nitrophenyl) N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate*** (80 mg, 121.25 umol, 1 *eq.*) in DCM (1 mL). The mixture was stirred at 80°C for 1 hr, then concentrated and the residue was purified by prep-HPLC (Column: Nano-Micro UniSil 5-100 C18 ULTRA 100*250mm 5um;mobile phase: [water(0.1%TFA)-ACN]; B%: 55%-80%,11min) to give ***trans-[(1S)-1-phenylethyl] N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate*** (12.19 mg, 18.77 umol, 15.48% yield, 99% purity) as a white solid. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.35 (br s, 1H), 7.81 - 7.65 (m, 2H), 7.48 - 7.36 (m, 5H), 7.31 (br d, J=6.8 Hz, 1H), 5.89 (br d, J=6.2 Hz, 1H), 4.85 (br d, J=5.5 Hz, 1H), 3.47 (br s, 1H), 3.03 (br s, 1H), 2.24 (br d, J=11.2 Hz, 2H), 2.08 (br d, J=11.0 Hz, 2H), 1.71 (q, J=11.9 Hz, 2H), 1.61 (br d, J=6.4 Hz, 3H), 1.49 - 1.37 (m, 2H), 1.24 (br d, J=5.4 Hz, 6H), 1.13 (s, 9H). ESI [M+H] = 643.2

Example 72. Synthesis of N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino) cyclohexyl]thiazol-5-yl]phenyl]carbamate.

Scheme 31:



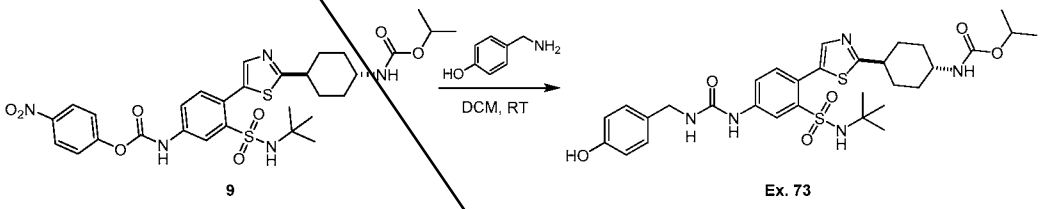
Preparation of Ex. 72.

To a solution of 2-pyridylmethanol (26.5 mg, 242.5 umol, 2 *eq.*) and DIEA (47 mg, 363.8umol, 3 *eq.*) in MeCN (2 mL) was added a solution of ***trans-(4-nitrophenyl) N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate*** (80 mg, 121.3 umol, 1 *eq.*) in DCM (1 mL). The mixture was stirred at 80°C for 1 hr, then concentrated and the residue was purified by prep-HPLC (Column: Waters Xbridge 150*25 5u; Mobile phase: [water (0.04% NH_3H_2O + 10 mM NH_4HCO_3)-ACN]; B%: 35%-65%, 10 min) to give ***trans-2-pyridylmethyl N-[3-(tert-butylsulfamoyl)-4-***

[2-[4-(isopropoxycarbonylamino)cyclohexyl] thiazol-5-yl]phenyl]carbamate (18.59 mg, 28.82 umol, 23.77% yield, 97.65% purity) as a pale yellow solid. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.56 (br d, J=4.5 Hz, 1H), 8.38 (d, J=1.8 Hz, 1H), 7.94 - 7.87 (m, 1H), 7.78 - 7.72 (m, 2H), 7.59 (br d, J=7.8 Hz, 1H), 7.44 - 7.38 (m, 2H), 5.32 (s, 2H), 4.85 (td, J=5.9, 12.0 Hz, 1H), 3.47 (br t, J=11.8 Hz, 1H), 3.07 - 2.96 (m, 1H), 2.24 (br d, J=12.3 Hz, 2H), 2.13 - 2.04 (m, 2H), 1.77 - 1.65 (m, 2H), 1.48 - 1.37 (m, 2H), 1.24 (br d, J=6.1 Hz, 6H), 1.14 (s, 9H). ESI [M+H] = 630.2

Example 73. Synthesis of trans-isopropyl N-[4-[5-[2-(tert-butylsulfamoyl)-4-[(4-hydroxyphenyl)methylcarbamoylamino]phenyl]thiazol-2-yl]cyclohexyl]carbamate.

Scheme 32:

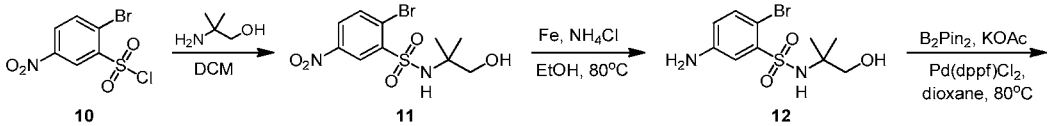


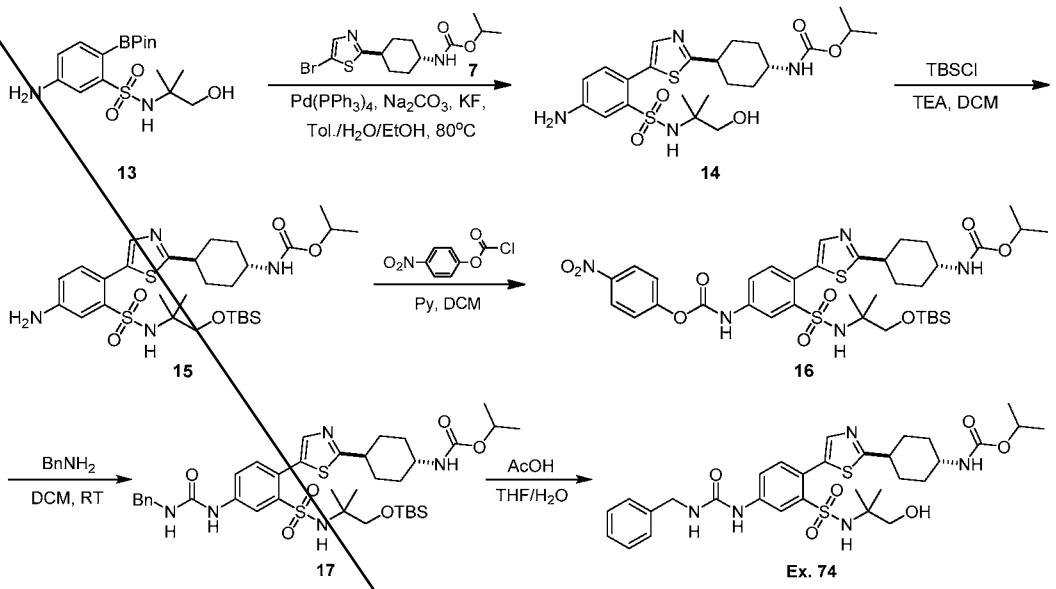
Preparation of Ex. 73.

To a solution 4-(aminomethyl) phenol (74.7 mg, 606.5 umol, 3 *eq.*) and DIEA (78.4 mg, 606.5 umol, 3 *eq.*) in DCM (2 mL) was added ***trans-(4-nitrophenyl) N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate*** (133.4 mg, 202.2 umol, 1 *eq.*) in DCM (2 mL). The mixture was stirred at 25°C for 1 hr, then concentrated and the residue was purified by prep-HPLC (TFA condition) to give ***trans-isopropyl N-[4-[5-[2-(tert-butylsulfamoyl)-4-[(4-hydroxyphenyl)methylcarbamoylamino]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (23.56 mg, 35.99 umol, 17.80% yield, 98.357% purity) as a white solid. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.24 (d, J=2.2 Hz, 1H), 7.77 (s, 1H), 7.69 (dd, J=2.2, 8.3 Hz, 1H), 7.36 (d, J=8.3 Hz, 1H), 7.16 (d, J=8.8 Hz, 2H), 6.75 (d, J=8.3 Hz, 2H), 4.85 - 4.74 (m, 1H), 4.30 (s, 2H), 3.45 (s, 1H), 3.15 - 2.97 (m, 1H), 2.23 (br d, J=12.3 Hz, 2H), 2.08 (br d, J=13.2 Hz, 2H), 1.79 - 1.63 (m, 2H), 1.48 - 1.34 (m, 2H), 1.22 (br d, J=6.1 Hz, 6H), 1.12 (s, 9H). ESI [M+H] = 644.2

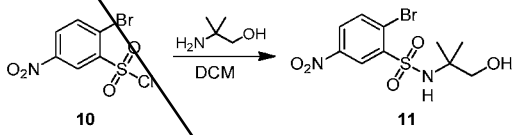
Example 74. Synthesis of trans-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-[(2-hydroxy-1,1-dimethyl-ethyl)sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate.

Scheme 33:



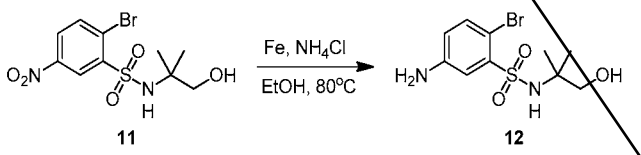


Preparation of compound 11.



To a mixture of ***2-amino-2-methyl-propan-1-ol*** (3 g, 33.7 mmol, 5.1 *eq.*) and DMAP (80 mg, 654.8 umol, 0.98 *eq.*) in DCM (50 mL) was added ***2-bromo-5-nitro- benzenesulfonyl chloride*** (2 g, 6.66 mmol, 1 *eq.*). The mixture was stirred at 20°C for 30 mins, then washed with 1N HCl (20 mL) and sat.aq.$NaHCO_3$ (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to give ***2-bromo-N-(2-hydroxy-1,1-dimethyl-ethyl)-5-nitro- benzenesulfonamide*** (1.7 g, 4.8 mmol, 72.3% yield) as a yellow gum. ESI [M+H] = 355.0/353.0

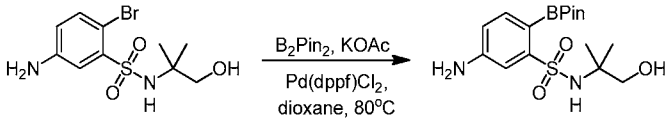
Preparation of compound 12.



A mixture of ***2-bromo-N-(2-hydroxy-1,1-dimethyl-ethyl)-5-nitro- benzenesulfonamide*** (1.7 g, 4.8 mmol, 1 *eq.*), Fe (1.5 g, 26.9 mmol, 5.6 *eq.*) and NH_4Cl (800 mg, 14.9 mmol, 3.1 *eq.*) in EtOH (15 mL)/H_2O (7.5 mL)/THF (7.5 mL) was stirred at 80°C for 2 hrs. The reaction mixture was concentrated to remove EtOH, diluted with H_2O (100 mL) and extracted with EtOAc (100 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate = 100:1 to 1:1) to give ***5-amino-2-bromo-N-(2-hydroxy-1,1-dimethyl-***

ethyl)benzenesulfonamide (1.1 g, 3.4 mmol, 70.7% yield) as a pale yellow solid. ESI [M+H] = 325.0/323.0

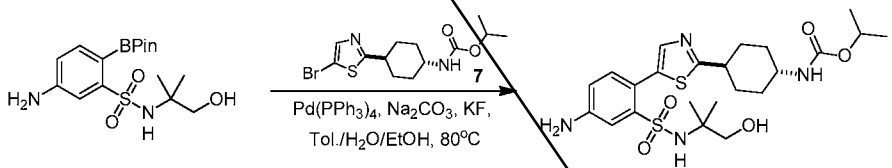
Preparation of compound 13.



12 13

A mixture of ***5-amino-2-bromo-N-(2-hydroxy-1,1-dimethyl-ethyl) benzenesulfonamide*** (400 mg, 1.24 mmol, 1 *eq.*), 4,4,5,5-tetramethyl-2-(4,4,5,5- tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (942.8 mg, 3.7 mmol, 3 *eq.*), $Pd(dppf)Cl_2$ (90.6 mg, 123.8 umol, 0.1 *eq.*) and KOAc (364.4 mg, 3.7 mmol, 3 *eq.*) in dioxane (4 mL) was stirred at 80°C for 12 hrs under N_2 atmosphere and then concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate = 50:1 to 3:1) to give ***5-amino-N-(2-hydroxy-1,1- dimethyl-ethyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide*** (201 mg, crude) as a white solid. ^1H NMR (400MHz, METHANOL-d4) δ = 7.53 (d, J=8.0 Hz, 1H), 7.28 (d, J=2.4 Hz, 1H), 6.75 (dd, J=6.4 Hz, 1H), 3.35 (s, 2H), 1.36 (s, 12H), 1.10 (s, 6H).

Preparation of compound 14.

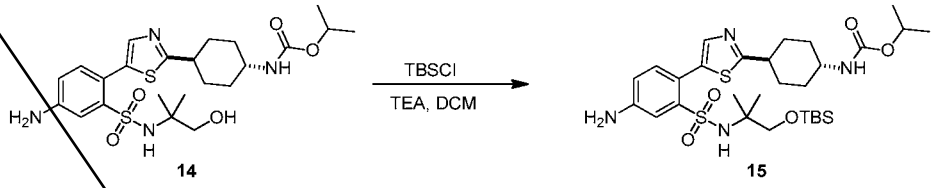


13 14

A mixture of ***5-amino-N-(2-hydroxy-1,1-dimethyl-ethyl)-2-(4,4,5,5-tetramethyl -1,3,2-dioxaborolan-2-yl)benzenesulfonamide*** (140.7 mg, 380.1 umol, 1.2 *eq.*), ***trans-isopropyl N-[4-(5-bromothiazol-2-yl)cyclohexyl]carbamate*** (110 mg, 316.7 umol, 1 *eq.*), Na_2CO_3 (100.7 mg, 950.3 umol, 3 *eq.*), KF (27.6 mg, 475.1 umol, 1.5 *eq.*) and $Pd(PPh_3)_4$ (36.6 mg, 31.7 umol, 0.1 *eq.*) in toluene(1 mL)/EtOH (1 mL)/H_2O (0.3 mL) was stirred at 80°C for 12 hrs under N_2 atmosphere. The reaction mixture was concentrated, diluted with H_2O (10 mL) and extracted with EtOAc (10 mL x 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by prep-TLC (SiO_2, Ethyl acetate) to give ***trans-isopropyl N-[4-[5-[4-amino-2-[(2-hydroxy-1,1-dimethyl-ethyl)sulfamoyl] phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (117 mg, crude) as a yellow solid. ^1H NMR (400MHz, METHANOL-d4) δ = 7.63 (d, J=7.0 Hz, 1H), 7.44 (d, J=2.2 Hz, 1H), 7.15 (d, J=8.3 Hz, 1H), 6.83 (dd, J=2.4, 8.1 Hz, 1H), 4.85 - 4.78 (m, 1H), 3.50 - 3.39 (m,

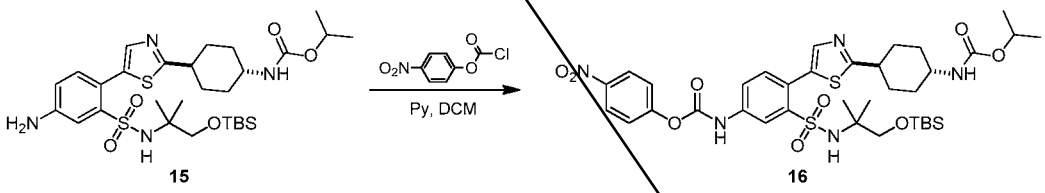
1H), 3.25 (s, 2H), 3.03 - 2.91 (m, 1H), 2.21 (br t, J=6.1 Hz, 2H), 2.12 - 2.00 (m, 2H), 1.75 - 1.60 (m, 2H), 1.45 - 1.33 (m, 2H), 1.28 - 1.19 (s, 6H), 1.03 (s, 6H)

Preparation of compound 15.



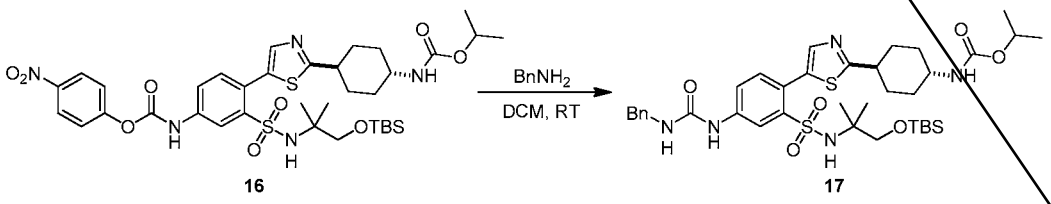
To a solution of ***trans-isopropyl N-[4-[5-[4-amino-2-[(2-hydroxy-1,1-dimethyl-ethyl) sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (90 mg, 176.3 umol, 1 *eq.*) in DCM (1 mL) were added TEA (53.5 mg, 528.7 umol, 3 *eq.*), DMAP (2.2 mg, 17.6 umol, 0.1 *eq.*) and TBSCl (66.4 mg, 440.6 umol, 2.5 *eq.*). The mixture was stirred at 30°C for 12 hrs and then concentrated. The residue was purified by prep-TLC (SiO_2, Petroleum ether: Ethyl acetate = 2:1) to give ***trans-isopropyl N-[4-[5-[4-amino-2-[[2-[tert-butyl(dimethyl)silyl]oxy-1,1-dimethyl-ethyl]sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (120 mg, crude) as a pale yellow solid. ESI [M+H] = 625.2

Preparation of compound 16.



To a solution of ***trans-isopropyl N-[4-[5-[4-amino-2-[[2-[tert-butyl(dimethyl)silyl] oxy-1,1-dimethyl-ethyl]sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (90 mg, 144 umol, 1 *eq.*) in DCM (1 mL) were added DMAP (1.8 mg, 14.4 umol, 0.1 *eq.*), pyridine (34.2 mg, 432 umol, 3 *eq.*) and (4-nitrophenyl) carbonochloridate (43.5 mg, 216 umol, 1.5 *eq.*). The mixture was stirred at 25°C for 0.5 hr and used into the next step directly without further purification. ESI [M+H] = 790.3

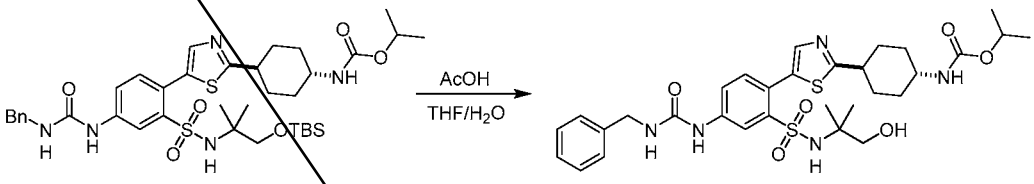
Preparation of compound 17.



To a solution of phenylmethanamine (36 mg, 336 umol, 3 *eq.*) in DCM (1 mL) was added a solution of ***trans-(4-nitrophenyl) N-[3-[[2-[tert-butyl(dimethyl)silyl] oxy-1,1-dimethyl-ethyl]sulfamoyl]-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-***

yl]phenyl]carbamate (88.5 mg, 112 umol, 1 *eq.*) in DCM (1 mL) and the mixture was stirred at 25°C for 0.5 hr. The reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc (10 mL x 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by prep-TLC (SiO_2, Petroleum ether: Ethyl acetate = 1:1) to give ***trans-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-[[2-[tert-butyl(dimethyl)silyl]oxy-1,1-dimethyl-ethyl]sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (93 mg, crude) as a yellow gum. ESI [M+H] = 758.4

Preparation of Ex. 74.



17 Ex. 74

A mixture of ***trans-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-[[2-[tert-butyl(dimethyl)silyl]oxy-1,1-dimethyl-ethyl]sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (84.9 mg, 112 umol, 1 *eq.*) in AcOH (0.5 mL)/THF (0.5 mL)/H_2O (0.5 mL) was stirred at 80°C for 0.5 hr. Then the mixture was concentrated and the residue was purified by prep-HPLC (TFA condition) to give ***trans-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-[(2-hydroxy-1,1-dimethyl-ethyl)sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (5.86 mg, 9.10 umol, 8.13% yield, 100% purity) as a yellow solid. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.25 (d, J=2.6 Hz, 1H), 7.81 - 7.66 (m, 2H), 7.40 - 7.30 (m, 5H), 7.28 - 7.22 (m, 1H), 4.82 (td, J=6.0, 12.5 Hz, 1H), 4.41 (s, 2H), 3.45 (br t, J=11.6 Hz, 1H), 3.28 (s, 2H), 3.00 (br t, J=11.8 Hz, 1H), 2.23 (br d, J=13.2 Hz, 2H), 2.11 - 2.00 (m, 2H), 1.77 - 1.60 (m, 2H), 1.48 - 1.32 (m, 2H), 1.22 (br d, J=6.1 Hz, 6H), 1.05 (s, 6H). ESI [M+H] = 644.3

Example 75. Compound Primary Screening

1. BACKGROUND

Primary screening was a phenotypic screen that utilized the synthetic lethal interaction between AID and RAD51 to identify compounds that were both potent and on target. AID expressing cells are dependent upon RAD51 for survival; inhibiting RAD51 in AID positive cells results in a cytotoxic effect. Based on such an effect, compounds that were potent in AID positive cells and were significantly less potent in AID negative cells were identified.

2. MATERIALS AND SUPPLIES

Plastic ware and consumables needed for this experiment include: Cell Culture media; Evaporation Buffer media; 100% DMSO; 96 well U-bottom sterile culture plates; 250mL bottle; 1.5mL Opaque amber epi tubes; Epi Tube rack; 300mL reservoirs; 25mL reservoir; 25mL serological pipette tips; 5mL serological pipette tips P1000 Pipette Tips; and P200 Pipette Tips.

Equipment needed for this experiment include: Viaflo 384 liquid handler; Eppendorf serological pipette; Eppendorf P1000 Pipette; and Eppendorf P200 Pipette

Daudi Cell Culture and WI-38 Cell Cultures were also needed for this experiment.

Lastly, compounds (*e.g.*, the compounds of this disclosure) to be tested are needed.

3. PROCEDURE

All steps were performed in a sterile environment inside the Biosafety cabinet.

The first step was to set up a cell killing assay in the Daudi cell line (AID positive). A 96 well u-bottom plate was prepared by writing the experiment number, plate number, date and initials in the top right corner of the plate lid. With a sterile 300ml reservoir, and 25ml serological pipette, evaporation buffer media was pipetted into reservoir in 25ml increments. Using the liquid handler,150ul of evaporation buffer media was pipetted from reservoir into rows A and H, and Columns 1 and 12 of the 96 well u-bottom plate. Cell cultures were counted to obtain the density of cells per ml, and the culture viability. The cell density information was used to obtain 1,000,000 cells from culture using a 5mL serological pipette into an epi tube. The cell density information from the culture was used to calculate the number of cells and volume of media needed for the assay to seed 1250 cells in 130ul of media per available culture well in the 96 well u-bottom plate. Rows B through F were used for cells (50 wells in total), with row G left for an empty media control. The calculation was overestimated by 10mL to account for the dead volume in the 300ml reservoir. Once the media volume was calculated, the appropriate volume of media was pipetted in 25mL increments into the 250mL bottle using a 25mL serological pipette. The 250ml bottle was capped tightly, and placed into a 37°C water bath for 2 minutes. While the culture media was warming, 10mL of fresh media was pipetted from the 500mL culture media bottle into a sterile 25mL reservoir. Using the Eppendorf multichannel pipette, 130ul of media was pipetted from the 25mL reservoir into row G of the 96 well u-bottom plate. Once the 250mL bottle of media was warmed, the volume of culture needed was pipetted into the bottle, and mixed gently with a 25mL serological pipette as to not create bubbles, and then the contents of the bottle were pipetted into a new 300mL reservoir. Using the liquid handler, 130ul of

culture was pipetted from the 300mL reservoir into rows B through F of the 96 well u-bottom plate. Once the culture was added, the plate was placed into a 37°C incubator until the compound master plate was prepared for use.

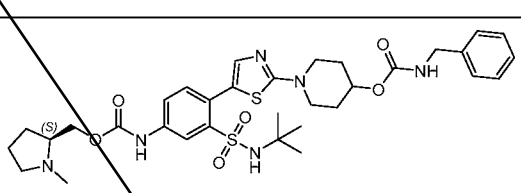
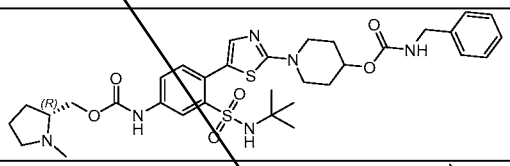
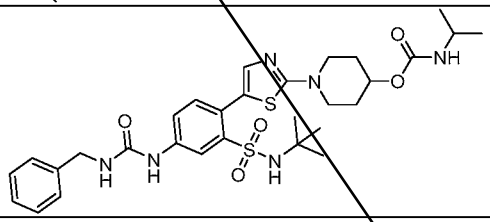
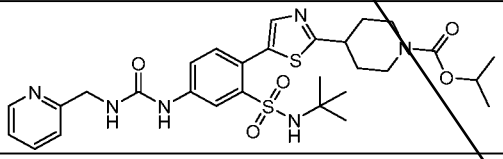
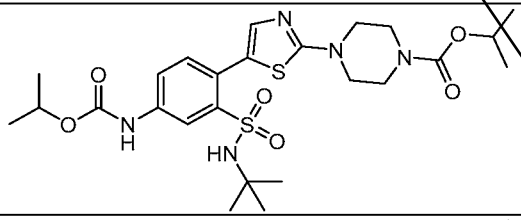
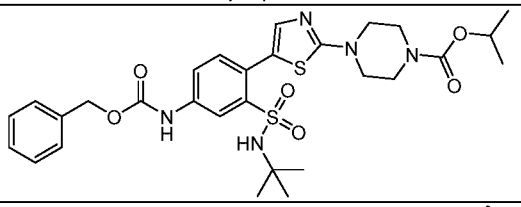
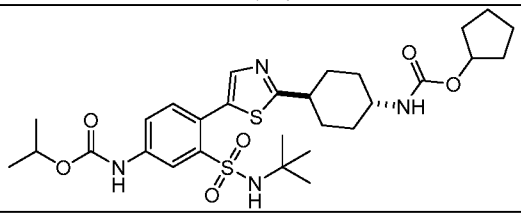
Two 96 well u-bottom plates were prepared by writing the master plate name in the upper right corner of the plate lid. Labeling one DMSO master and the other Media Master. The compounds of interest were obtained from the laboratory freezer, and placed into a 25 well storage box with a lid, and set the box aside. The compounds were vortexed after thawing but before use. Using an automatic multichannel pipette, 20ul of 100% DMSO was pipetted into wells B3-B11 through G3-G11 of the DMSO master plate. For each compound on the master plate, 50ul of the compound were pipetted in the appropriate well of row 2 (reference plate map to determine appropriate well). A serial dilution was prepared beginning by aspirating 20ul from row 2 and mixing with row 3, repeating until row 11 was reached. Using the liquid handler, 194ul of Daudi media was dispensed into wells B2-B11 through G2-G11 of the Media master plate. Using the liquid handler, 6ul from the DMSO master plate was aspirated and dispensed into the media master plate, mixing 100ul twice.

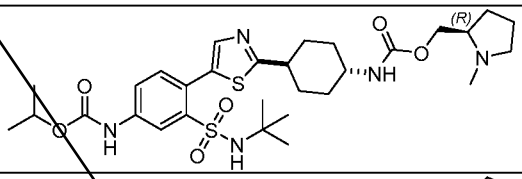
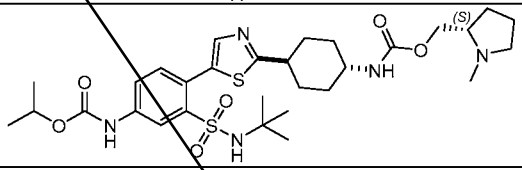
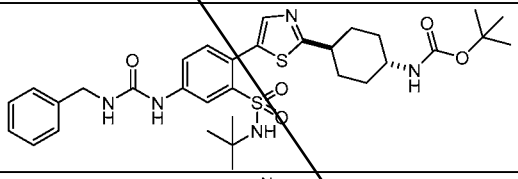
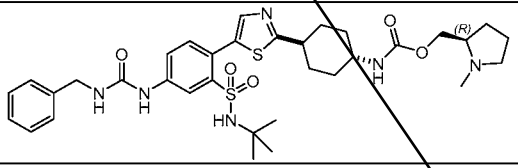
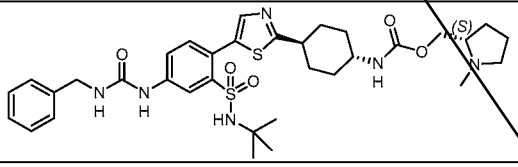
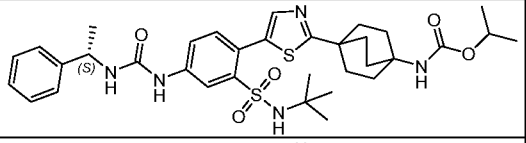
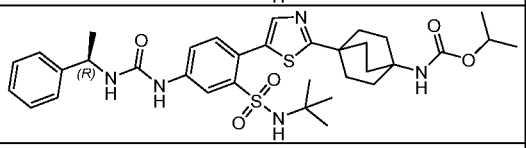
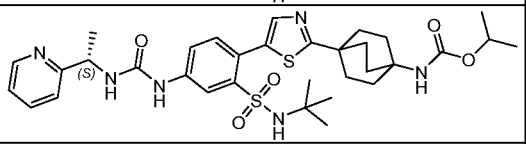
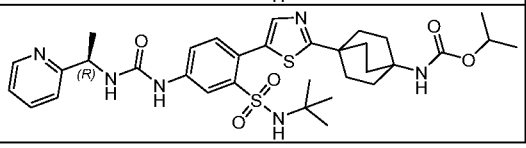
Compounds from master plate were then added to the culture plate. The culture plates were removed from the incubator, and set inside the biosafety cabinet. Using a liquid handler, 20ul from wells B2 to B11 through G2 to G11 of master plate were aspirated, and dispensed into wells B2 to B11 through G2 to G11 of culture plate. This set was continued with each culture plate. Once the culture plates acquired their 20ul of compound dilutions, they were placed back into the incubator, until their reads on Day 7 of experiment. Cell death was measured on Day 7 of the experiment using Cell-Titer Glo and a Promega Plate reader.

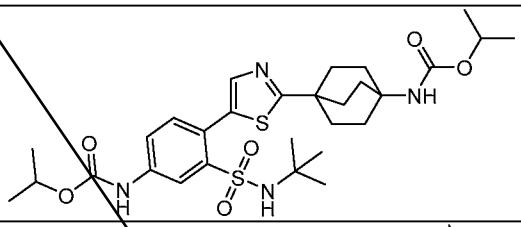
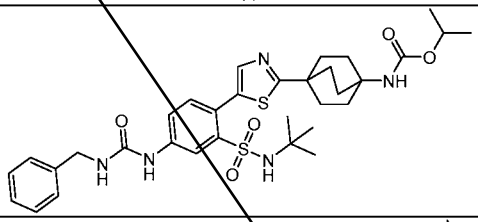
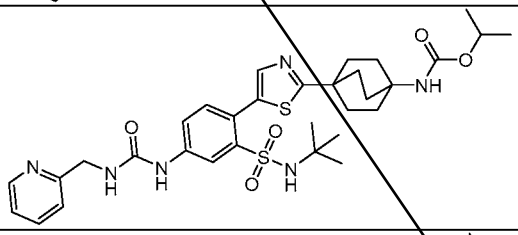
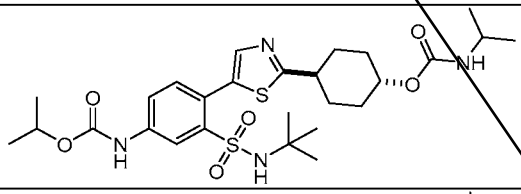
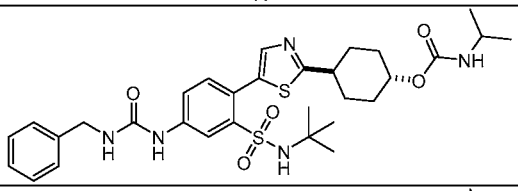
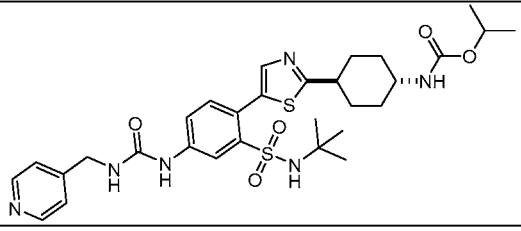
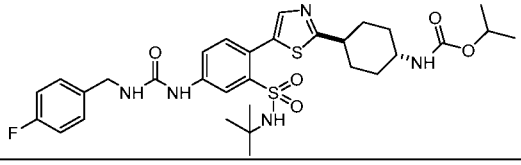
Percent cell death and EC_{50} values were calculated by comparing the cell viability of the compound treated wells to the non-treated wells. Normalized RLU values were obtained by subtracting the media well values from each of the wells in the same column, and then dividing that value by the DMSO treated cells values. The percent kill was then calculated by subtracting the normalized RLU value from 1 and multiplying by 100. The average normalized percent kill value and standard error of the mean was then calculated. The kill values were then inputted into Prism with the corresponding standard errors. In Prism a non-linear regression line was plotted with the data points using a semi-log scale, and the EC_{50} value was calculated. For compounds that showed good potency in the Daudi cell line, the assay was repeated using WI-38 cells (AID negative).

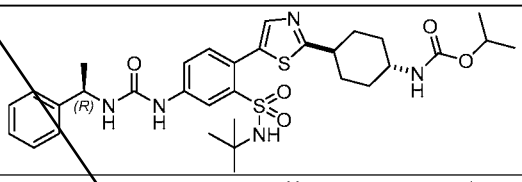
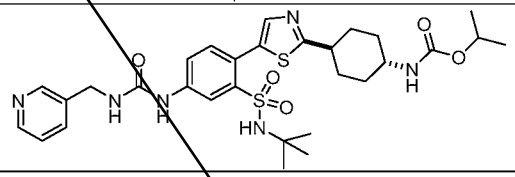
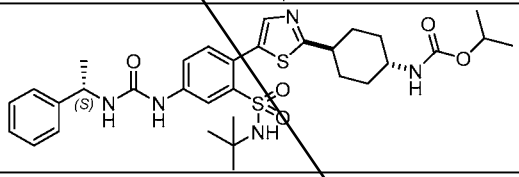
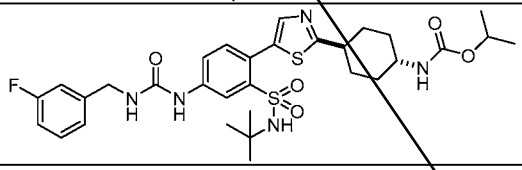
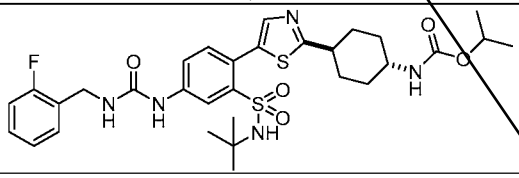
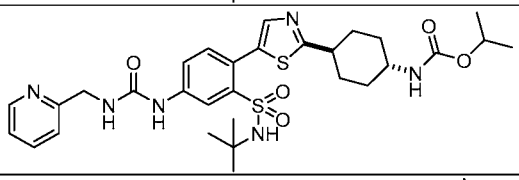
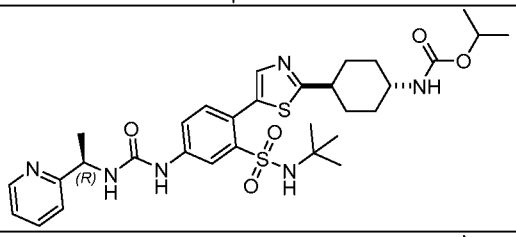
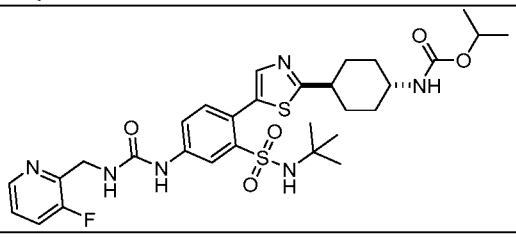
Screening Data

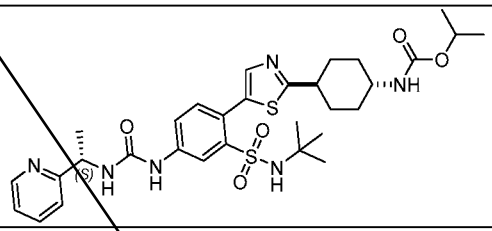
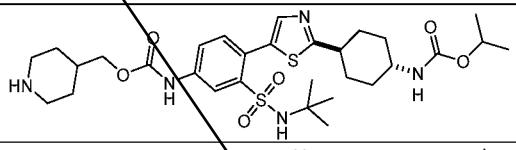
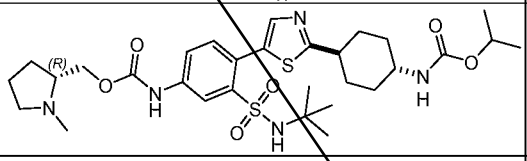
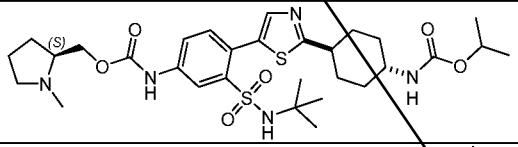
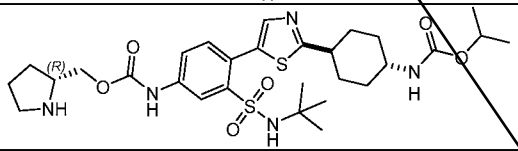
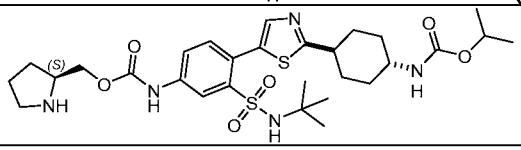
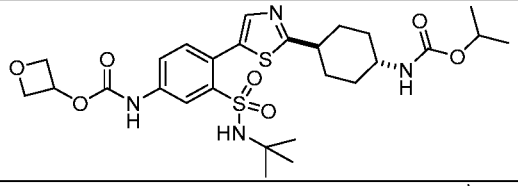
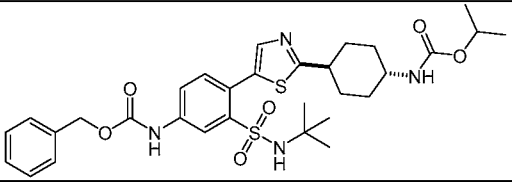
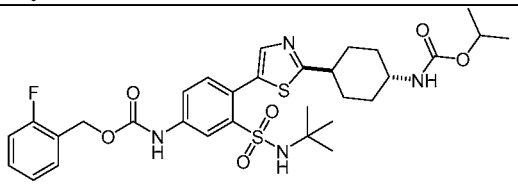
Table 1

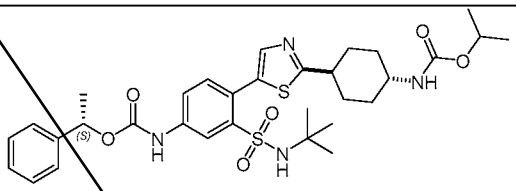
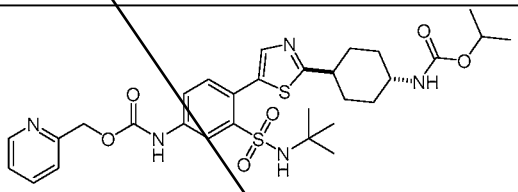
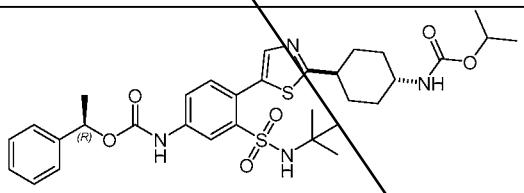
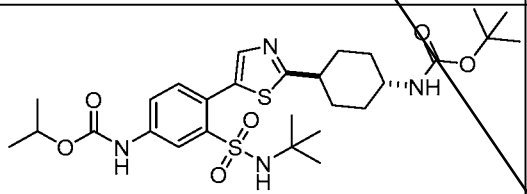
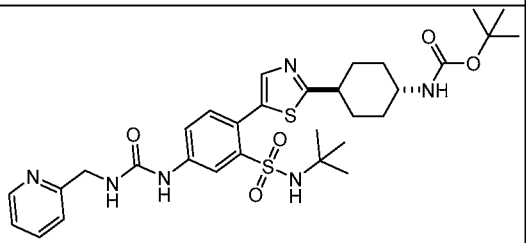
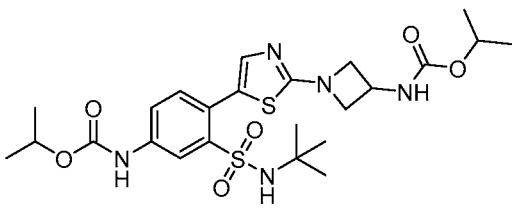
Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 1		C	ND
Ex. 2		C	ND
Ex. 3		A	C
Ex. 4		C	C
Ex. 5		C	ND
Ex. 6		C	ND
Ex. 7		B	ND

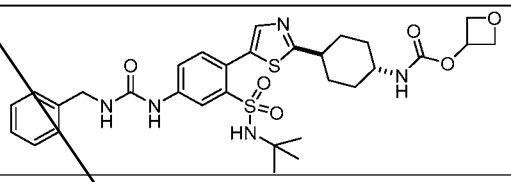
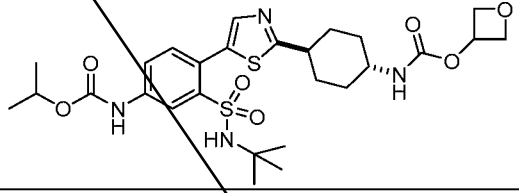
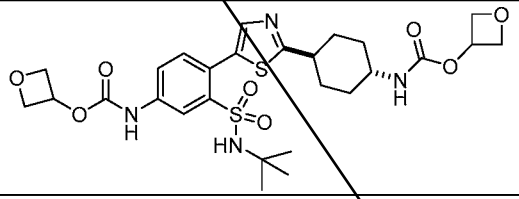
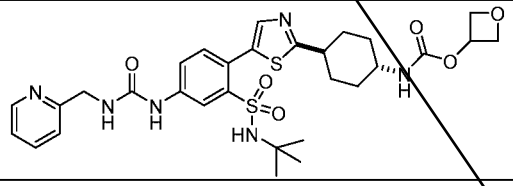
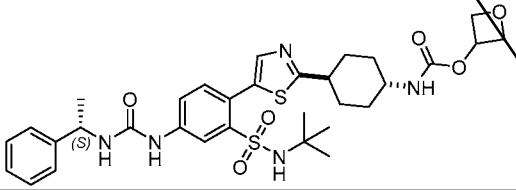
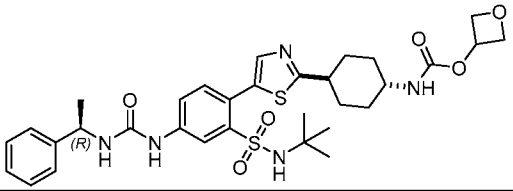
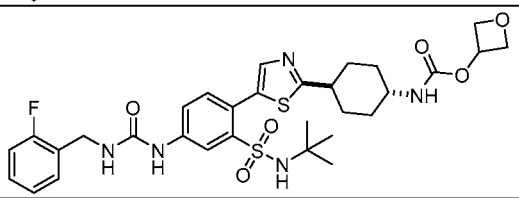
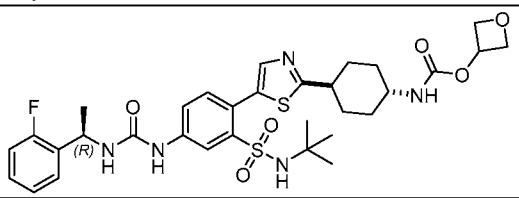
Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 8		C	ND
Ex. 9		B	ND
Ex. 10		A	C
Ex. 11		B	ND
Ex. 12		B	ND
Ex. 13		A	C
Ex. 14		A	C
Ex. 15		A	C
Ex. 16		A	C

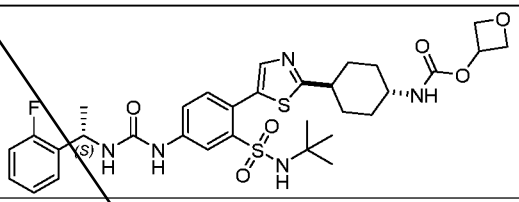
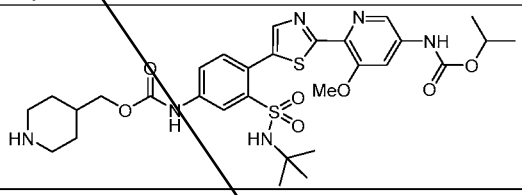
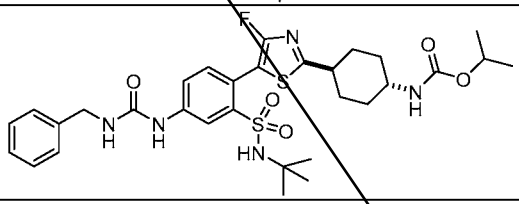
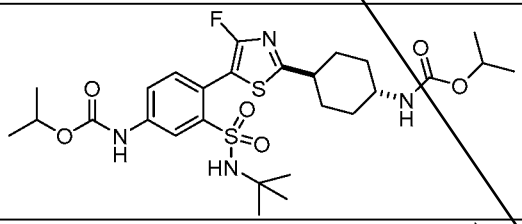
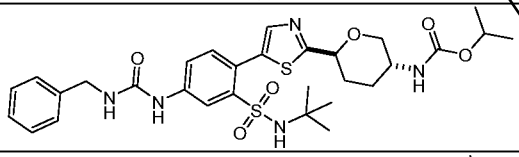
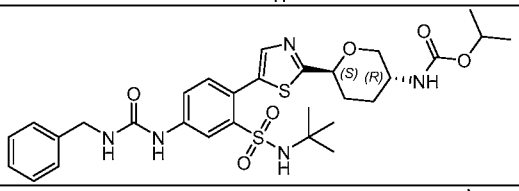
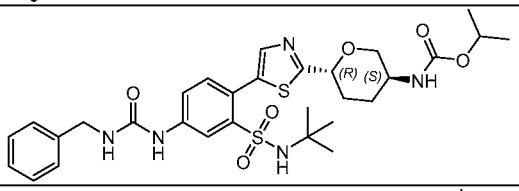
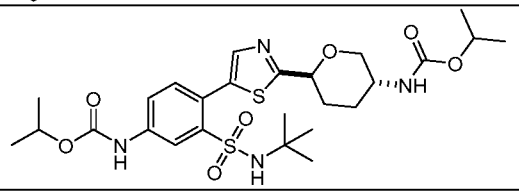
Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 17		B	C
Ex. 18		A	C
Ex. 19		A	C
Ex. 20		C	ND
Ex. 21		A	ND
Ex. 22		B	ND
Ex. 23		A	ND

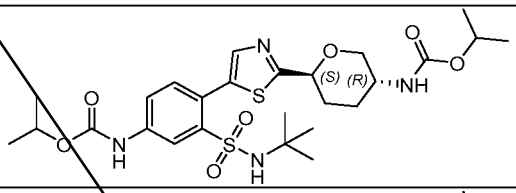
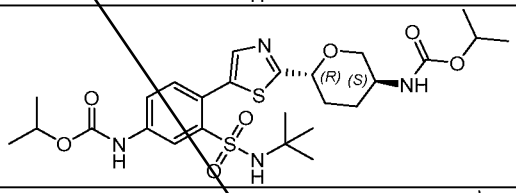
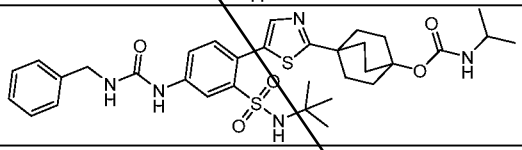
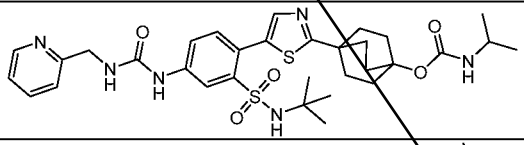
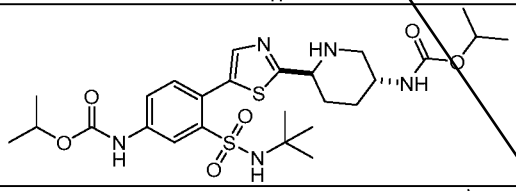
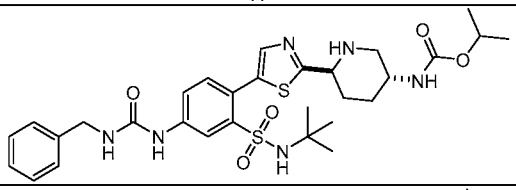
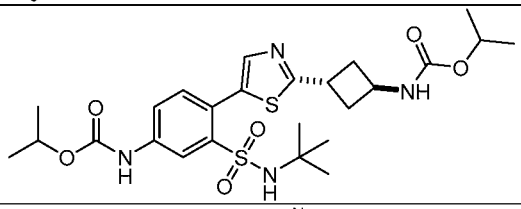
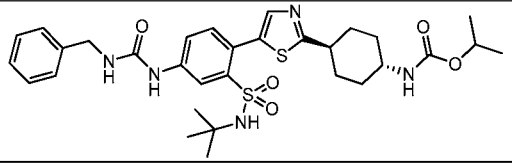
Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 24		A	C
Ex. 25		A	ND
Ex. 26		A	C
Ex. 27		A	C
Ex. 28		A	ND
Ex. 29		A	C
Ex. 30		A	C
Ex. 31		A	C

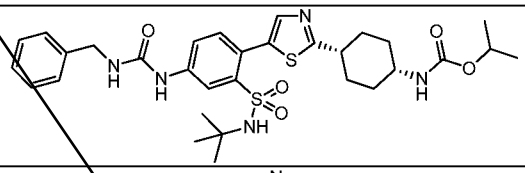
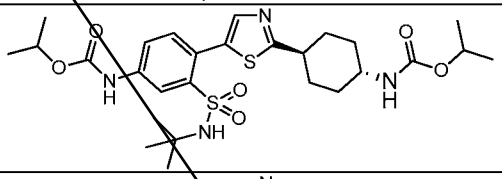
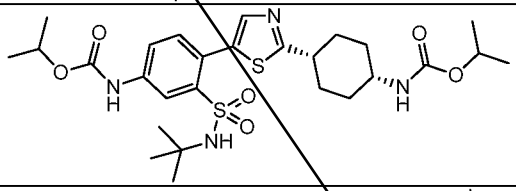
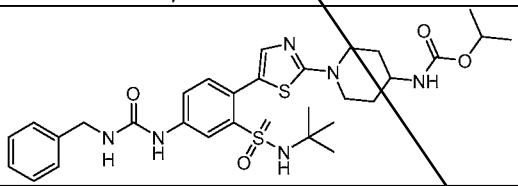
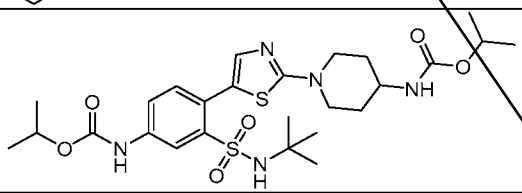
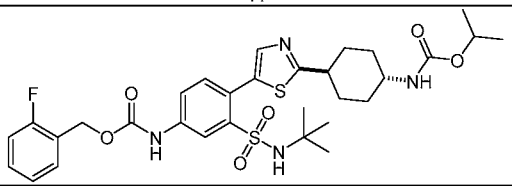
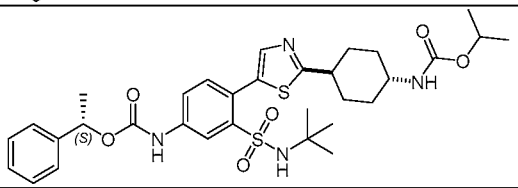
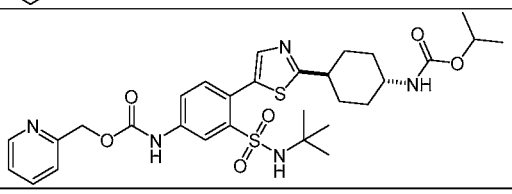
Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 32		A	C
Ex. 33		C	ND
Ex. 34		B	ND
Ex. 35		B	ND
Ex. 36		B	ND
Ex. 37		B	ND
Ex. 38		A	C
Ex. 39		A	C
Ex. 40		A	C

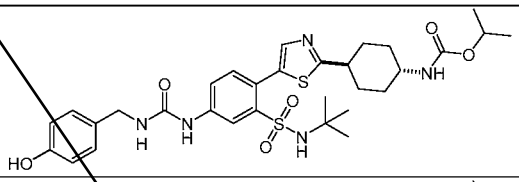
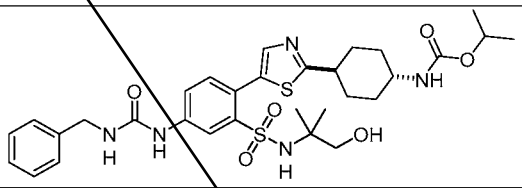
Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 41		A	C
Ex. 42		A	C
Ex. 43		B	C
Ex. 44		C	ND
Ex. 45		A	ND
Ex. 46		C	C
Ex. 47		A	C

Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
			
Ex. 48		B	C
Ex. 49		B	C
Ex. 50		A	C
Ex. 51		A	C
Ex. 52		A	C
Ex. 53		A	C
Ex. 54		A	C

Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 55		A	C
Ex. 56		B	ND
Ex. 57		A	C
Ex. 58		B	C
Ex. 59		A	C
Ex. 59A		B	C
Ex. 59B		A	C
Ex. 60		B	C

Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 60A		C	C
Ex. 60B		B	C
Ex. 61		C	C
Ex. 62		C	C
Ex. 63		B	ND
Ex. 64		A	C
Ex. 65		B	C
Ex. 66A		A	C

Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 66B		B	ND
Ex. 67A		B	C
Ex. 67B		C	ND
Ex. 68		B	ND
Ex. 69		C	ND
Ex. 70		A	C
Ex. 71		A	C
Ex. 72		A	C

Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 73		A	C
Ex. 74		A	C

Example 76. Bi-directional Caco-2 permeability

Bi-directional Caco-2 permeability was assayed. Caco-2 cells were seeded onto permeable polycarbonate supports and allowed to differentiate for about 3 weeks prior to being used in the assays. The cells were then exposed to the compounds from either the apical or basolateral sides and incubated at 37C for up to 90 minutes under light agitation. Compound transport was then measured using LC/MS/MS analysis at 30, 60, and 90 minutes.

Table 2

Example No.	Caco-2 Results				
	AB Papp (cm/sec x 10^6)	BA Papp (cm/sec x 10^6)	BA/AB Ratio	AB Recovery %	BA Recovery %
Ex. 5	5.9	2.2	0.4	34.7	80.8
Ex. 7	1.7	2.1	1.2	55.9	92
Ex. 10	1.2	1.5	1.3	59.9	87.2
Ex. 17	2.8	2.1	0.8	47.2	81.9
Ex. 20	9.4	10.4	1.1	44.8	96.6
Ex. 21	3.6	10.7	3	58.6	78.3
Ex. 22	1.1	21	18.7	83.1	89
Ex. 27	0.7	0.5	0.7	92	107.1
Ex. 28	1.9	3.3	1.7	66.9	81

Example No.	Caco-2 Results				
	AB Papp (cm/sec x 10^6)	BA Papp (cm/sec x 10^6)	BA/AB Ratio	AB Recovery %	BA Recovery %
Ex. 29	10.9	24.1	2.2	93.9	90.9
Ex. 30	11.5	15.7	1.4	79.8	115.6
Ex. 31	12.8	13.6	1.1	70.5	92.3
Ex. 34	0.4	45.2	103.9	98.2	96.8
Ex. 35	0.4	41	99.1	98.8	107.5
Ex. 38	17.9	22.9	1.3	73.3	82.3
Ex. 44	3.2	4.2	1.3	36.2	69.4
Ex. 45	7.6	10.8	1.4	73.6	84.4
Ex. 47	8.7	12.4	1.4	65.3	80.7
Ex. 48	23.1	16	0.7	80.1	90.3
Ex. 50	2	29.1	14.6	87.7	98.1
Ex. 51	9.9	10.5	1.1	65	85.7
Ex. 52	7.4	10.9	1.5	64.1	91.7
Ex. 53	9.3	9.7	1	63.1	90.9
Ex. 55	6.5	7	1.1	65.2	83.8
Ex. 57	1.3	3.5	2.7	61.9	86.8
Ex. 58	5.1	3.4	0.7	52.3	83.5
Ex. 59	3.1	15	4.8	61.5	91.5
Ex. 59A	3.1	9.6	3.1	55.6	85.5
Ex. 59B	4.6	9.7	2.1	56.2	82
Ex. 60	15.6	13.3	0.9	59.8	90.8
Ex. 60A	12	11.2	0.9	55.3	86
Ex. 60B	11.6	13.5	1.2	51.2	84.3
Ex. 63	10.5	26.7	2.5	83.6	90

Example No.	Caco-2 Results				
	AB Papp (cm/sec x 10^6)	BA Papp (cm/sec x 10^6)	BA/AB Ratio	AB Recovery %	BA Recovery %
Ex. 64	2.2	27.4	12.5	79.7	95.9
Ex. 66A	2.9	3.9	1.4	64.9	81.2
Ex. 67A	10.5	6.9	0.7	50.7	78.6

Example 77. Human Liver Microsome Stability

The stability of the claimed compounds was determined in the presences of human liver microsomes. The compounds were incubated with the microsomes at 37 °C for 45 minutes. Samples were analyzed using LC-MS/MS. Data analysis included half-life, clearance rate, and the percentage of hepatic blood flow (%QH) for each of the compounds in the different species. Below are liver microsome assay data of representative compounds, which show that the claimed compounds have superior metabolic stability.

Table 3

	Human Liver Microsome Stability		
	Half Life (min)	Clearance (µg/min/mg)	% QH
Ex. 29	20.5	68.0	78.8
Ex. 31	22.4	61.9	77.3
Ex. 66A	77.6	18.2	49.6
Ex. 67A	>300	<4.6	<20.3

Example 78. Cell Line Screen

The activity of the claimed compounds was measured in a variety of cell lines with different expression levels of activation induced cytidine deaminase (AICDA). The potency assay was repeated in all of the listed cell lines and the EC_{50} values recorded.

Table 4

Cell Line (Cancer Type)	**AICDA Expression**	**EC_{50} (nM) Ex. 29**	**EC_{50} (nM) Ex. 31**	**EC_{50} (nM) Ex. 66A**	**EC_{50} (nM) Ex. 67A**
Daudi (Lymphoma)	High	43	20	18	311
WSU-FSCCL (Lymphoma)	Negative	67	<40	25	344
U-698-M (Lymphoma)	High	113	31	88	791
CCRF-SB (Leukemia)	High	1283	2164	183	932
KYSE-70 (Head and Neck)	Low	4660	4701	2639	2629
SNU-1	Negative	n.d.	n.d.	609	2927

Cell Line (Cancer Type)	AICDA Expression	EC_{50} (nM) Ex. 29	EC_{50} (nM) Ex. 31	EC_{50} (nM) Ex. 66A	EC_{50} (nM) Ex. 67A
(Gastric)					
KG-1 (Leukemia)	Negative	>10000	8785	3067	2995
KYSE-510 (Head and Neck)	Negative	>10000	>10000	4516	3403
SNU-5 (Gastric)	Low	n.d.	n.d.	2941	3845
TOV-1120D (Ovary)	Negative	9172	n.d.	2377	4924
OV56 (Ovary)	Low	9086	n.d.	5944	7228
ARPE19/HPV16 (HPV Immortalized RPE)	Negative	>10000	>10000	>10000	>10000
WI-38 (Normal Human Lung Fibroblast)	Negative	>10000	>10000	>10000	>10000

n.d. not determined

Example 79. Pharmacokinetic (PK)

PK studies in mice were used to determine the fate of the compounds in a whole organism. Rats were treated with the compounds either orally of via IV at the indicated doses and followed for up to 24 hours. Plasma samples were taken at different time points and analyzed by LC-MS.

Table 5

po @ 80 mg/kg (Formulation: 30% PEG400, 10% Vitamin E TPGS in water)		Ex. 29	Ex. 31	Ex. 66A	Ex. 67A
Rat Female	$T_{1/2}$ (hr)	4.66	4.75	2.59	11.5
	F (%) @ 5 mg/kg	8.39	2.77	3.31	86.5
Rat Male	$T_{1/2}$ (hr)	3.97	3.79	1.86	6.46
	F (%) @ 5 mg/kg	3.69	1.49	2.55	46.9

Example 80. Compound 67A Activity in Pancreatic Cancer Cell Lines

Cells were harvested during the logarithmic growth period and cells were counted using Count-star. The cell concentrations were adjusted to 4.44×10^4 cells/mL with respective culture medium. 90 µL cell suspensions was added to two 96-well plates (plates A and B) with the final cell density of 4×10^3 cells/well (cell concentration were adjusted according to the data base or density optimization assay). One plate was used for the day 0 reading, the second plate was treated with compound and incubated for seven days with medium replacement at 72 hours.

For the day 0 reading, 10 µL of culture medium was added to each well of plate A. The vehicle control was 0.25 % v/v DMSO. The plate was equilibrated and its content was at room temperature for approximately 30 minutes. 50 µL CellTiter-Glo reagent was added to each well. The contents were mixed for 5 minutes on an orbital shaker to induce cell lysis. The plate was allowed to incubate at room temperature for 20 minutes to stabilize luminescent signal. Luminescence (for day 0) was recorded using EnVision Multi Label Reader.

For the day 7 reading, a 10×solution of test article was prepared (100 µM of test article in media with 3.16-fold serial dilutions was used to achieve 9 dose levels). 10 µL (10×) of Compound 67A was dispensed into each well (triplicate for each drug concentration) of plate B. The DMSO final concentration in culture medium was 0.25% [v/v]).

The test plate B was incubated for 72 hours in the humidified incubator at 37°C with 5% CO_2. 80 µL of media containing Compound 67A was aspirated with liquid handling Biomek FXP. 80 µL fresh culture media containing 1× Compound 67A solutions was added to each well. The mixture was incubated for an additional 96 hours in a humidified incubator at 37°C with 5% CO_2 (168 hours total), and then measured by CTG assay. The plate was equilibrated and its content brought to room temperature for approximately 30 minutes. 50 µL of CellTiter-Glo reagent was added to each well. The contents were mixed for 5 minutes on an orbital shaker to induce cell lysis. The plates were allowed to incubate at room temperature for 20 minutes to stabilize the luminescent signal. Luminescence was then recorded.

The data was then displayed graphically using GraphPad Prism 5.0. Absolute IC_{50} (EC_{50}), was calculated by fitting a dose-response curve using a nonlinear regression model with a sigmoidal dose response. The formula for calculating surviving rate is shown below and the absolute IC_{50} (EC_{50}) was then calculated according to the dose-response curve generated by GraphPad Prism 5.0.

$$\text{The surviving rate (\%)} = \frac{(\text{LumTest article} - \text{LumMedium control})}{(\text{LumNontreated} - \text{LumMedium control})}\ x\ 100\%$$

For IC_{50} values shown in Table 6, “A” means IC_{50} < 10 µM; “B” means IC_{50} ranging between 10 µM and 25 µM; “C” means IC_{50} ranging between 25 µM and 35 µM; “D” means IC_{50} ranging between 35 µM and 50 µM; “E” means IC_{50} > 50 µM; “Nd” means Not determined.

Table 6

Cell Line	IC50 (μM)
AsPC-1	C
BxPC-3	B
Capan-1	E
Capan-2	A
CFPAC-1	C
HPAF-II	B
HuP-T4	B
KP4	C
MIA PaCa-2	B
Panc 05.04	C
Panc 10.05	D
PANC-1	D
PL45	C
SU.86.86	Nd
SW1990	E

Example 81. PDX model 1

Activity of Compound 67A ("67A") was assessed in 3 different mouse PDX models with various levels of AID expression (low, medium, and high) (Figure 1). All models showed greater than 50% tumor growth inhibition with 67A treatment at 80 mg/kg QD (Low: 63%, Medium: 104%, high: 79%). For the medium expressing PDX model, the activity of 67A at two additional doses (1 mg/kg and 40 mg/kg) was examined. A response of 36% TGI at 1 mg/kg QD and a response of 96% TGI at 40 mg/kg QD was observed.

Next, a cross over experiment (Table 7) was performed to determine the activity of 67A in large, established tumors. On day 46 of treatment, vehicle treated and 1 mg/kg QD treated mice from the medium expressing PDX model were re-randomized into three treatment groups: vehicle, 1 mg/kg QD, and 80 mg/kg QD (Figure 2A). Tumor regression in the now 80 mg/kg treated cohort with a %TGI of 137 was observed.

The 40 and 80 mg/kg treatment groups were also re-randomized into three new groups: 80 mg/kg QD, 14 day dosing holiday followed by 80 mg/kg QD, and 21 day dosing holiday followed by 80 mg/kg QD (Figure 2B). During drug holiday, the tumors steadily began to grow at the same doubling rate as the vehicle control, and tumor growth was observed in the animals with tumors. Once treatment resumed with Compound 67A, the growth of the tumors was suppressed. The 80 mg/kg QD treatment group continued to show repressed tumor growth with a final %TGI of 96.

Table 7

Model	Tumor Characterization	Passage Number	AICDA Fold Gene Expression*
Model 1	Primary; Adenocarcinoma; Poorly Differentiated; Stage II	6	0.86
Model 2	Primary; Adenocarcinoma; Moderately Differentiated; Stage II	6	5.85
Model 3	Primary; Adenocarcinoma; Moderately Differentiated; Stage IV	5	9.85

*Fold change in expression determined by comparing the individual model RPKM score to the mean RPKM score of the entire PDX collection.

Compound 67A anti-tumor activity in Model 1

Engrafted mice were treated with either vehicle or Compound 67A at 80 mg/kg QD for 52 days. Tumor volumes were measured twice weekly and followed over the course of the experiment. The results demonstrated that Compound 67A treatment was successful (see Figures 3A and 3B). Percent tumor growth inhibition was calculated for each Compound 67A treated animal on day 52.

Compound 67A anti-tumor activity in Model 2, Part 1

Engrafted mice were treated with either vehicle or Compound 67A at 1, 40, or 80 mg/kg QD for 45 days. Tumor volumes were measured twice weekly and followed over the course of the experiment. The results demonstrated that Compound 67A treatment was successful (see Figures 4A and 4B). Percent tumor growth inhibition was calculated for each Compound 67A treated animal on day 45.*Compound 67A anti-tumor activity in Model 2, Part 2*

Animals treated with vehicle and 1 mg/kg QD from Model 2, Part 1 were re-randomized and treated with either vehicle or Compound 67A at 1 mg/kg QD or 80 mg/kg QD (Figure 2A). Tumor volumes were measured twice weekly and followed for 49 days (Figure 6A). Change in tumor volume on day 49 as compared to day 0 was calculated for vehicle and Compound 67A at 80 mg/kg treated animals (Figure 6B). Progression free

survival was measured as stasis of tumor volume and graphed for vehicle and Compound 67A at 80 mg/kg treated animals (Figure 6C).

Compound 67A anti-tumor activity after dosing holidays in Model 2, Part 3

Animals treated with Compound 67A at 40 and 80 mg/kg QD from Model 2, Part 1 were re-randomized and treated with either vehicle or Compound 67A at 80 mg/kg QD continuously, Compound 67A at 80 mg/kg after a 14 day dosing holiday, or Compound 67A at 80 mg/kg after a 21 day dosing holiday (Figure 2B). Tumor volumes were measured twice weekly and followed for 49 days. The results demonstrated that Compound 67A treatment was successful (see Figures 7A-7C).

Compound 67A anti-tumor activity in Model 3

Engrafted mice were treated with either vehicle or Compound 67A at 80 mg/kg QD for 33 days. Tumor volumes were measured twice weekly and followed over the course of the experiment. The results demonstrated that Compound 67A treatment was successful (see Figures 3A and 3B). Percent tumor growth inhibition was calculated for each Compound 67A treated animal on day 33.

Compound 67A showed anti-cancer activity in all Models (i.e. Model 1, Model 2, and Model 3) of pancreatic cancer tested with a mean % TGI ranging from 62% to 104%. Compound 67A is also active in large established tumors, and dosing holidays did not lead to the development of resistance. In Model 2 one partial responder and one tumor-free responder was observed. The data demonstrate that 67A has anti-cancer activity in multiple preclinical models of pancreatic cancer.

Example 82. PDX model 2

Activity of Compound 67A ("67A") was assessed in 3 different mouse PDX models of pancreatic cancer engrafted onto the flanks of immunocompromised mice. The three models tested were Model A (CTG-0955), Model B (CTG-1462), and Model C (CTG-1295). Tumors were allowed to reach a mean tumor volume of 500 mm^3 prior to dosing. The dosing groups were as follows:

- Vehicle;
- Compound 67A at 80 mg/kg orally, once daily;
- Compound 67A at 80 mg/kg orally, once daily for four weeks, followed by a dosing holiday until tumors reach 1000 mm^3, at which point administration of Compound 67A at 80 mg/kg orally, once daily is reinitiated;
- Compound 67A at 80 mg/kg orally, daily every other week; and

- Compound 67A at 80 mg/kg orally, every other day.

All models were measured twice per week and showed anti-tumor activity in larger tumors, similar to the observations of Example 80. Dosing of Compound 67A daily resulted in greater anti-tumor activity in comparison to every other day dosing. Reinitiating treatment with Compound 67A after a dosing holiday resulted in slowed tumor growth or tumor shrinkage, depending on the initial sensitivity of the model.

Compound 67A anti-tumor activity in Model A

The dosing groups were analyzed to determining %TGI relative to continuous dosing in comparison to every other day dosages (FIG. 8A), as well as comparison of tumor volume (FIG. 8B). Tumor regression in the cohort treated with a continuous dosage of Compound 67A at 80 mg/kg with a %TGI of 52 was observed. Tumor regression in the cohort treated with an every other day dosage of Compound 67A at 80 mg/kg with a %TGI of 20 was observed. Tumor regression in the cohort with a 200mm^3 tumor treated daily with Compound 67A with a %TGI of 64 was observed. Tumor regression in the cohort with a 500mm^3 tumor treated with Compound 67A with a %TGI of 52 was observed.

Compound 67A anti-tumor activity in Model B

The dosing groups were analyzed to determing %TGI relative to continuous dosing in comparison to every other day dosages (FIG. 9A), comparison of tumor volume (FIG. 9B), and analysis of post holiday dosing (FIG. 9C). Tumor regression in the cohort treated with a continuous dosage of Compound 67A at 80 mg/kg with a %TGI of 68 was observed. Tumor regression in the cohort treated with an every other day dosage of Compound 67A at 80 mg/kg with a %TGI of 54 was observed. Tumor regression in the cohort with a 200mm^3 tumor treated daily with Compound 67A with a %TGI of 79 was observed. Tumor regression in the cohort with a 500mm^3 tumor treated with Compound 67A with a %TGI of 68 was observed.

Treatment with oral Compound 67A administered daily for four weeks at 80 mg/kg showed a decrease in tumor growth. After four weeks, a dosing holiday was implemented until the tumor growth reached 1000 mm^3 (i.e., about 6 days for Model B). At this point, administration of Compound 67A daily at 80 mg/kg was resumed. Post dosing holiday treatment exhibited a decrease in tumor progression (FIG. 9D).

Compound 67A anti-tumor activity in Model C

The dosing groups were analyzed to determing %TGI relative to continuous dosing in comparison to every other day dosages (FIG. 10A), comparison of tumor volume (FIG. 10B),

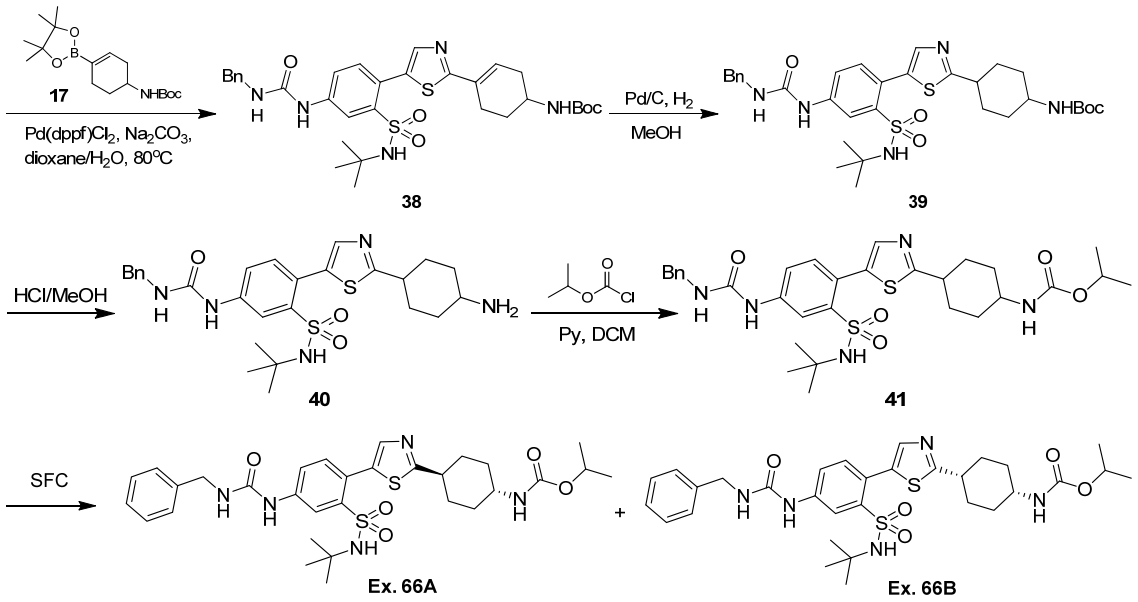
and analysis of post holiday dosing (FIG. 10C). Tumor regression in the cohort treated with a continuous dosage of Compound 67A at 80 mg/kg with a %TGI of 93 was observed. Tumor regression in the cohort treated with an every other day dosage of Compound 67A at 80 mg/kg with a %TGI of 65 was observed. Tumor regression in the cohort with a 200mm^3 tumor treated daily with Compound 67A with a %TGI of 104 was observed. Tumor regression in the cohort with a 500mm^3 tumor treated with Compound 67A with a %TGI of 93 was observed.

Treatment with oral Compound 67A administered daily for four weeks at 80 mg/kg showed a decrease in tumor growth. After four weeks, a dosing holiday was implemented until the tumor growth reached 1000 mm^3 (i.e., about 48 days for Model C). At this point, administration of Compound 67A daily at 80 mg/kg was resumed. Post dosing holiday treatment exhibited a decrease in tumor progression (FIG. 10D).

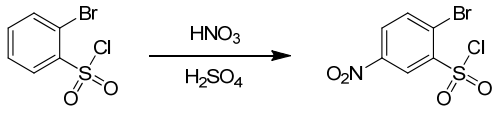
EQUIVALENTS

The details of one or more embodiments of the disclosure are set forth in the accompanying description above. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. Other features, objects, and advantages of the disclosure will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. All patents and publications cited in this specification are incorporated by reference.

The foregoing description has been presented only for the purposes of illustration and is not intended to limit the disclosure to the precise form disclosed, but by the claims appended hereto.

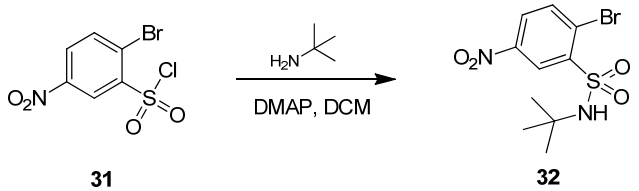


Preparation of compound 31.



To a solution of ***2-bromobenzenesulfonyl chloride*** (100.00 g, 391.36 mmol, 1.00 *eq.*) in H_2SO_4 (1.0 L) was added a solution of HNO_3 (79.95 g, 1.21 mol, 57.11 mL, 95% purity, 3.08 *eq.*) in H_2SO_4 (0.50 L) drop-wise at 0°C. The mixture was stirred at 26°C for 2 hrs. TLC (Petroleum ether: EtOAc =10:1, R_f=0.40) showed the reaction was complete. The mixture was added slowly to ice water (5 L) with vigorous stirring and then filtered. The filter cake was washed with H_2O (1 L X 3) and dried to give ***2-bromo-5-nitro-benzenesulfonyl chloride*** (105.00 g, crude) as a yellow solid. ^{1}H NMR (400MHz, CHLOROFORM-d) δ = 8.98 (d, J=2.4 Hz, 1H), 8.37 (dd, J=2.5, 8.7 Hz, 1H), 8.09 (d, J=8.6 Hz, 1H).

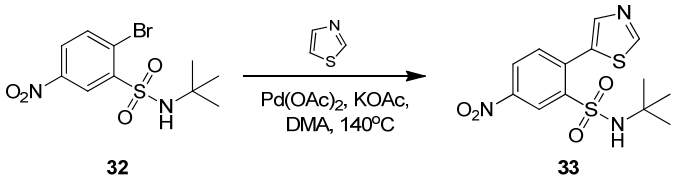
Preparation of compound 32.



To a mixture of ***2-methylpropan-2-amine*** (200 g, 2.73 mol, 287.36 mL, 3.29 *eq.*) and DMAP (10 g, 81.85 mmol, 0.1 *eq.*) in DCM (2 L) was added ***2-bromo-5-nitro-benzenesulfonyl chloride*** (250 g, 831.91 mmol, 1 *eq.*) portionwise at 0°C. The mixture was warmed to 15°C and stirred for 1 hr. LCMS showed the reaction was complete. The mixture was washed

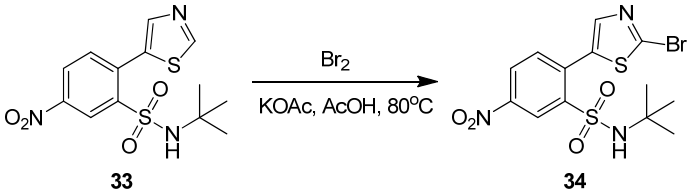
with HCl (1 N, 2 L), sat.aq. $NaHCO_3$ (500 mL) and brine (500 mL), dried over Na_2SO_4, filtered and concentrated to give ***2-bromo-N-tert-butyl-5-nitro-benzenesulfonamide*** (240 g, crude) as a gray solid. ESI [M+H] = 336.9/338.9.

Preparation of compound 33.



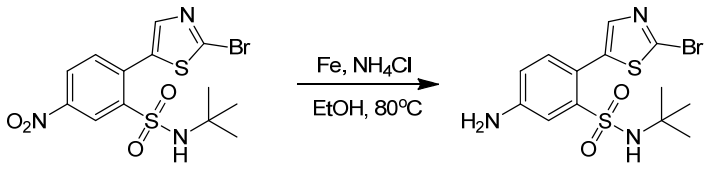
To a solution of ***2-bromo-N-tert-butyl-5-nitro-benzenesulfonamide*** (35 g, 103.80 mmol, 1 *eq*.) in DMA (300 mL), were added thiazole (26.51 g, 311.40 mmol, 3 *eq*.), $Pd(OAc)_2$ (2.33 g, 10.38 mmol, 0.1 *eq*.) and KOAc (30.56 g, 311.40 mmol, 3 *eq*.). The mixture was stirred at 140°C for 16 hrs under N_2. LCMS showed the reaction was complete, the mixture was poured into water (3 L) and filtered. The filter cake was washed with water (200 mL x 3) and then dried to give ***N-tert-butyl-5-nitro-2-thiazol-5-yl-benzenesulfonamide*** (25.1 g, crude) as a black brown solid. ^1H NMR (400MHz, DMSO-d6) δ = 9.30 (s, 1H), 8.81 (d, J=2.4 Hz, 1H), 8.46 (dd, J=2.4, 8.3 Hz, 1H), 8.12 (s, 1H), 7.83 (d, J=8.3 Hz, 1H), 7.60 (s, 1H), 1.07 (s, 9H). ESI [M+H] = 342.0.

Preparation of compound 34.



To a solution of ***N-tert-butyl-5-nitro-2-thiazol-5-yl-benzenesulfonamide*** (15 g, 43.94 mmol, 1 *eq*.) in AcOH (200 mL), were added KOAc (21.56 g, 219.68 mmol, 5 *eq*.) and Br_2 (35.11 g, 219.68 mmol, 5 *eq*.). The mixture was stirred at 80°C for 3 hrs. LCMS showed the reaction was complete, the mixture was quenched by sat.aq.Na_2CO_3 (1 L) and extracted with EtOAc (300 mL x 3). The combined organic phase was dried over Na_2SO_4, filtered and concentrated to give ***2-(2-bromothiazol-5-yl)-N-tert-butyl-5-nitro-benzenesulfonamide*** (18 g, crude) as a green solid which was used without any purification.

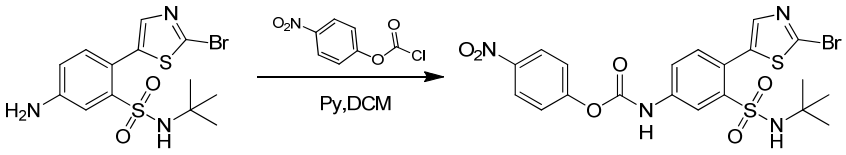
Preparation of compound 35.



34 35

To a solution of ***2-(2-bromothiazol-5-yl)-N-tert-butyl-5-nitro-benzenesulfonamide*** (8.5 g, 20.22 mmol, 1 *eq*.) in EtOH (70 mL), THF (40 mL) and H_2O (20 mL), were added Fe (3.39 g, 60.67 mmol, 3 *eq*.) and NH_4Cl (3.25 g, 60.67 mmol, 2.12 mL, 3 *eq*.). The mixture was stirred at 90°C for 1 hr. LCMS showed the reaction was complete. The mixture was filtered, the filtrate was concentrated to remove organic solvent and extracted with DCM (200 mL x 2). The organic phase was dried over Na_2SO_4, filtered and concentrated to afford ***5-amino-2-(2-bromothiazol-5-yl)-N-tert-butyl- benzenesulfonamide*** (6 g, crude) as a yellow solid. ^{1}HNMR showed the structure was correct. 1H NMR (400MHz, DMSO-d6) δ = 7.58 - 7.52 (m, 1H), 7.30 (d, J=2.4 Hz, 1H), 7.11 (d, J=8.3 Hz, 1H), 6.99 (s, 1H), 6.73 (dd, J=2.4, 8.3 Hz, 1H), 5.94 (s, 2H), 1.07 (s, 9H). ESI [M+H] = 390.0/392.0.

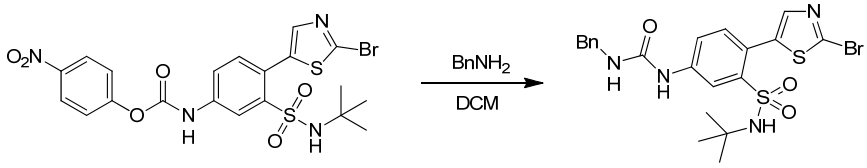
Preparation of compound 36.



35 36

General method D, ***(4-nitrophenyl) N-[4-(2-bromothiazol-5-yl)-3-(tert-butylsulfamoyl)phenyl]carbamate*** as DCM solution ESI [M+H] = 555.0/557.0

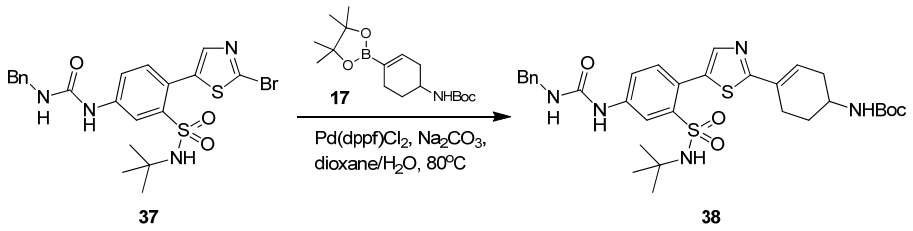
Preparation of compound 37.



36 37

General method H, ***1-benzyl-3-[4-(2-bromothiazol-5-yl)-3-(tert-butylsulfamoyl) phenyl]urea***. ESI [M+H] = 523.0/525.0.

Preparation of compound 38.

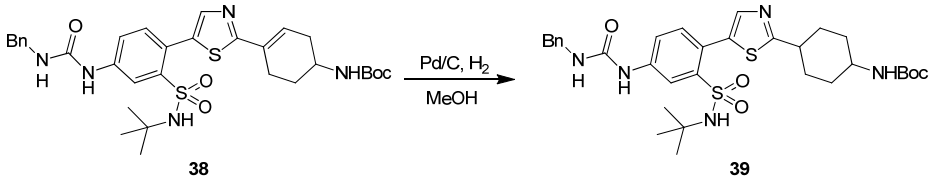


37 38

General method B (*Suzuki reaction)*, ***tert-butyl-N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl]thiazol-2-yl]cyclohex-3-en-1-yl]carbamate (Compound 38)***.

To a solution of ***Compound 17*** (1 *eq*.) in dioxane and H_2O, were added $Pd(dppf)Cl_2$ (0.1 *eq*.), ***Compound 37*** (0.9 *eq*.) and Na_2CO_3 (3 *eq*.). The mixture was stirred at 80°C for 12 hrs under N_2. LCMS showed the reaction was complete. The mixture was concentrated and the residue was purified by prep-TLC (PE/EtOAc=1:1) to yield product **38**. ESI [M+H] = 640.5

Preparation of compound 39.

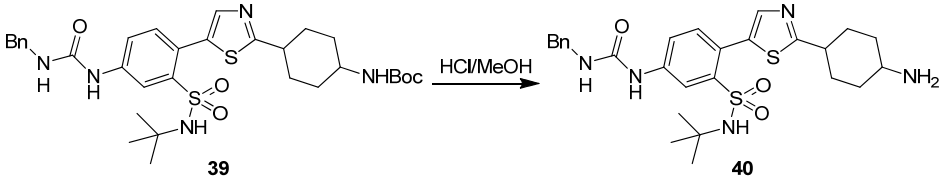


38 39

tert-butyl N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert- butylsulfamoyl)phenyl]thiazol-2-yl]cyclohexyl]carbamate.

To a solution of ***Compound 38*** (1 *eq*.) and AcOH (0.1 *eq*.) in EtOAc was added Pd/C (10% purity, 1.00 *eq*.). The mixture was stirred under H_2 (15 psi) at 40°C for 1 hr. LCMS showed the reaction was complete and then the mixture was filtered and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate =10/1 to 1:1) to give ***Compound 39.*** ESI [M+H] = 642.5

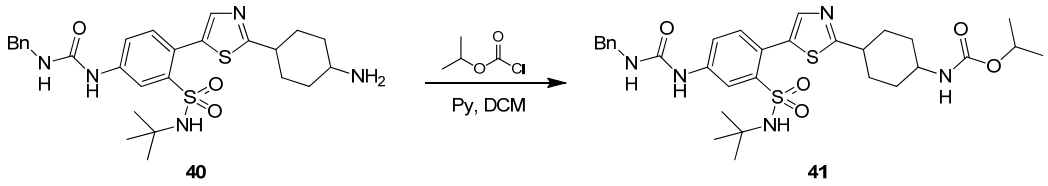
Preparation of compound 40.



39 40

General method F, ***1-[4-[2-(4-aminocyclohexyl)thiazol-5-yl]-3-(tert-butylsulfamoyl) phenyl]-3-benzyl-urea***. ESI [M+H] = 542.5.

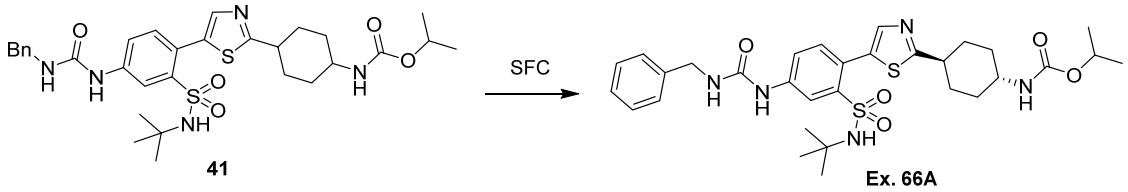
Preparation of compound 41



40 41

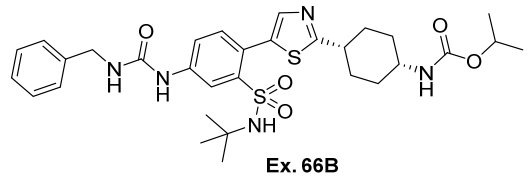
General method D, ***isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl]thiazol-2-yl]cyclohexyl]carbamate***, ESI [M+H] = 628.3.

Preparation of Compound Ex. 66A and Ex. 66B.



41 Ex. 66A

+



Ex. 66B

Compound 41 was separated by SFC (Instrument: Thar SFC80 preparative SFC; Column: Chiralpak AD-H 250*30mm i.d. 5u; Mobile phase: A for CO_2 and B for IPA(0.1%NH_3H_2O); Gradient: B%=42%; Flow rate:70 g/min; Wavelength:220 nm; Column temperature: 40℃; System back pressure: 100 bar; Cycle time:20 min; Injection amount: 4 mg per injection), and then purified by prep-HPLC (Column: Luna C18 100*30 5u; mobile phase: [water(0.1%TFA)-ACN];B%: 35%-75%,5min). ***Ex. 66A*** (11.54 mg, 18.38 umol, 5.53% yield, 100% purity) and ***Ex. 66B*** (9.38 mg, 14.94 umol, 4.50% yield, 100% purity) were obtained.

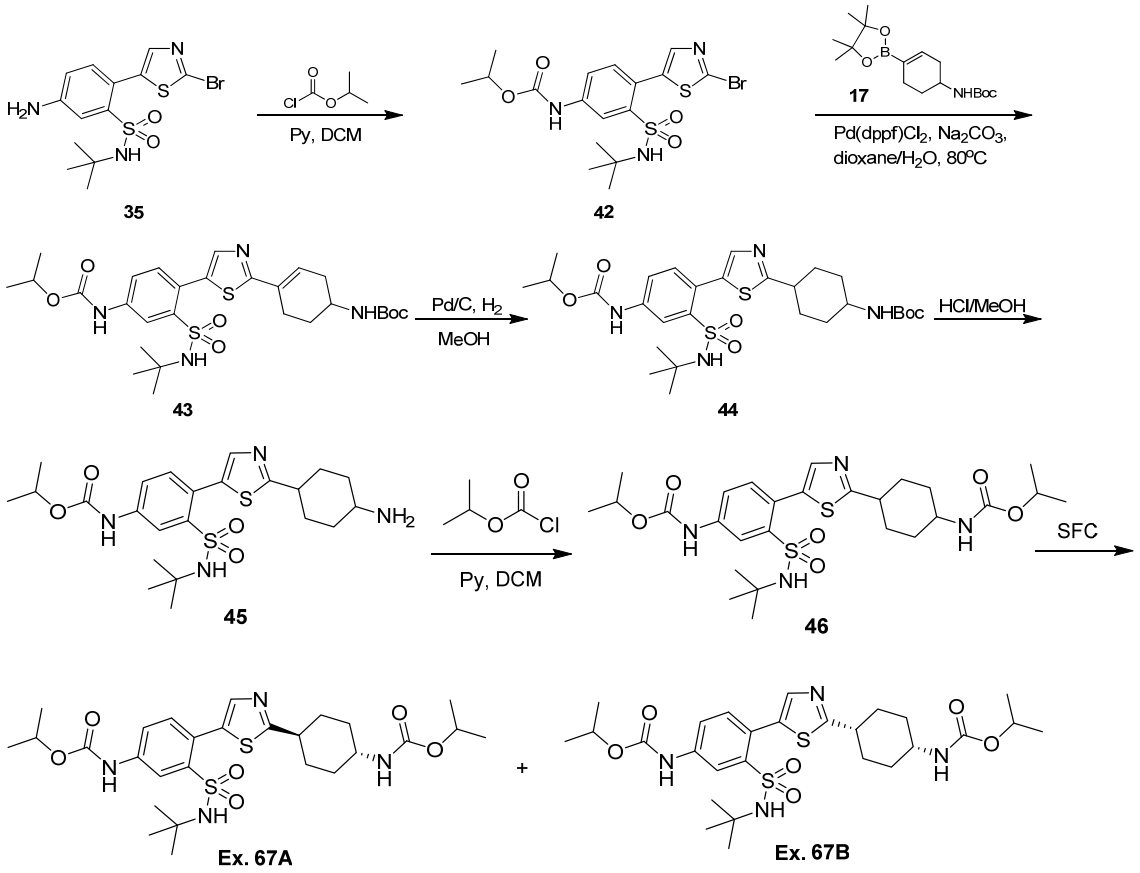
Trans-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl]thiazol-2-yl]cyclohexyl]carbamate, ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.24 (d, J=2.2 Hz, 1H), 7.72 (s, 1H), 7.69 (dd, J=2.6, 8.3 Hz, 1H), 7.37 - 7.30 (m, 5H), 7.27 - 7.22 (m, 1H), 4.85 - 4.78 (m, 1H), 4.41 (s, 2H), 3.50 - 3.39 (m, 1H), 3.06 - 2.96 (m, 1H), 2.22 (br d, J=11.8 Hz, 2H), 2.07 (br d, J=10.1 Hz, 2H), 1.76 - 1.62 (m, 2H), 1.41 (dq, J=3.1, 12.7 Hz, 2H), 1.22 (br d, J=6.1 Hz, 6H), 1.11 (s, 9H). ESI [M+H] = 628.2.

Cis-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl]thiazol-2-yl]cyclohexyl]carbamate. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.25 (d, J=2.2 Hz, 1H), 7.74 (s, 1H), 7.70 (dd, J=2.2, 8.3 Hz, 1H), 7.38 - 7.30 (m, 5H), 7.28 - 7.22 (m, 1H),

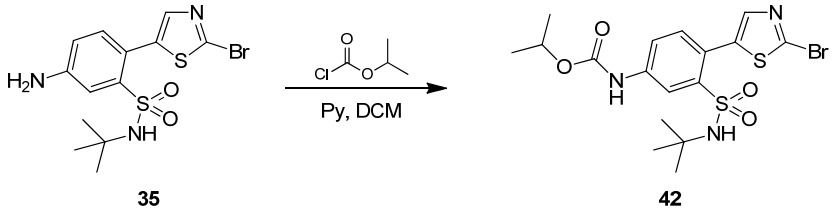
4.82 (br d, J=6.1 Hz, 1H), 4.41 (s, 2H), 3.74 (br s, 1H), 3.20 - 3.13 (m, 1H), 2.03 - 1.95 (m, 4H), 1.86 - 1.72 (m, 4H), 1.23 (d, J=6.1 Hz, 6H), 1.12 (s, 9H). ESI [M+H] = 628.2.

Examples 67A and 67B

Scheme 26:

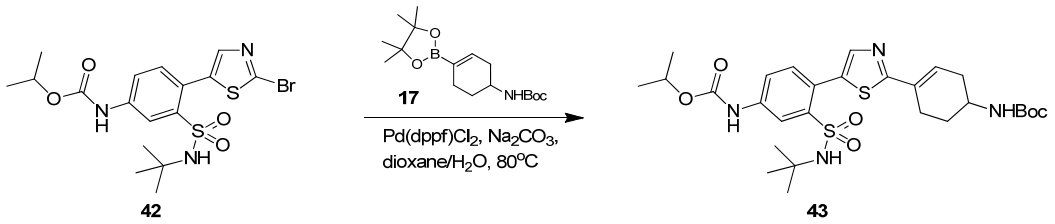


Preparation of compound 42.



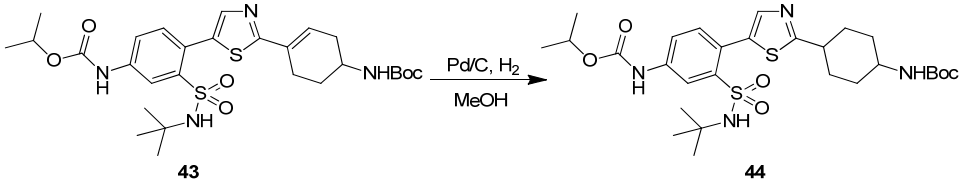
General method D, ***isopropyl N-[4-(2-bromothiazol-5-yl) -3-(tert-butylsulfamoyl) phenyl]carbamate.*** ESI [M+H] =476.0/478.0.

Preparation of compound 43



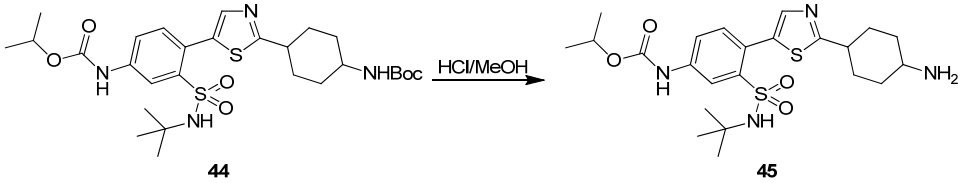
General method B, ***isopropyl N-[4-[2-[4-(tert -butoxycarbonylamino)cyclohexen-1-yl] thiazol-5-yl]-3-(tert-butylsulfamoyl)phenyl]carbamate***. ESI [M+H] =593.3.

Preparation of compound 44.



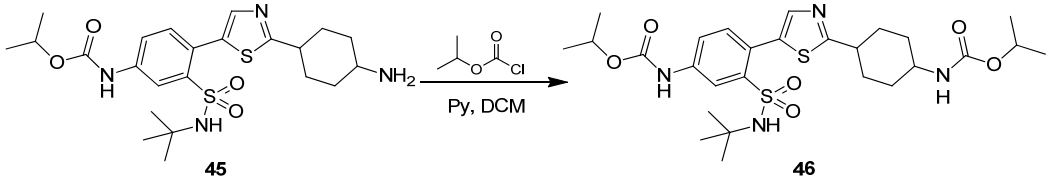
General method I, ***isopropyl N-[4-[2-[4-(tert-butoxycarbonylamino)cyclohexyl] thiazol-5-yl]-3-(tert-butylsulfamoyl)phenyl]carbamate***. ESI [M+H] =595.3.

Preparation of compound 45.



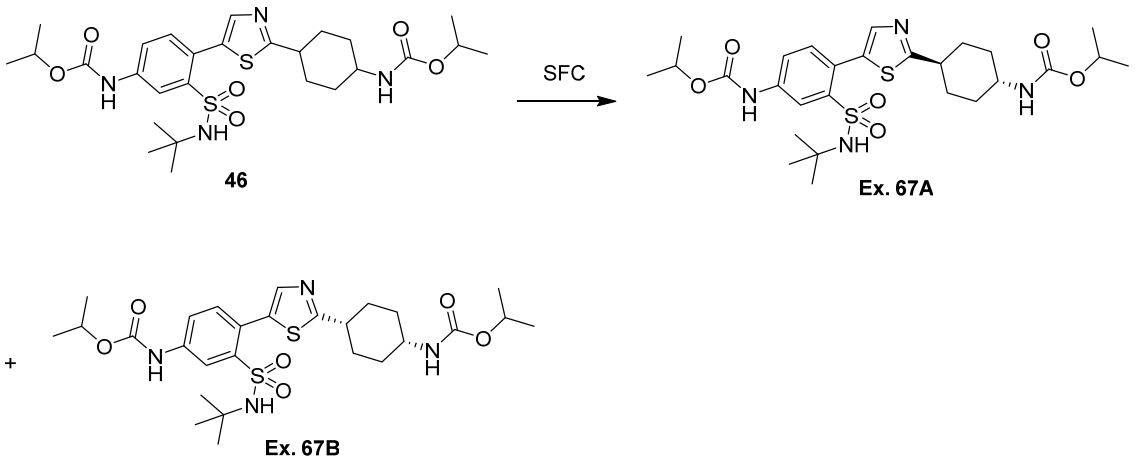
General method F, ***isopropyl N-[4-[2-(4-aminocyclohexyl) thiazol-5-yl]-3-(tert-butylsulfamoyl)phenyl]carbamate***. ESI [M+H] =495.2.

Preparation of compound 46.



General method D, ***isopropyl N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino) cyclohexyl]thiazol-5-yl]phenyl]carbamate***. ESI [M+H] =581.2.

Preparation of Ex. 67A and Ex. 67B.



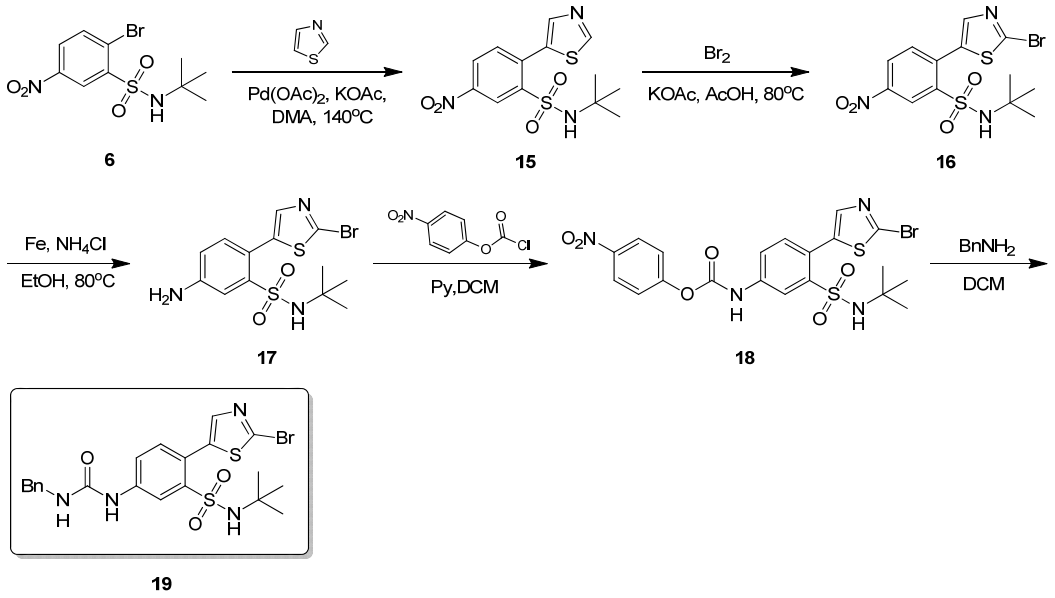
Compound 46 was separated by SFC (Instrument: Thar SFC80 preparative SFC; Column: ChiralpakAD-H 250*30mm i.d. 5u; Mobile phase: A for CO_2 and B for IPA(0.1%NH_3H_2O); Gradient: B%=30%; Flow rate:70 g/min; Wavelength:220 nm; Column temperature: 40℃; System back pressure: 100 bar; Cycle time:8 min; Injection amount: 3 mg per injection); and then purified by prep-HPLC (Column: Agela Durashell C18 150*25 5u; mobile phase: [water(0.1%TFA)-ACN];B%: 55%-85%,12min), ***Ex. 67A*** (5.76 mg, 100% purity) and ***Ex. 67B*** (3.95 mg , 100% purity) ***were obtained as a pale yellow solid.***

Trans-isopropylN-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl] thiazol-5-yl]phenyl]carbamate (Compound S12). ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.37 (d, J=2.3 Hz, 1H), 7.75 (s, 1H), 7.69 (dd, J=2.2, 8.4 Hz, 1H), 7.40 (d, J=8.3 Hz, 1H), 5.01 (td, J=6.3, 12.5 Hz, 1H), 4.86 - 4.82 (m, 1H), 3.52 - 3.42 (m, 1H), 3.04 (tt, J=3.5, 12.0 Hz, 1H), 2.35 - 2.19 (m, 2H), 2.15 - 1.99 (m, 2H), 1.72 (dq, J=3.0, 12.9 Hz, 2H), 1.43 (dq, J=3.3, 12.6 Hz, 2H), 1.34 (d, J=6.2 Hz, 6H), 1.25 (br d, J=6.1 Hz, 6H), 1.14 (s, 9H). ESI [M+H] = 581.2.

Cis-isopropylN-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate (Compound S13). ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.37 (d, J=2.2 Hz, 1H), 7.76 (s, 1H), 7.70 (dd, J=2.2, 8.3 Hz, 1H), 7.40 (d, J=8.3 Hz, 1H), 5.01 (td, J=6.3, 12.5 Hz, 1H), 4.84 (br s, 1H), 3.77 (br s, 1H), 3.23 - 3.15 (m, 1H), 2.05 - 1.98 (m, 4H), 1.89 - 1.72 (m, 4H), 1.34 (d, J=6.2 Hz, 6H), 1.25 (d, J=6.1 Hz, 6H), 1.16 (s, 9H). ESI [M+H] = 581.2

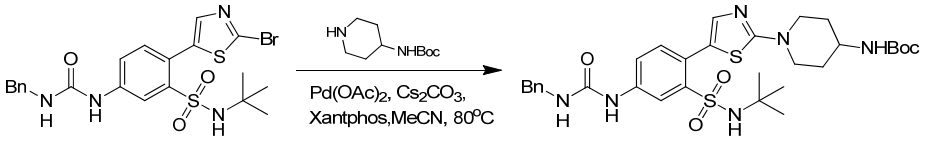
Example 68. Synthesis of *isopropyl N-[1-[5-[4-(benzylcarbamoylamino)-2-(tert-butyl sulfamoyl)phenyl]thiazol-2-yl]-4-piperidyl]carbamate.*

Scheme 27:



6 15 16 17 18 19

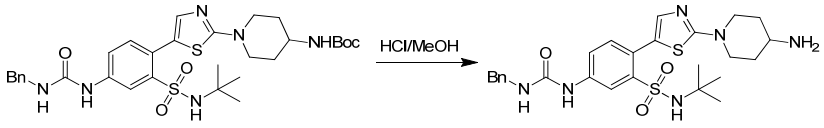
Preparation of compound 24.



19 24

A mixture of ***1-benzyl-3-[4-(2-bromothiazol-5-yl)-3-(tert-butylsulfamoyl)phenyl]urea*** (0.06 g, 114.62 umol, 1.0 eq.), tert-butyl N-(4-piperidyl)carbamate (40 mg, 199.72 umol, 1.74 eq.), Xantphos (6.63 mg, 11.46 umol, 0.1 eq.), $Pd(OAc)_2$ (2.57 mg, 11.46 umol, 0.1 eq.) and Cs_2CO_3 (112.04 mg, 343.86 umol, 3.0 eq.) in ACN (3 mL) was heated to 80°C for 12 hrs under N_2. LCMS showed the reaction was complete and the mixture was concentrated. The residue was purified by prep-TLC (Petroleum ether: Ethyl acetate = 1:1) to give ***tert-butyl N-[1-[5-[4-(benzylcarbamoylamino) -2-(tert-butylsulfamoyl)phenyl]thiazol-2-yl]-4-piperidyl]carbamate*** (0.03 g, crude) as a yellow solid. ESI [M+H] = 643.1.

Preparation of compound 25.

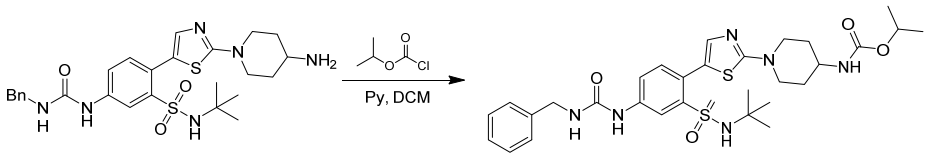


24 25

Tert-butyl N-[1-[5-[4-(benzylcarbamoylamino)-2-(tert-butylsulfamoyl)phenyl] thiazol-2-yl]-4-piperidyl]carbamate (30 mg, 46.67 umol, 1.0 eq.) was dissolved into HCl/MeOH (4M, 1 mL) and the mixture was stirred at 20°C for 0.5 hr. LCMS showed the reaction was complete and the mixture was concentrated to give ***1-[4-[2-(4-amino-1-piperidyl)thiazol-5-yl]-3-(tert-***

butylsulfamoyl)phenyl]-3-benzyl-urea (27 mg, crude, HCl salt) as a yellow solid. ESI [M+H] = 543.2.

Preparation of Ex. 68.

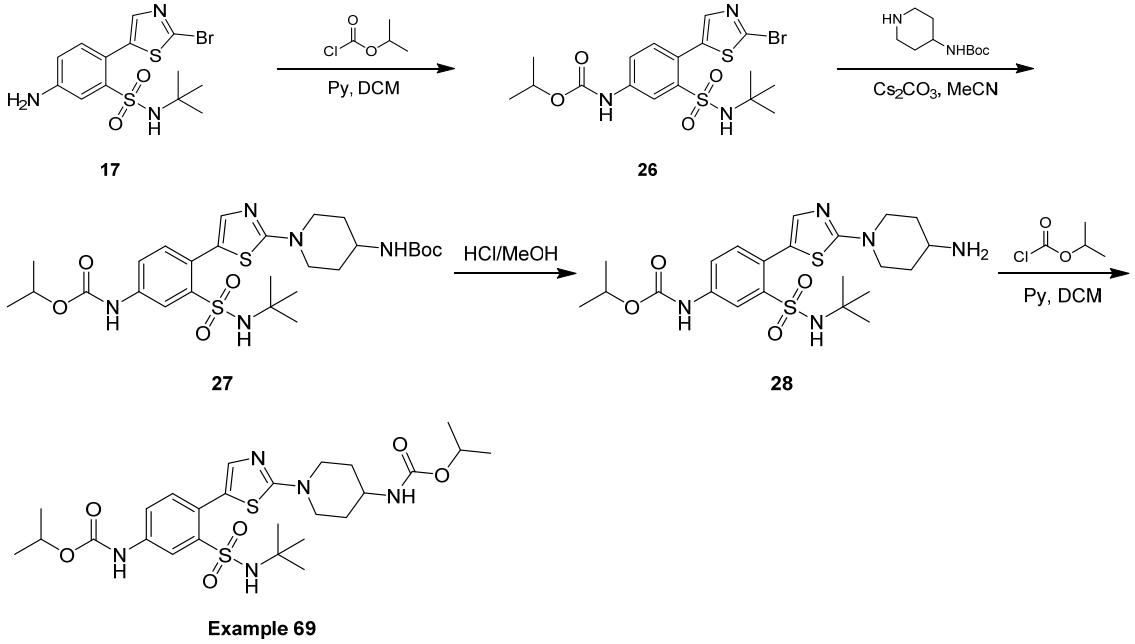


25 Ex. 68

General method D, ***isopropyl N-[1-[5-[4-(benzylcarbamoylamino)-2-(tert-butyl sulfamoyl)phenyl]thiazol-2-yl]-4-piperidyl]carbamate***. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.24 (d, J=2.2 Hz, 1H), 7.69 (dd, J=2.4, 8.4 Hz, 1H), 7.39 (d, J=8.4 Hz, 1H), 7.36 - 7.19 (m, 6H), 4.82 - 4.73 (m, 1H), 4.40 (s, 2H), 3.92 (br d, J=13.7 Hz, 2H), 3.75 - 3.68 (m, 1H), 3.43 (br t, J=11.0 Hz, 2H), 2.10 - 2.02 (m, 2H), 1.71 - 1.60 (m, 2H), 1.22 (br d, J=6.2 Hz, 6H), 1.18 (s, 9H). ESI [M+H] = 629.2.

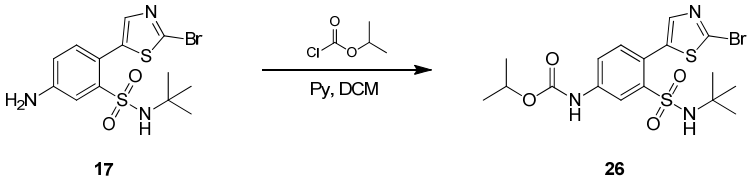
Example 69. Synthesis of *isopropyl N-[1-[5-[2-(tert-butylsulfamoyl)-4-(isopropoxy carbonylamino)phenyl]thiazol-2-yl]-4-piperidyl]carbamate.*

Scheme 28:



17 26 27 28 Example 69

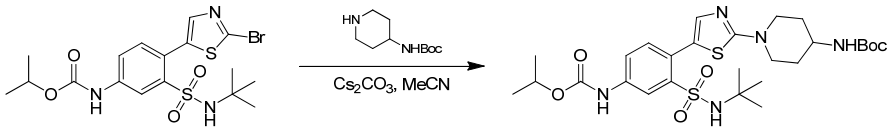
Preparation of compound 26.



17 26

General method D, ***isopropyl N-[4-(2-bromothiazol-5-yl)-3-(tert-butylsulfamoyl) phenyl]carbamate***. ESI [M+H] = 478.0/476.0.

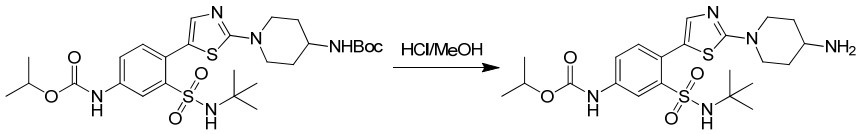
Preparation of compound 27.



26 27

To a solution of ***isopropyl N-[4-(2-bromothiazol-5-yl)-3-(tert-butylsulfamoyl) phenyl]carbamate*** (0.05 g, 104.95 umol, 1.0 eq.) in MeCN (2 mL) were added Cs_2CO_3 (102.59 mg, 314.86 umol, 3.0 eq.), KI (17.42 mg, 104.95 umol, 1.0 eq.) and tert-butyl N-(4-piperidyl)carbamate (105.10 mg, 524.76 umol, 5.0 eq.). The mixture was stirred at 95°C for 12 hrs and then concentrated. The residue was purified by prep-TLC (SiO_2, Petroleum ether: Ethyl acetate = 4:3) to give ***N-[4-[2-[4-(tert-butoxycarbonylamino)-1-piperidyl]thiazol-5-yl]-3-(tert-butylsulfamoyl) phenyl]carbamate*** (0.025 g, crude) as a white solid. ESI [M+H] = 596.2.

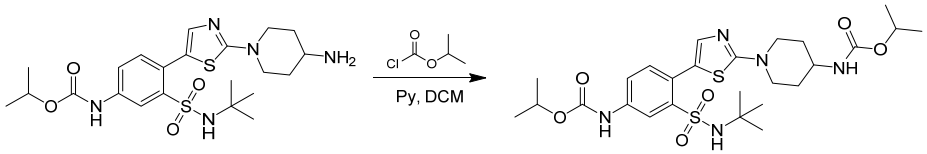
Preparation of compound 28.



27 28

Isopropyl N-[4-[2-[4-(tert-butoxycarbonylamino)-1-piperidyl]thiazol-5-yl]-3-(tert-butylsulfamoyl)phenyl]carbamate (0.025 g, 41.96 umol, 1.0 eq.) was dissolved into HCl/MeOH (4M, 1.5 mL) and the mixture was stirred at 20°C for 0.5 hr. LCMS showed the reaction was complete and the mixture was concentrated to give the ***N-[4-[2-(4-amino-1-piperidyl)thiazol-5-yl]-3-(tert-butylsulfamoyl)phenyl] carbamate*** (0.02 g, crude, HCl salt) as a white solid. ESI [M+H] = 496.2.

Preparation of Ex. 69.



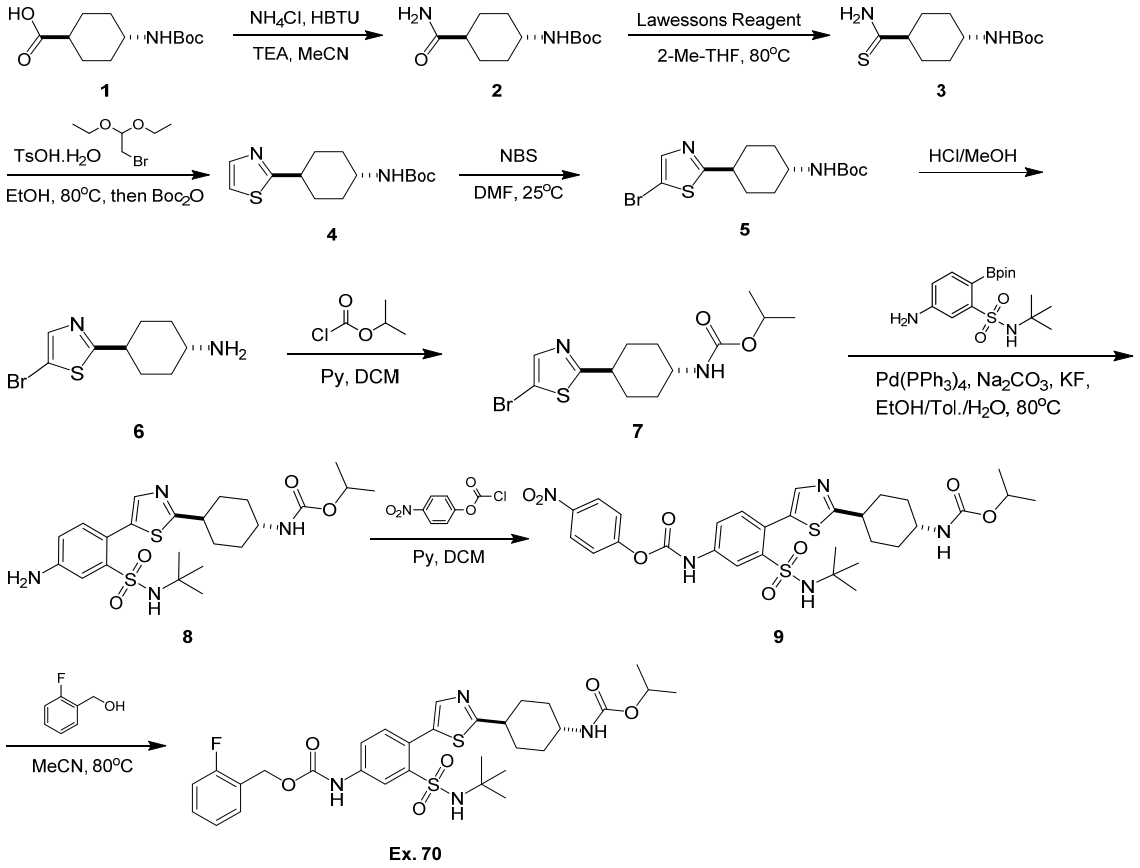
28 Ex. 69

General method D, ***isopropyl N-[1-[5-[2-(tert-butylsulfamoyl)-4-(isopropoxy carbonylamino)phenyl]thiazol-2-yl]-4-piperidyl]carbamate***. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.35 (s, 1H), 7.66 (br d, J=8.4 Hz, 1H), 7.42 (d, J=8.4 Hz, 1H), 7.31 (s, 1H), 4.98 (td, J=6.2, 12.5 Hz, 1H), 4.84 - 4.77 (m, 1H), 3.93 (br d, J=13.5 Hz, 2H), 3.77 -

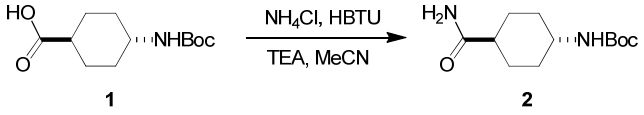
3.67 (m, 1H), 3.44 (br t, J=12.3 Hz, 2H), 2.06 (br dd, J=3.1, 13.2 Hz, 2H), 1.71 - 1.61 (m, 2H), 1.31 (d, J=6.4 Hz, 6H), 1.23 (br d, J=6.2 Hz, 6H), 1.19 (s, 9H). ESI [M+H] = 582.2.

Example 70. Synthesis of trans-isopropyl N-[4-[5-[2-(tert-butylsulfamoyl)-4-[(2-fluorophenyl)methoxy carbonylamino]phenyl]thiazol-2-yl]cyclohexyl]carbamate.

Scheme 29:

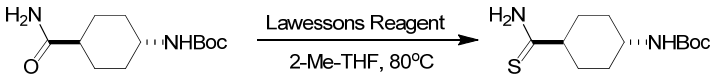


Preparation of compound 2.



To a mixture of ***trans-4-(tert-butoxycarbonylamino)cyclohexanecarboxylic acid*** (65.0 g, 267.2 mmol, 1.0 *eq.*), NH_4Cl (21.4 g, 400.7 mmol, 1.5 *eq.*) and TEA (801.5 mmol, 111.6 mL, 3 *eq.*) in MeCN (1.3 L) was added HBTU (111.5 g, 293.9 mmol, 1.1 *eq.*) and the mixture was stirred at 25°C for 3 hrs. The mixture was filtered and then the filter cake was washed with petroleum ether (200 mL) and dried to give ***trans-tert-butyl N-(4-carbamoylcyclohexyl)carbamate*** (140 g, crude，2 batches) as a white solid. ^1H NMR (METHANOL-d4, 400MHz) δ = 3.25-3.34 (m, 1H), 2.14 (tt, J=12.3, 3.5 Hz, 1H), 1.83-1.99 (m, 4H), 1.52 (qd, J=13.1, 2.9 Hz, 2H), 1.42 (s, 9H), 1.21 (qd, J=12.7, 3.5 Hz, 2H)

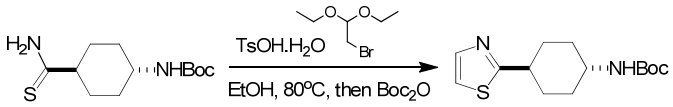
Preparation of compound 3.



2 3

A mixture of ***trans-tert-butyl N-(4-carbamoylcyclohexyl)carbamate*** (90.0 g, 371.4 mmol, 1.0 *eq.*), Na_2CO_3 (39.4 g, 371.4 mmol, 1.0 *eq.*) and Lawesson's reagent (82.6 g, 204.3 mmol, 0.55 *eq.*) in 2-Me-THF (600 mL) was stirred at 80°C for 2 hrs and then the reaction mixture was poured into H_2O (200 mL). The aqueous phase was extracted with EtOAc (500 mL x 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to give ***trans-tert-butyl N-(4-carbamothioylcyclohexyl)carbamate*** (180 g, crude，2 batches) as a white solid. ^{1}H NMR (METHANOL-d4, 400MHz) δ = 3.35-3.46 (m, 1H), 2.87-3.00 (m, 1H), 2.09-2.20 (m, 2H), 1.99-2.09 (m, 2H), 1.54-1.68 (m, 2H), 1.26-1.45 (m, 2H), 1.14-1.25 (m, 9H)

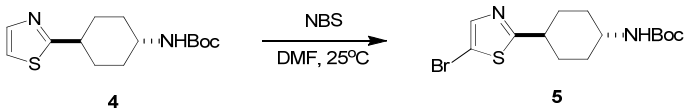
Preparation of compound 4.



3 4

A mixture of ***trans-tert-butyl N-(4-carbamothioylcyclohexyl)carbamate*** (180.0 g, 696.7 mmol, 1.0 *eq.*), 2-bromo-1,1-diethoxy-ethane (137.3 g, 696.7 mmol, 1.0 *eq.*) and $TsOH.H_2O$ (265 g, 1.4 mol, 2 *eq.*) in EtOH (2.0 L) was stirred at 80°C for 6 hrs. Then the mixture was cooled to RT and adjusted to PH=9 with aq.sat.Na_2CO_3 and Boc_2O (152 g, 696.7 mmol, 1 *eq.*) was added. The mixture was stirred at 30°C for 3 hrs, then concentrated and diluted with H_2O (2 L). The mixture was extracted with EtOAc (800 mL x 3) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was triturated with petroleum ether (1.5 L) to give ***trans-tert-butyl N-(4-thiazol-2-ylcyclohexyl) carbamate*** (70 g, 247.87 mmol, 35.58% yield) as a white solid. ^{1}H NMR (METHANOL-d4, 400MHz) δ = 7.67 (d, J=3.1 Hz, 1H), 7.44 (d, J=3.5 Hz, 1H), 3.33-3.44 (m, 1H), 2.93-3.06 (m, 1H), 2.12-2.21 (m, 2H), 2.00-2.09 (m, 2H), 1.57-1.71 (m, 2H), 1.41-1.48 (m, 9H), 1.29-1.38 (m, 1H), 1.13-1.28 (m, 1H).

Preparation of compound 5.



4 5

A mixture of ***trans-tert-butyl N-(4-thiazol-2-ylcyclohexyl)carbamate*** (68 g, 240.8 mmol, 1 *eq.*) and NBS (47.1 g, 264.9 mmol, 1.1 *eq.*) in DMF (500 mL) was stirred at 25°C for 10 hrs and then poured into H_2O (2 L) and extracted with EtOAc (500 mL x 3). The combined organic layers were washed with brine (300 mL x 5), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate = 20:1 to 10:1) to give ***trans-tert-butyl N-[4-(5-bromothiazol-2-yl)cyclohexyl]carbamate*** (78 g, crude) as a yellow solid. ESI [M+H] = 363.0/361.0

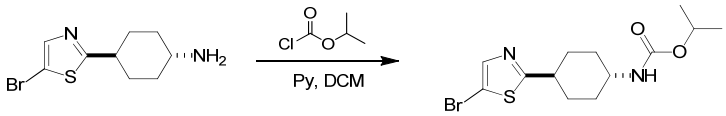
Preparation of compound 6.



5 6

A mixture of ***trans-tert-butyl N-[4-(5-bromothiazol-2-yl)cyclohexyl]carbamate*** (50 g, 138.4 mmol, 1 *eq.*) in HCl/MeOH (4 M, 700 mL) was stirred at 25°C for 0.5 hr and then concentrated to give ***trans-4-(5-bromothiazol-2-yl) cyclohexanamine*** (45 g, crude, HCl salt) as a yellow solid. ESI [M+H] = 263.0/261.0

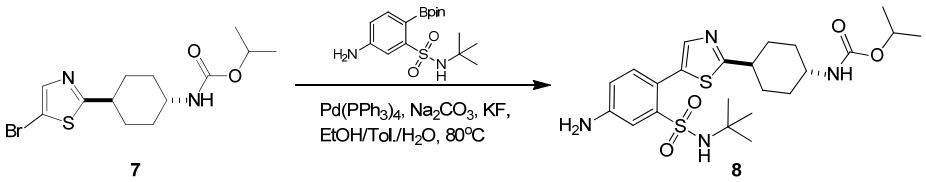
Preparation of compound 7.



6 7

To a solution of ***trans-4-(5-bromothiazol-2-yl)cyclohexanamine*** (45 g, HCl salt, 172.3 mmol, 1 *eq.*), Pyridine (61.5 mmol, 69.5 mL, 5 *eq.*) and DMAP (4.2 g, 34.5 mmol, 0.2 *eq.*) in DCM (300 mL) was added isopropyl carbonochloridate (258.5 mmol, 35.9 mL, 1.5 *eq.*) dropwise at 0°C. The mixture was stirred at 25°C for 0.5 hr and then washed with HCl (1N, 1 L) and sat.aq.Na_2CO_3 (1 L). The organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate =15:1 to 10:1) to give ***trans-isopropyl N-[4-(5-bromothiazol-2-yl)cyclohexyl]carbamate*** (37 g, 106.55 mmol, 61.84% yield) as a yellow solid. ^{1}H NMR (METHANOL-d4, 400MHz) δ = 7.60 (s, 1H), 4.81 (dt, J=12.2, 6.2 Hz, 1H), 3.41 (tt, J=11.6, 4.0 Hz, 1H), 2.88-3.00 (m, 1H), 2.10-2.20 (m, 2H), 1.98-2.07 (m, 2H), 1.55-1.68 (m, 2H), 1.30-1.43 (m, 2H), 1.21 (br d, J=6.2 Hz, 6H).

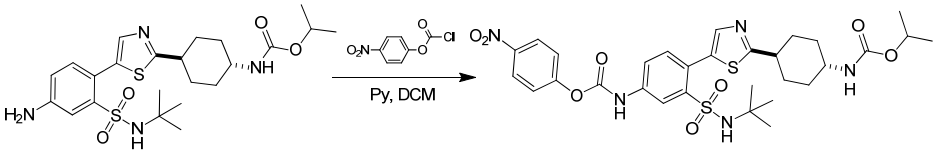
Preparation of compound 8.



7 8

A mixture of ***trans-isopropyl N-[4-(5-bromothiazol-2-yl)cyclohexyl]carbamate*** (16.2 g, 46.7 mmol, 1 *eq.*), ***5-amino-N-tert-butyl-2-(4,4,5,5-tetramethyl-1,3,2- dioxaborolan-2-yl)benzenesulfonamide*** (19.9 g, 56.1 mmol, 1.2 *eq.*), KF (4.1 g, 70.1 mmol, 1.5 *eq.*), Na_2CO_3 (14.9 g, 140.2 mmol, 3 *eq.*) and $Pd(PPh_3)_4$ (1.6 g, 1.4 mmol, 0.03 *eq.*) in toluene (150 mL), EtOH (150 mL) and H_2O (50 mL) stirred at 80°C for 6 hrs under N_2 atmosphere. The reaction mixture was concentrated and the residue was diluted with H_2O (100 mL) and extracted with EtOAc (100 mL x 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate= 20:1 to 1:1) to give ***trans-isopropyl N-[4-[5-[4-amino-2-(tert-butylsulfamoyl) phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (13 g, 26.3 mmol, 56.2% yield) as a white solid. ^1H NMR (400MHz, METHANOL-d4) δ = 7.65 - 7.60 (m, 1H), 7.43 (d, J=2.2 Hz, 1H), 7.13 (d, J=8.3 Hz, 1H), 6.83 (dd, J=2.6, 8.3 Hz, 1H), 4.81 (br s, 1H), 3.76 - 3.71 (m, 1H), 3.05 - 2.86 (m, 1H), 2.20 (br d, J=12.3 Hz, 2H), 2.06 (br d, J=10.5 Hz, 2H), 1.75 - 1.60 (m, 2H), 1.47 - 1.34 (m, 2H), 1.22 (br d, J=6.1 Hz, 6H), 1.09 (s, 9H).

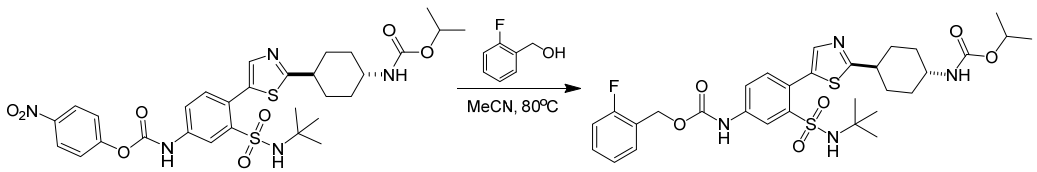
Preparation of compound 9.



8 9

To a solution of ***trans-isopropyl N-[4-[5-[4-amino-2-(tert-butylsulfamoyl)phenyl] thiazol-2-yl]cyclohexyl]carbamate*** (250 mg, 505.4 umol, 1 *eq.*) in DCM (4 mL) were added DMAP (6.2 mg, 50.5 umol, 0.1 *eq.*)， Pyridine (120 mg, 1.5 mmol, 3 *eq.*) and (4-nitrophenyl) carbonochloridate (153 mg, 758 umol, 1.5 *eq.*). The mixture was stirred at 25°C for 0.5 hr and used directly for the next step. ESI [M+H] = 660.2

Preparation of compound Ex. 70.

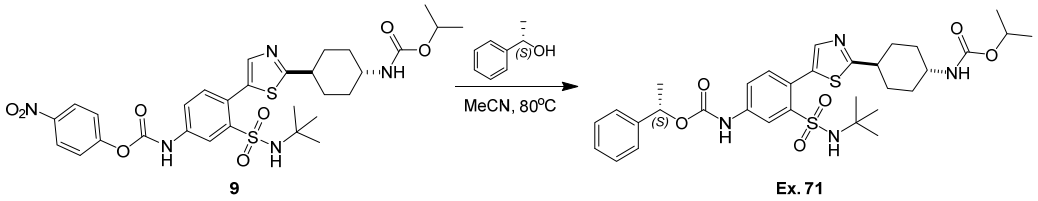


9 Ex. 70

To a solution of (2-fluorophenyl)methanol (45.9 mg, 363.8 umol, 3 *eq.*) and DIEA (47 mg, 363.8 umol, 3 *eq.*) in MeCN (1 mL) was added the above solution (1 mL). The mixture was stirred at 80°C for 1 hr, then concentrated and the residue was purified by prep-HPLC (Column: Waters Xbridge 150*25 5u; Mobile phase: [water (10 mM NH_4HCO_3)-ACN]; B%: 42%-72%, 12 min) to give ***trans-isopropyl N-[4-[5-[2-(tert-butylsulfamoyl)-4-[(2-fluorophenyl)methoxy carbonylamino]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (16.34 mg, 25.26 umol, 20.83% yield, 100% purity) as a yellow solid. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.36 (s, 1H), 7.77 - 7.63 (m, 2H), 7.52 (t, J=7.0 Hz, 1H), 7.44 - 7.31 (m, 2H), 7.23 - 7.08 (m, 2H), 5.29 (s, 2H), 4.83 (br s, 1H), 3.46 (br d, J=11.8 Hz, 1H), 3.00 (br t, J=11.8 Hz, 1H), 2.22 (br d, J=12.7 Hz, 2H), 2.07 (br d, J=11.4 Hz, 2H), 1.76 - 1.62 (m, 2H), 1.47 - 1.35 (m, 2H), 1.22 (br d, J=6.1 Hz, 6H), 1.12 (s, 9H). ESI [M+H] = 647.2

Example 71. Synthesis of trans-[(1S)-1-phenylethyl] N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate.

Scheme 30:

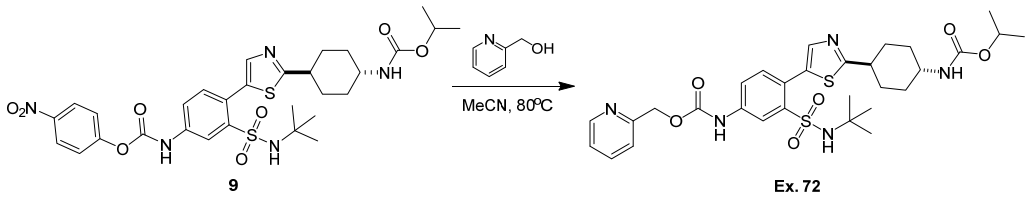


Preparation of Ex. 71.

To a solution of (1S)-1-phenylethanol (29.6 mg, 242.5 umol, 2 *eq.*) and DIEA (47 mg, 363.8 umol, 3 *eq.*) in MeCN (2 mL) was added a solution of ***trans-(4-nitrophenyl) N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate*** (80 mg, 121.25 umol, 1 *eq.*) in DCM (1 mL). The mixture was stirred at 80°C for 1 hr, then concentrated and the residue was purified by prep-HPLC (Column: Nano-Micro UniSil 5-100 C18 ULTRA 100*250mm 5um;mobile phase: [water(0.1%TFA)-ACN]; B%: 55%-80%,11min) to give ***trans-[(1S)-1-phenylethyl] N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate*** (12.19 mg, 18.77 umol, 15.48% yield, 99% purity) as a white solid. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.35 (br s, 1H), 7.81 - 7.65 (m, 2H), 7.48 - 7.36 (m, 5H), 7.31 (br d, J=6.8 Hz, 1H), 5.89 (br d, J=6.2 Hz, 1H), 4.85 (br d, J=5.5 Hz, 1H), 3.47 (br s, 1H), 3.03 (br s, 1H), 2.24 (br d, J=11.2 Hz, 2H), 2.08 (br d, J=11.0 Hz, 2H), 1.71 (q, J=11.9 Hz, 2H), 1.61 (br d, J=6.4 Hz, 3H), 1.49 - 1.37 (m, 2H), 1.24 (br d, J=5.4 Hz, 6H), 1.13 (s, 9H). ESI [M+H] = 643.2

Example 72. Synthesis of N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino) cyclohexyl]thiazol-5-yl]phenyl]carbamate.

Scheme 31:



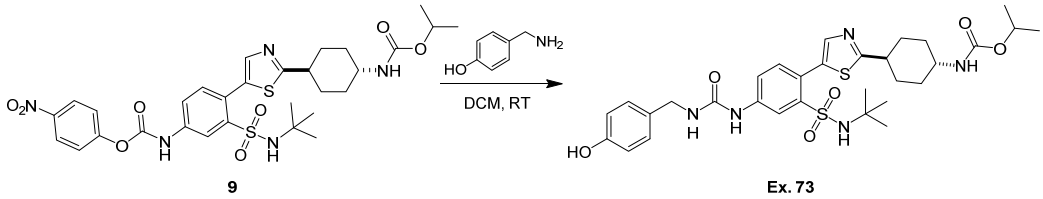
9 Ex. 72

Preparation of Ex. 72.

To a solution of 2-pyridylmethanol (26.5 mg, 242.5 umol, 2 *eq.*) and DIEA (47 mg, 363.8umol, 3 *eq.*) in MeCN (2 mL) was added a solution of ***trans-(4-nitrophenyl) N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate*** (80 mg, 121.3 umol, 1 *eq.*) in DCM (1 mL). The mixture was stirred at 80°C for 1 hr, then concentrated and the residue was purified by prep-HPLC (Column: Waters Xbridge 150*25 5u; Mobile phase: [water (0.04% NH_3H_2O + 10 mM NH_4HCO_3)-ACN]; B%: 35%-65%, 10 min) to give ***trans-2-pyridylmethyl N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl] thiazol-5-yl]phenyl]carbamate*** (18.59 mg, 28.82 umol, 23.77% yield, 97.65% purity) as a pale yellow solid. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.56 (br d, J=4.5 Hz, 1H), 8.38 (d, J=1.8 Hz, 1H), 7.94 - 7.87 (m, 1H), 7.78 - 7.72 (m, 2H), 7.59 (br d, J=7.8 Hz, 1H), 7.44 - 7.38 (m, 2H), 5.32 (s, 2H), 4.85 (td, J=5.9, 12.0 Hz, 1H), 3.47 (br t, J=11.8 Hz, 1H), 3.07 - 2.96 (m, 1H), 2.24 (br d, J=12.3 Hz, 2H), 2.13 - 2.04 (m, 2H), 1.77 - 1.65 (m, 2H), 1.48 - 1.37 (m, 2H), 1.24 (br d, J=6.1 Hz, 6H), 1.14 (s, 9H). ESI [M+H] = 630.2

Example 73. Synthesis of trans-isopropyl N-[4-[5-[2-(tert-butylsulfamoyl)-4-[(4-hydroxyphenyl)methylcarbamoylamino]phenyl]thiazol-2-yl]cyclohexyl]carbamate.

Scheme 32:



9 Ex. 73

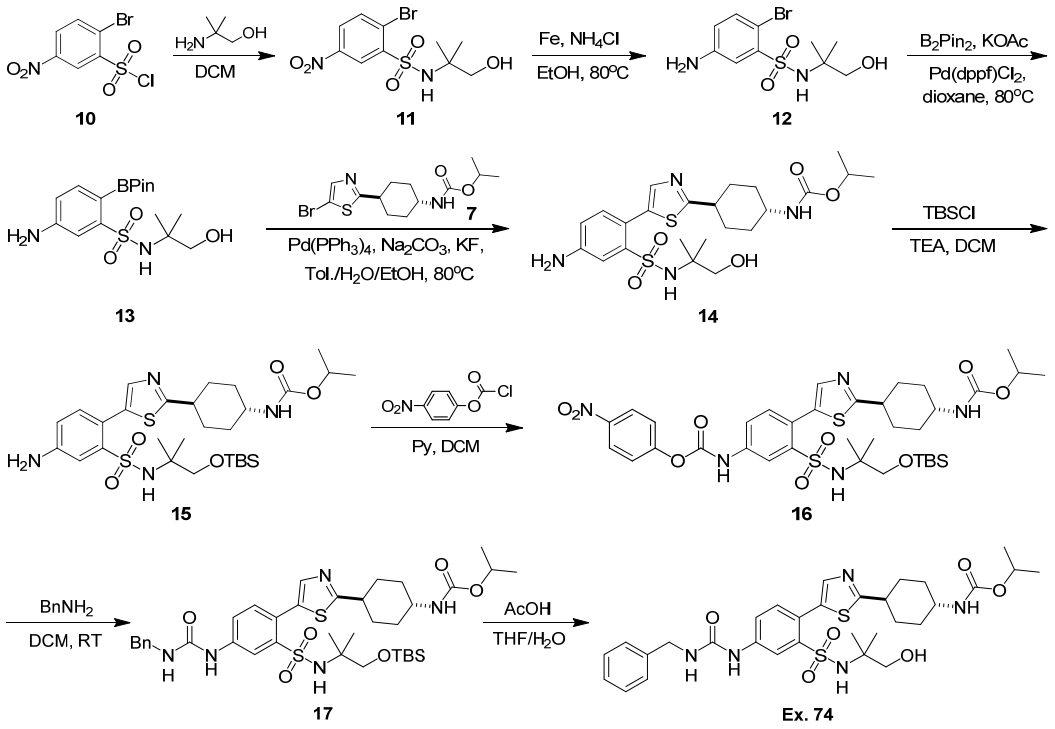
Preparation of Ex. 73.

To a solution 4-(aminomethyl) phenol (74.7 mg, 606.5 umol, 3 *eq.*) and DIEA (78.4 mg, 606.5 umol, 3 *eq.*) in DCM (2 mL) was added ***trans-(4-nitrophenyl) N-[3-(tert-butylsulfamoyl)-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-***

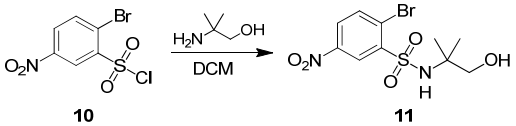
yl]phenyl]carbamate (133.4 mg, 202.2 umol, 1 *eq.*) in DCM (2 mL). The mixture was stirred at 25°C for 1 hr, then concentrated and the residue was purified by prep-HPLC (TFA condition) to give ***trans-isopropyl N-[4-[5-[2-(tert-butylsulfamoyl)-4-[(4-hydroxyphenyl)methylcarbamoylamino]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (23.56 mg, 35.99 umol, 17.80% yield, 98.357% purity) as a white solid. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.24 (d, J=2.2 Hz, 1H), 7.77 (s, 1H), 7.69 (dd, J=2.2, 8.3 Hz, 1H), 7.36 (d, J=8.3 Hz, 1H), 7.16 (d, J=8.8 Hz, 2H), 6.75 (d, J=8.3 Hz, 2H), 4.85 - 4.74 (m, 1H), 4.30 (s, 2H), 3.45 (s, 1H), 3.15 - 2.97 (m, 1H), 2.23 (br d, J=12.3 Hz, 2H), 2.08 (br d, J=13.2 Hz, 2H), 1.79 - 1.63 (m, 2H), 1.48 - 1.34 (m, 2H), 1.22 (br d, J=6.1 Hz, 6H), 1.12 (s, 9H). ESI [M+H] = 644.2

Example 74. Synthesis of trans-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-[(2-hydroxy-1,1-dimethyl-ethyl)sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate.

Scheme 33:



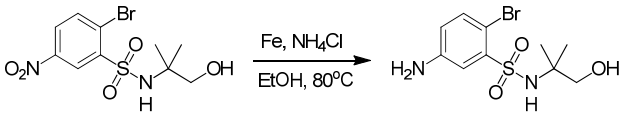
Preparation of compound 11.



To a mixture of ***2-amino-2-methyl-propan-1-ol*** (3 g, 33.7 mmol, 5.1 *eq.*) and DMAP (80 mg, 654.8 umol, 0.98 *eq.*) in DCM (50 mL) was added ***2-bromo-5-nitro- benzenesulfonyl***

chloride (2 g, 6.66 mmol, 1 *eq.*). The mixture was stirred at 20°C for 30 mins, then washed with 1N HCl (20 mL) and sat.aq.$NaHCO_3$ (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to give ***2-bromo-N-(2-hydroxy-1,1-dimethyl-ethyl)-5-nitro- benzenesulfonamide*** (1.7 g, 4.8 mmol, 72.3% yield) as a yellow gum. ESI [M+H] = 355.0/353.0

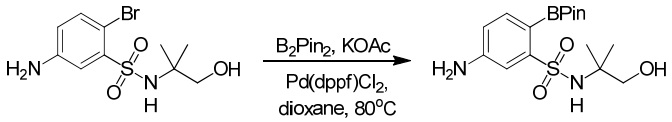
Preparation of compound 12.



11 12

A mixture of ***2-bromo-N-(2-hydroxy-1,1-dimethyl-ethyl)-5-nitro- benzenesulfonamide*** (1.7 g, 4.8 mmol, 1 *eq.*), Fe (1.5 g, 26.9 mmol, 5.6 *eq.*) and NH_4Cl (800 mg, 14.9 mmol, 3.1 *eq.*) in EtOH (15 mL)/H_2O (7.5 mL)/THF (7.5 mL) was stirred at 80°C for 2 hrs. The reaction mixture was concentrated to remove EtOH, diluted with H_2O (100 mL) and extracted with EtOAc (100 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate = 100:1 to 1:1) to give ***5-amino-2-bromo-N-(2-hydroxy-1,1-dimethyl-ethyl)benzenesulfonamide*** (1.1 g, 3.4 mmol, 70.7% yield) as a pale yellow solid. ESI [M+H] = 325.0/323.0

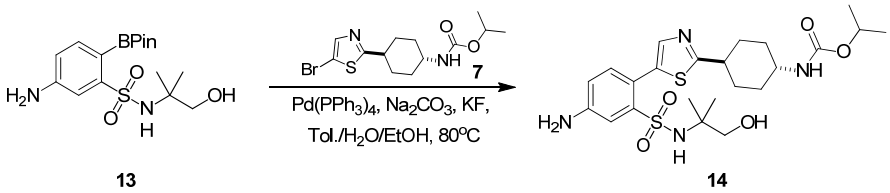
Preparation of compound 13.



12 13

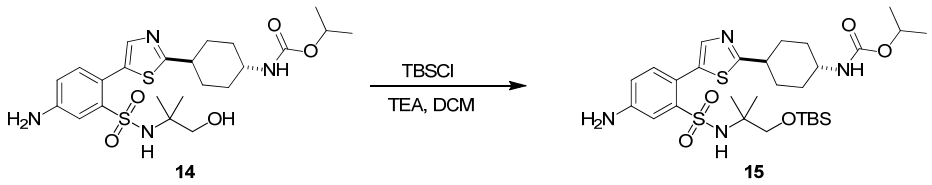
A mixture of ***5-amino-2-bromo-N-(2-hydroxy-1,1-dimethyl-ethyl) benzenesulfonamide*** (400 mg, 1.24 mmol, 1 *eq.*), 4,4,5,5-tetramethyl-2-(4,4,5,5- tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (942.8 mg, 3.7 mmol, 3 *eq.*), $Pd(dppf)Cl_2$ (90.6 mg, 123.8 umol, 0.1 *eq.*) and KOAc (364.4 mg, 3.7 mmol, 3 *eq.*) in dioxane (4 mL) was stirred at 80°C for 12 hrs under N_2 atmosphere and then concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate = 50:1 to 3:1) to give ***5-amino-N-(2-hydroxy-1,1- dimethyl-ethyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide*** (201 mg, crude) as a white solid. ^1H NMR (400MHz, METHANOL-d4) δ = 7.53 (d, J=8.0 Hz, 1H), 7.28 (d, J=2.4 Hz, 1H), 6.75 (dd, J=6.4 Hz, 1H), 3.35 (s, 2H), 1.36 (s, 12H), 1.10 (s, 6H).

Preparation of compound 14.



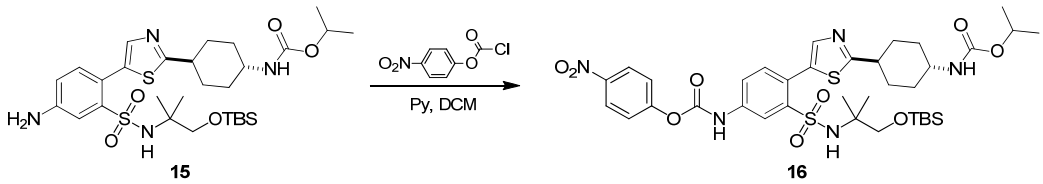
A mixture of ***5-amino-N-(2-hydroxy-1,1-dimethyl-ethyl)-2-(4,4,5,5-tetramethyl -1,3,2-dioxaborolan-2-yl)benzenesulfonamide*** (140.7 mg, 380.1 umol, 1.2 *eq.*), ***trans-isopropyl N-[4-(5-bromothiazol-2-yl)cyclohexyl]carbamate*** (110 mg, 316.7 umol, 1 *eq.*), Na_2CO_3 (100.7 mg, 950.3 umol, 3 *eq.*), KF (27.6 mg, 475.1 umol, 1.5 *eq.*) and $Pd(PPh_3)_4$ (36.6 mg, 31.7 umol, 0.1 *eq.*) in toluene(1 mL)/EtOH (1 mL)/H_2O (0.3 mL) was stirred at 80°C for 12 hrs under N_2 atmosphere. The reaction mixture was concentrated, diluted with H_2O (10 mL) and extracted with EtOAc (10 mL x 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by prep-TLC (SiO_2, Ethyl acetate) to give ***trans-isopropyl N-[4-[5-[4-amino-2-[(2-hydroxy-1,1-dimethyl-ethyl)sulfamoyl] phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (117 mg, crude) as a yellow solid. ^{1}H NMR (400MHz, METHANOL-d4) δ = 7.63 (d, J=7.0 Hz, 1H), 7.44 (d, J=2.2 Hz, 1H), 7.15 (d, J=8.3 Hz, 1H), 6.83 (dd, J=2.4, 8.1 Hz, 1H), 4.85 - 4.78 (m, 1H), 3.50 - 3.39 (m, 1H), 3.25 (s, 2H), 3.03 - 2.91 (m, 1H), 2.21 (br t, J=6.1 Hz, 2H), 2.12 - 2.00 (m, 2H), 1.75 - 1.60 (m, 2H), 1.45 - 1.33 (m, 2H), 1.28 - 1.19 (s, 6H), 1.03 (s, 6H)

Preparation of compound 15.



To a solution of ***trans-isopropyl N-[4-[5-[4-amino-2-[(2-hydroxy-1,1-dimethyl-ethyl) sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (90 mg, 176.3 umol, 1 *eq.*) in DCM (1 mL) were added TEA (53.5 mg, 528.7 umol, 3 *eq.*), DMAP (2.2 mg, 17.6 umol, 0.1 *eq.*) and TBSCl (66.4 mg, 440.6 umol, 2.5 *eq.*). The mixture was stirred at 30°C for 12 hrs and then concentrated. The residue was purified by prep-TLC (SiO_2, Petroleum ether: Ethyl acetate = 2:1) to give ***trans-isopropyl N-[4-[5-[4-amino-2-[[2-[tert-butyl(dimethyl)silyl]oxy-1,1-dimethyl-ethyl]sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (120 mg, crude) as a pale yellow solid. ESI [M+H] = 625.2

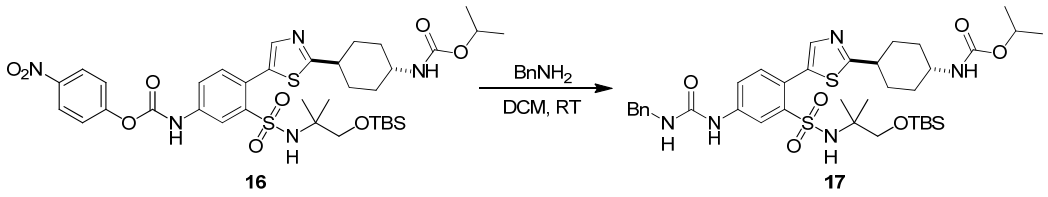
Preparation of compound 16.



15 16

To a solution of ***trans-isopropyl N-[4-[5-[4-amino-2-[[2-[tert-butyl(dimethyl)silyl] oxy-1,1-dimethyl-ethyl]sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (90 mg, 144 umol, 1 *eq.*) in DCM (1 mL) were added DMAP (1.8 mg, 14.4 umol, 0.1 *eq.*), pyridine (34.2 mg, 432 umol, 3 *eq.*) and (4-nitrophenyl) carbonochloridate (43.5 mg, 216 umol, 1.5 *eq.*). The mixture was stirred at 25°C for 0.5 hr and used into the next step directly without further purification. ESI [M+H] = 790.3

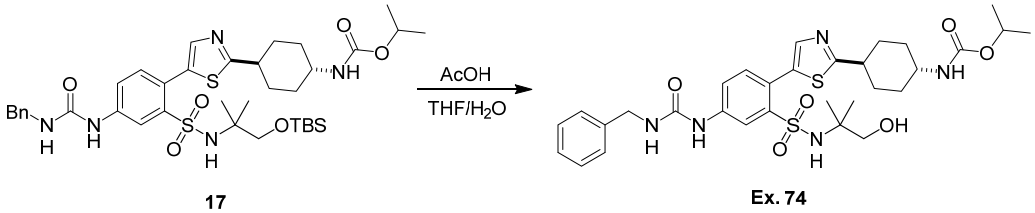
Preparation of compound 17.



16 17

To a solution of phenylmethanamine (36 mg, 336 umol, 3 *eq.*) in DCM (1 mL) was added a solution of ***trans-(4-nitrophenyl) N-[3-[[2-[tert-butyl(dimethyl)silyl] oxy-1,1-dimethyl-ethyl]sulfamoyl]-4-[2-[4-(isopropoxycarbonylamino)cyclohexyl]thiazol-5-yl]phenyl]carbamate*** (88.5 mg, 112 umol, 1 *eq.*) in DCM (1 mL) and the mixture was stirred at 25°C for 0.5 hr. The reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc (10 mL x 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by prep-TLC (SiO_2, Petroleum ether: Ethyl acetate = 1:1) to give ***trans-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-[[2-[tert-butyl(dimethyl)silyl]oxy-1,1-dimethyl-ethyl]sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (93 mg, crude) as a yellow gum. ESI [M+H] = 758.4

Preparation of Ex. 74.



17 Ex. 74

A mixture of ***trans-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-[[2-[tert-butyl(dimethyl)silyl]oxy-1,1-dimethyl-ethyl]sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (84.9 mg, 112 umol, 1 *eq.*) in AcOH (0.5 mL)/THF (0.5 mL)/H_2O

(0.5 mL) was stirred at 80°C for 0.5 hr. Then the mixture was concentrated and the residue was purified by prep-HPLC (TFA condition) to give ***trans-isopropyl N-[4-[5-[4-(benzylcarbamoylamino)-2-[(2-hydroxy-1,1-dimethyl-ethyl)sulfamoyl]phenyl]thiazol-2-yl]cyclohexyl]carbamate*** (5.86 mg, 9.10 umol, 8.13% yield, 100% purity) as a yellow solid. ^{1}H NMR (400MHz, METHANOL-d4) δ = 8.25 (d, J=2.6 Hz, 1H), 7.81 - 7.66 (m, 2H), 7.40 - 7.30 (m, 5H), 7.28 - 7.22 (m, 1H), 4.82 (td, J=6.0, 12.5 Hz, 1H), 4.41 (s, 2H), 3.45 (br t, J=11.6 Hz, 1H), 3.28 (s, 2H), 3.00 (br t, J=11.8 Hz, 1H), 2.23 (br d, J=13.2 Hz, 2H), 2.11 - 2.00 (m, 2H), 1.77 - 1.60 (m, 2H), 1.48 - 1.32 (m, 2H), 1.22 (br d, J=6.1 Hz, 6H), 1.05 (s, 6H). ESI [M+H] = 644.3

Example 75. Compound Primary Screening

1. BACKGROUND

Primary screening was a phenotypic screen that utilized the synthetic lethal interaction between AID and RAD51 to identify compounds that were both potent and on target. AID expressing cells are dependent upon RAD51 for survival; inhibiting RAD51 in AID positive cells results in a cytotoxic effect. Based on such an effect, compounds that were potent in AID positive cells and were significantly less potent in AID negative cells were identified.

2. MATERIALS AND SUPPLIES

Plastic ware and consumables needed for this experiment include: Cell Culture media; Evaporation Buffer media; 100% DMSO; 96 well U-bottom sterile culture plates; 250mL bottle; 1.5mL Opaque amber epi tubes; Epi Tube rack; 300mL reservoirs; 25mL reservoir; 25mL serological pipette tips; 5mL serological pipette tips P1000 Pipette Tips; and P200 Pipette Tips.

Equipment needed for this experiment include: Viaflo 384 liquid handler; Eppendorf serological pipette; Eppendorf P1000 Pipette; and Eppendorf P200 Pipette

Daudi Cell Culture and WI-38 Cell Cultures were also needed for this experiment.

Lastly, compounds (*e.g.*, the compounds of this disclosure) to be tested are needed.

3. PROCEDURE

All steps were performed in a sterile environment inside the Biosafety cabinet.

The first step was to set up a cell killing assay in the Daudi cell line (AID positive). A 96 well u-bottom plate was prepared by writing the experiment number, plate number, date and initials in the top right corner of the plate lid. With a sterile 300ml reservoir, and 25ml serological pipette, evaporation buffer media was pipetted into reservoir in 25ml increments.

Using the liquid handler,150ul of evaporation buffer media was pipetted from reservoir into rows A and H, and Columns 1 and 12 of the 96 well u-bottom plate. Cell cultures were counted to obtain the density of cells per ml, and the culture viability. The cell density information was used to obtain 1,000,000 cells from culture using a 5mL serological pipette into an epi tube. The cell density information from the culture was used to calculate the number of cells and volume of media needed for the assay to seed 1250 cells in 130ul of media per available culture well in the 96 well u-bottom plate. Rows B through F were used for cells (50 wells in total), with row G left for an empty media control. The calculation was overestimated by 10mL to account for the dead volume in the 300ml reservoir. Once the media volume was calculated, the appropriate volume of media was pipetted in 25mL increments into the 250mL bottle using a 25mL serological pipette. The 250ml bottle was capped tightly, and placed into a 37°C water bath for 2 minutes. While the culture media was warming, 10mL of fresh media was pipetted from the 500mL culture media bottle into a sterile 25mL reservoir. Using the Eppendorf multichannel pipette, 130ul of media was pipetted from the 25mL reservoir into row G of the 96 well u-bottom plate. Once the 250mL bottle of media was warmed, the volume of culture needed was pipetted into the bottle, and mixed gently with a 25mL serological pipette as to not create bubbles, and then the contents of the bottle were pipetted into a new 300mL reservoir. Using the liquid handler, 130ul of culture was pipetted from the 300mL reservoir into rows B through F of the 96 well u-bottom plate. Once the culture was added, the plate was placed into a 37°C incubator until the compound master plate was prepared for use.

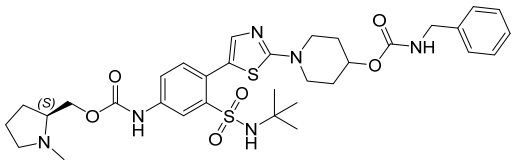
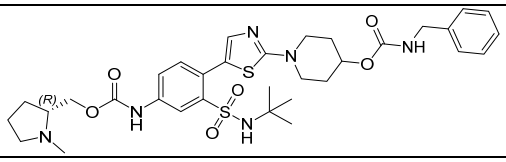
Two 96 well u-bottom plates were prepared by writing the master plate name in the upper right corner of the plate lid. Labeling one DMSO master and the other Media Master. The compounds of interest were obtained from the laboratory freezer, and placed into a 25 well storage box with a lid, and set the box aside. The compounds were vortexed after thawing but before use. Using an automatic multichannel pipette, 20ul of 100% DMSO was pipetted into wells B3-B11 through G3-G11 of the DMSO master plate. For each compound on the master plate, 50ul of the compound were pipetted in the appropriate well of row 2 (reference plate map to determine appropriate well). A serial dilution was prepared beginning by aspirating 20ul from row 2 and mixing with row 3, repeating until row 11 was reached. Using the liquid handler, 194ul of Daudi media was dispensed into wells B2-B11 through G2-G11 of the Media master plate. Using the liquid handler, 6ul from the DMSO master plate was aspirated and dispensed into the media master plate, mixing 100ul twice.

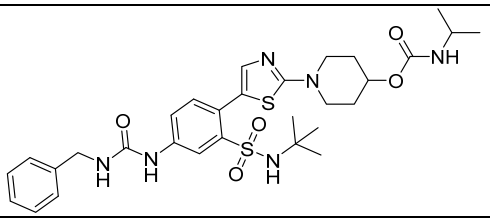
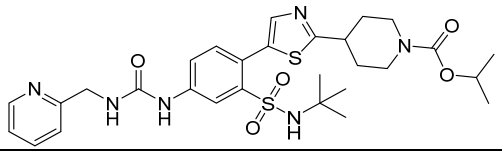
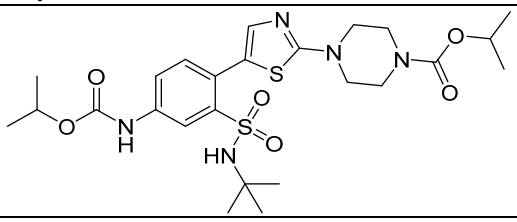
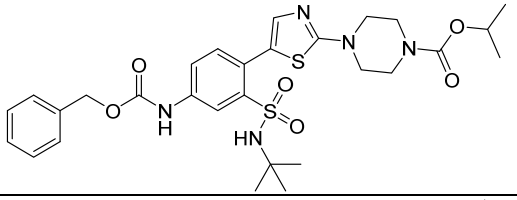
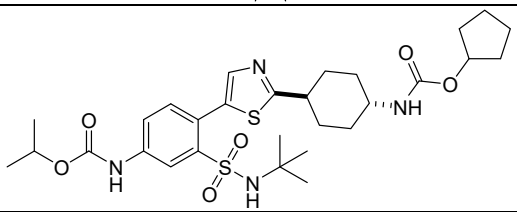
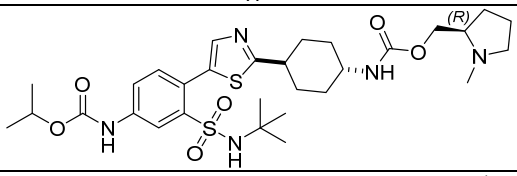
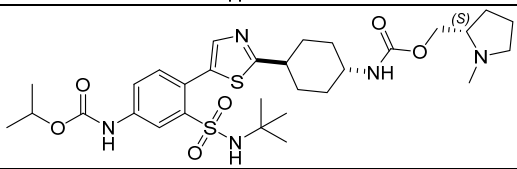
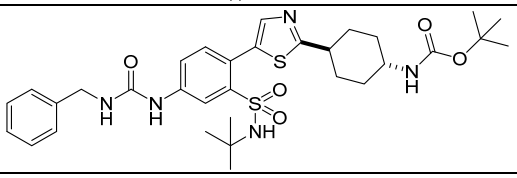
Compounds from master plate were then added to the culture plate. The culture plates were removed from the incubator, and set inside the biosafety cabinet. Using a liquid handler, 20ul from wells B2 to B11 through G2 to G11 of master plate were aspirated, and dispensed into wells B2 to B11 through G2 to G11 of culture plate. This set was continued with each culture plate. Once the culture plates acquired their 20ul of compound dilutions, they were placed back into the incubator, until their reads on Day 7 of experiment. Cell death was measured on Day 7 of the experiment using Cell-Titer Glo and a Promega Plate reader.

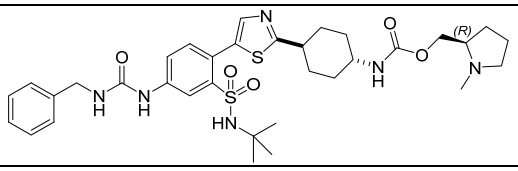
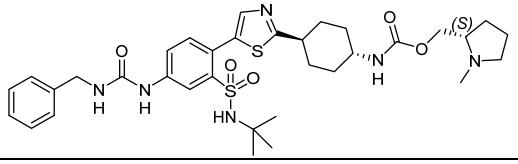
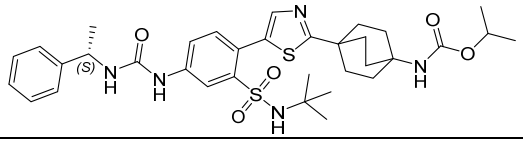
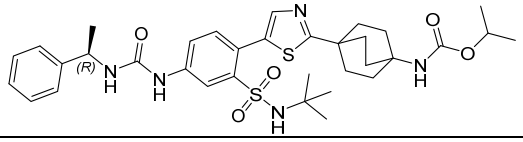
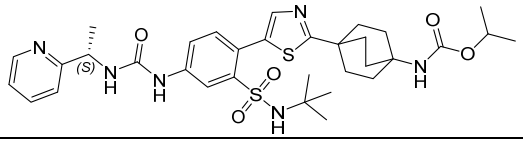
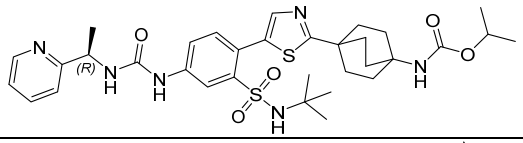
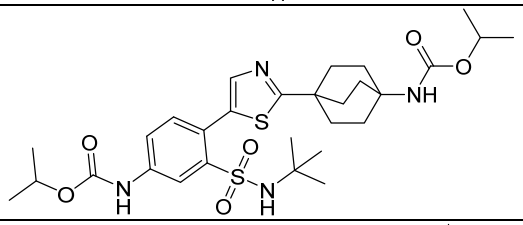
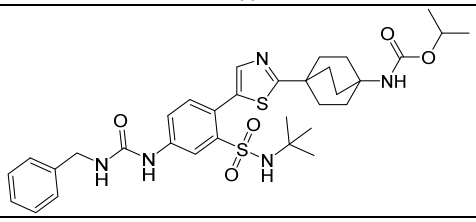
Percent cell death and EC_{50} values were calculated by comparing the cell viability of the compound treated wells to the non-treated wells. Normalized RLU values were obtained by subtracting the media well values from each of the wells in the same column, and then dividing that value by the DMSO treated cells values. The percent kill was then calculated by subtracting the normalized RLU value from 1 and multiplying by 100. The average normalized percent kill value and standard error of the mean was then calculated. The kill values were then inputted into Prism with the corresponding standard errors. In Prism a non-linear regression line was plotted with the data points using a semi-log scale, and the EC_{50} value was calculated. For compounds that showed good potency in the Daudi cell line, the assay was repeated using WI-38 cells (AID negative).

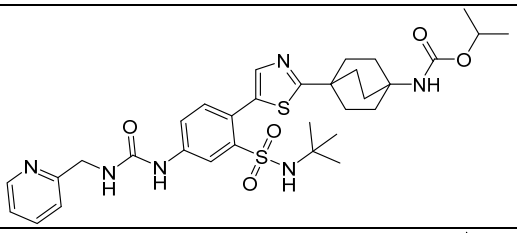
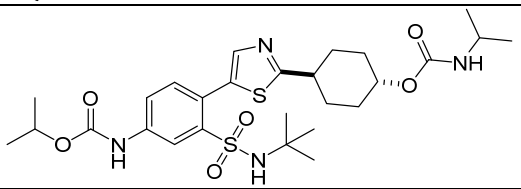
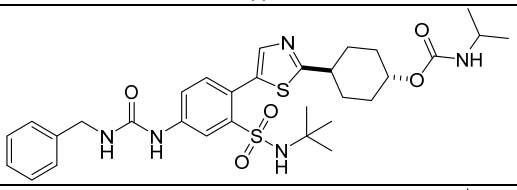
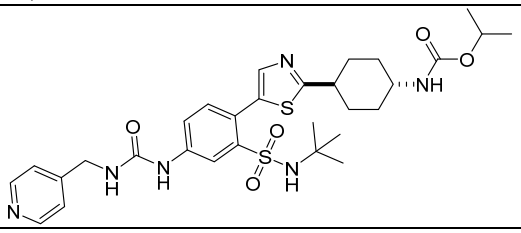
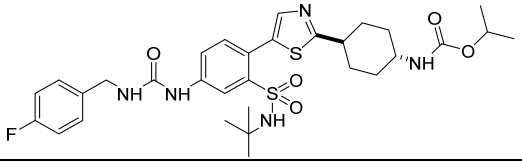
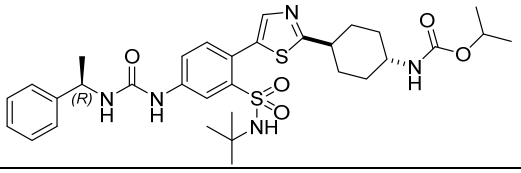
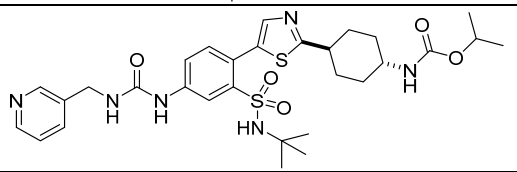
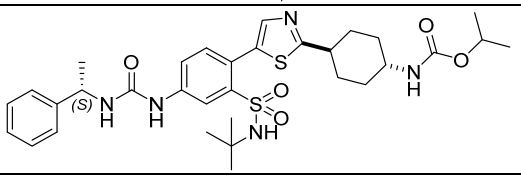
Screening Data

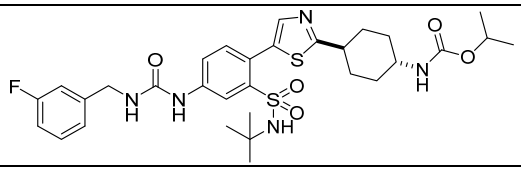
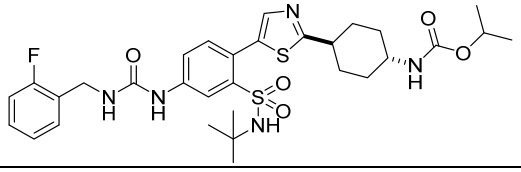
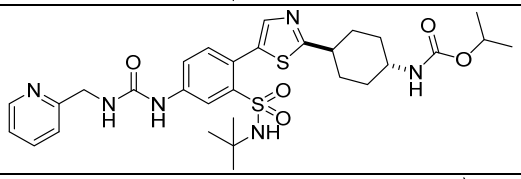
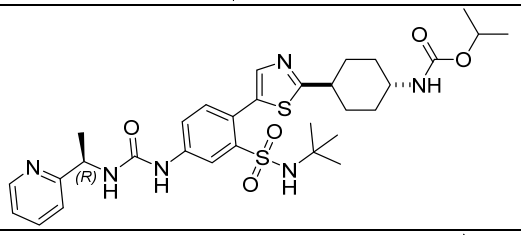
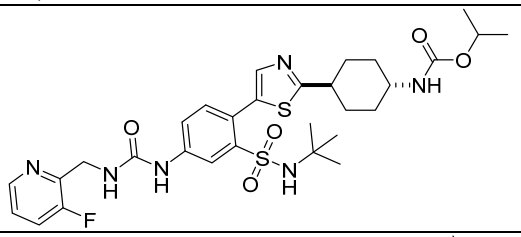
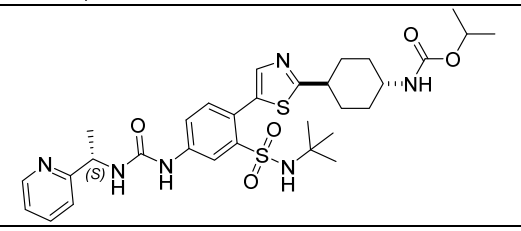
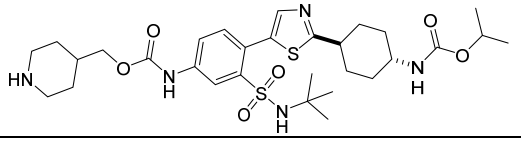
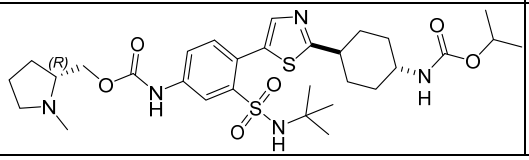
Table 1

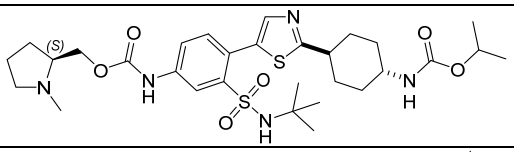
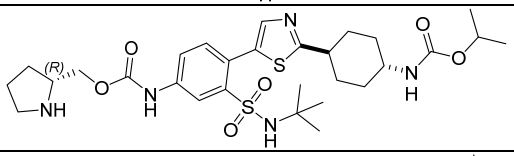
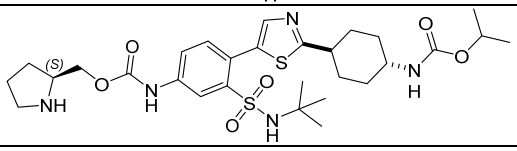
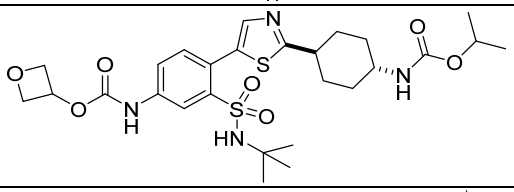
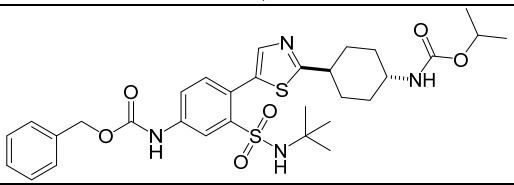
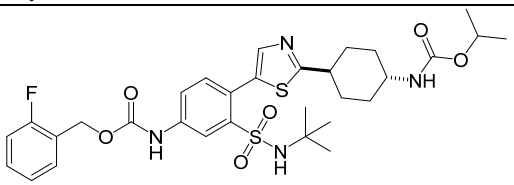
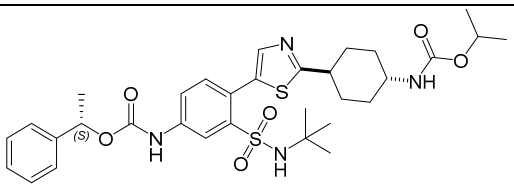
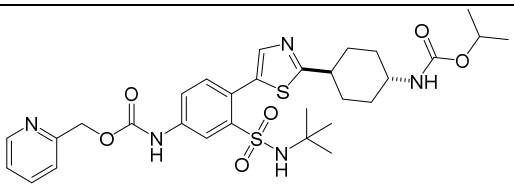
Compound No.	**Structure**	**AID+ EC_{50} (µM) A = ≤ 0.1 µM B = ≤ 1 µM C = > 1 µM ND = Not Determined**	**AID- EC_{50} (µM) A = ≤ 0.1 µM B = ≤ 1 µM C = > 1 µM ND = Not Determined**
Ex. 1		C	ND
Ex. 2		C	ND

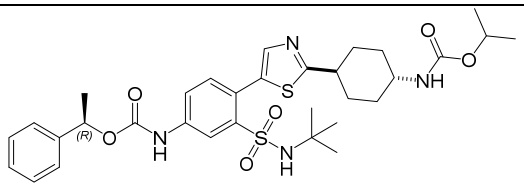
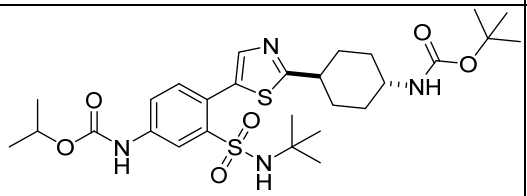
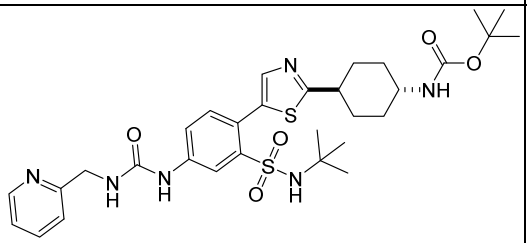
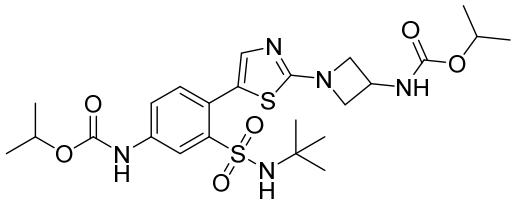
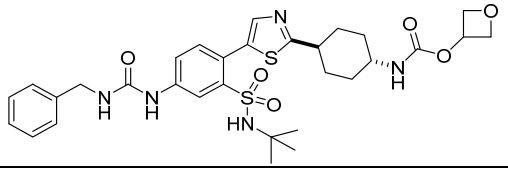
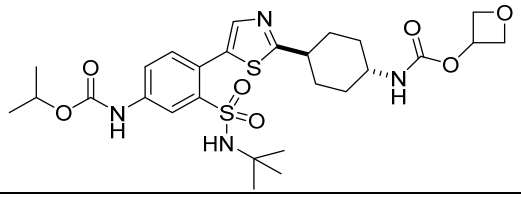
Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 3		A	C
Ex. 4		C	C
Ex. 5		C	ND
Ex. 6		C	ND
Ex. 7		B	ND
Ex. 8		C	ND
Ex. 9		B	ND
Ex. 10		A	C

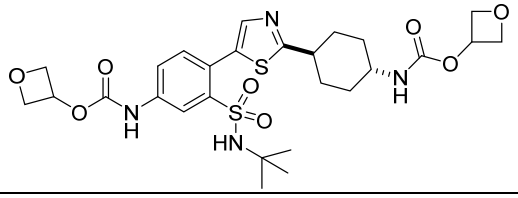
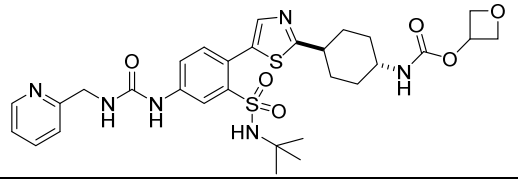
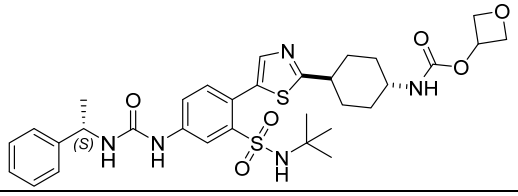
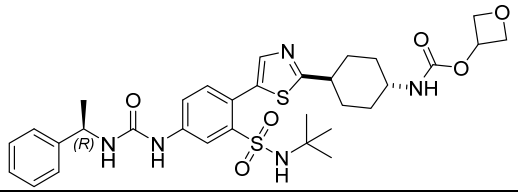
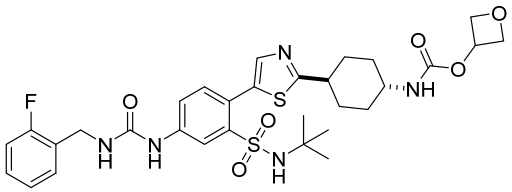
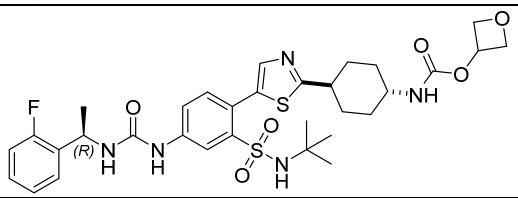
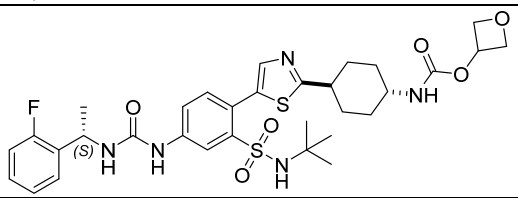
Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 11		B	ND
Ex. 12		B	ND
Ex. 13		A	C
Ex. 14		A	C
Ex. 15		A	C
Ex. 16		A	C
Ex. 17		B	C
Ex. 18		A	C

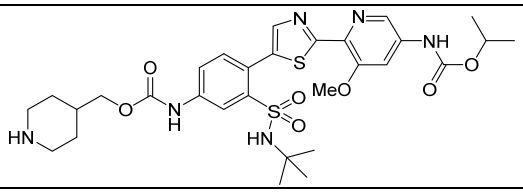
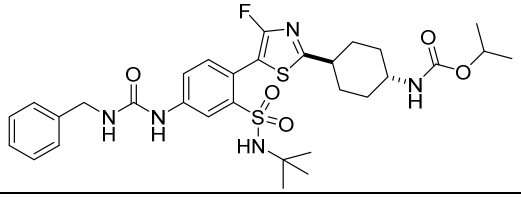
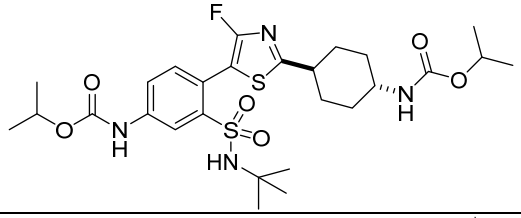
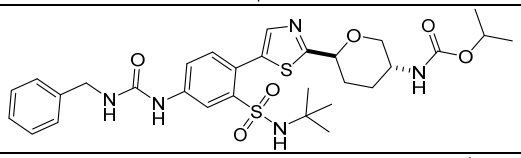
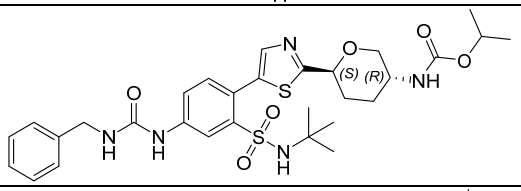
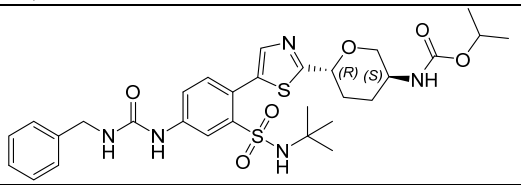
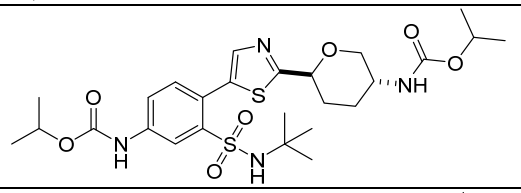
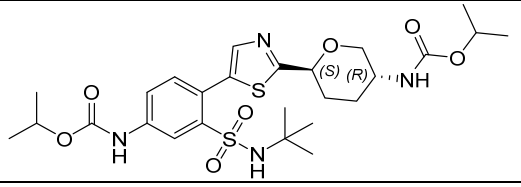
Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 19		A	C
Ex. 20		C	ND
Ex. 21		A	ND
Ex. 22		B	ND
Ex. 23		A	ND
Ex. 24		A	C
Ex. 25		A	ND
Ex. 26		A	C

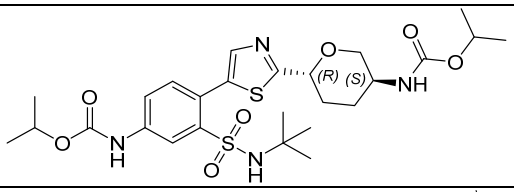
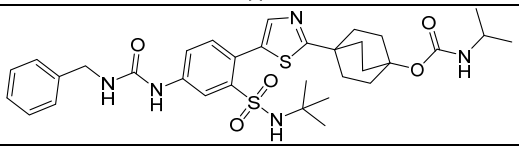
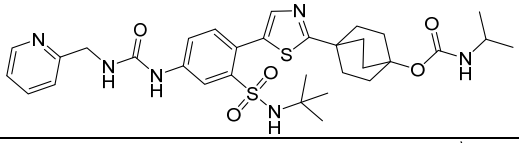
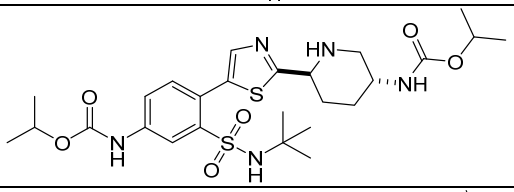
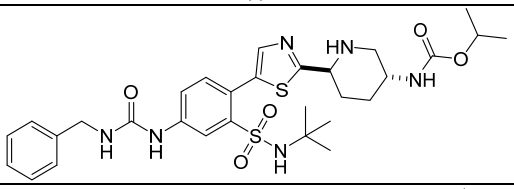
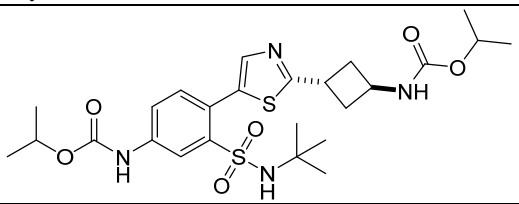
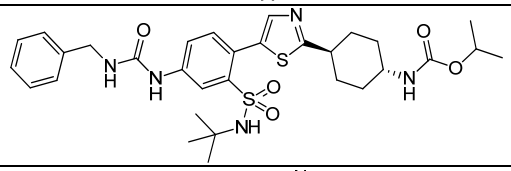
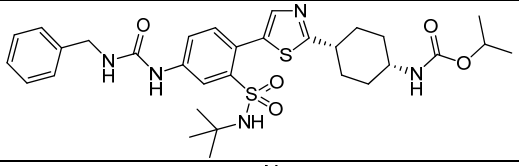
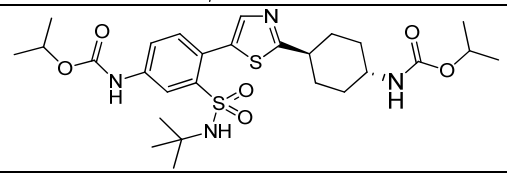
Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 27		A	C
Ex. 28		A	ND
Ex. 29		A	C
Ex. 30		A	C
Ex. 31		A	C
Ex. 32		A	C
Ex. 33		C	ND
Ex. 34		B	ND

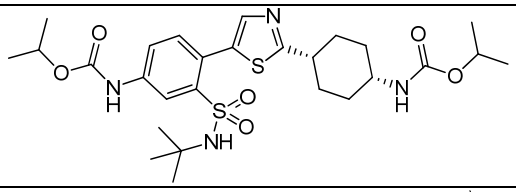
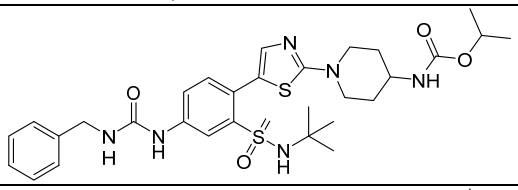
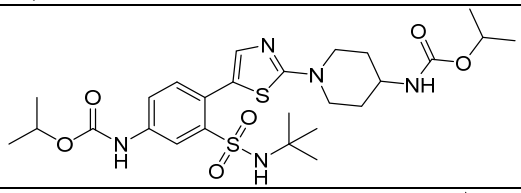
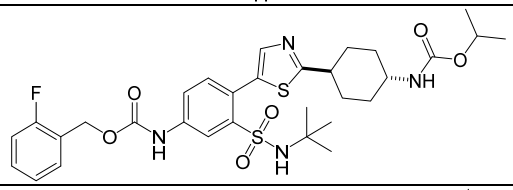
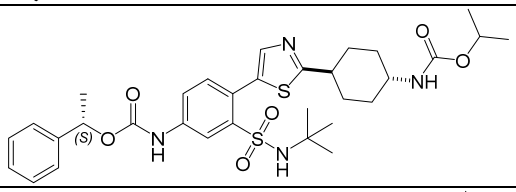
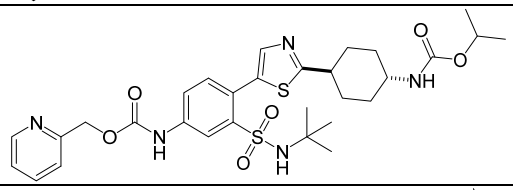
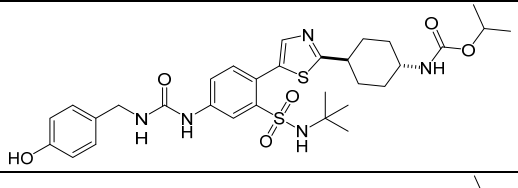
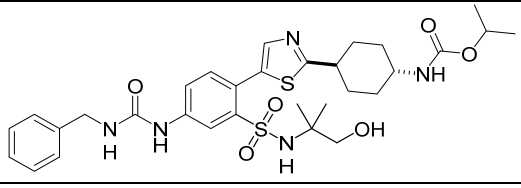
Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 35		B	ND
Ex. 36		B	ND
Ex. 37		B	ND
Ex. 38		A	C
Ex. 39		A	C
Ex. 40		A	C
Ex. 41		A	C
Ex. 42		A	C

Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 43		B	C
Ex. 44		C	ND
Ex. 45		A	ND
Ex. 46		C	C
Ex. 47		A	C
Ex. 48		B	C

Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 49		B	C
Ex. 50		A	C
Ex. 51		A	C
Ex. 52		A	C
Ex. 53		A	C
Ex. 54		A	C
Ex. 55		A	C

Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 56		B	ND
Ex. 57		A	C
Ex. 58		B	C
Ex. 59		A	C
Ex. 59A		B	C
Ex. 59B		A	C
Ex. 60		B	C
Ex. 60A		C	C

Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 60B		B	C
Ex. 61		C	C
Ex. 62		C	C
Ex. 63		B	ND
Ex. 64		A	C
Ex. 65		B	C
Ex. 66A		A	C
Ex. 66B		B	ND
Ex. 67A		B	C

Compound No.	Structure	AID+ EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined	AID- EC_{50} (μM) A = ≤ 0.1 μM B = ≤ 1 μM C = > 1 μM ND = Not Determined
Ex. 67B		C	ND
Ex. 68		B	ND
Ex. 69		C	ND
Ex. 70		A	C
Ex. 71		A	C
Ex. 72		A	C
Ex. 73		A	C
Ex. 74		A	C

Example 76. Bi-directional Caco-2 permeability

Bi-directional Caco-2 permeability was assayed. Caco-2 cells were seeded onto permeable polycarbonate supports and allowed to differentiate for about 3 weeks prior to being used in the assays. The cells were then exposed to the compounds from either the apical or basolateral sides and incubated at 37C for up to 90 minutes under light agitation. Compound transport was then measured using LC/MS/MS analysis at 30, 60, and 90 minutes.

Table 2

Example No.	Caco-2 Results				
	AB Papp (cm/sec x 10^6)	BA Papp (cm/sec x 10^6)	BA/AB Ratio	AB Recovery %	BA Recovery %
Ex. 5	5.9	2.2	0.4	34.7	80.8
Ex. 7	1.7	2.1	1.2	55.9	92
Ex. 10	1.2	1.5	1.3	59.9	87.2
Ex. 17	2.8	2.1	0.8	47.2	81.9
Ex. 20	9.4	10.4	1.1	44.8	96.6
Ex. 21	3.6	10.7	3	58.6	78.3
Ex. 22	1.1	21	18.7	83.1	89
Ex. 27	0.7	0.5	0.7	92	107.1
Ex. 28	1.9	3.3	1.7	66.9	81
Ex. 29	10.9	24.1	2.2	93.9	90.9
Ex. 30	11.5	15.7	1.4	79.8	115.6
Ex. 31	12.8	13.6	1.1	70.5	92.3
Ex. 34	0.4	45.2	103.9	98.2	96.8
Ex. 35	0.4	41	99.1	98.8	107.5
Ex. 38	17.9	22.9	1.3	73.3	82.3
Ex. 44	3.2	4.2	1.3	36.2	69.4
Ex. 45	7.6	10.8	1.4	73.6	84.4
Ex. 47	8.7	12.4	1.4	65.3	80.7
Ex. 48	23.1	16	0.7	80.1	90.3

Example No.	Caco-2 Results				
	AB Papp (cm/sec x 10^6)	BA Papp (cm/sec x 10^6)	BA/AB Ratio	AB Recovery %	BA Recovery %
Ex. 50	2	29.1	14.6	87.7	98.1
Ex. 51	9.9	10.5	1.1	65	85.7
Ex. 52	7.4	10.9	1.5	64.1	91.7
Ex. 53	9.3	9.7	1	63.1	90.9
Ex. 55	6.5	7	1.1	65.2	83.8
Ex. 57	1.3	3.5	2.7	61.9	86.8
Ex. 58	5.1	3.4	0.7	52.3	83.5
Ex. 59	3.1	15	4.8	61.5	91.5
Ex. 59A	3.1	9.6	3.1	55.6	85.5
Ex. 59B	4.6	9.7	2.1	56.2	82
Ex. 60	15.6	13.3	0.9	59.8	90.8
Ex. 60A	12	11.2	0.9	55.3	86
Ex. 60B	11.6	13.5	1.2	51.2	84.3
Ex. 63	10.5	26.7	2.5	83.6	90
Ex. 64	2.2	27.4	12.5	79.7	95.9
Ex. 66A	2.9	3.9	1.4	64.9	81.2
Ex. 67A	10.5	6.9	0.7	50.7	78.6

Example 77. Human Liver Microsome Stability

The stability of the claimed compounds was determined in the presences of human liver microsomes. The compounds were incubated with the microsomes at 37 ºC for 45 minutes. Samples were analyzed using LC-MS/MS. Data analysis included half-life, clearance rate, and the percentage of hepatic blood flow (%QH) for each of the compounds in the different species. Below are liver microsome assay data of representative compounds, which show that the claimed compounds have superior metabolic stability.

Table 3

	Human Liver Microsome Stability		
	Half Life (min)	Clearance (µg/min/mg)	% QH
Ex. 29	20.5	68.0	78.8
Ex. 31	22.4	61.9	77.3
Ex. 66A	77.6	18.2	49.6
Ex. 67A	>300	<4.6	<20.3

Example 78. Cell Line Screen

The activity of the claimed compounds was measured in a variety of cell lines with different expression levels of activation induced cytidine deaminase (AICDA). The potency assay was repeated in all of the listed cell lines and the EC_{50} values recorded.

Table 4

Cell Line (Cancer Type)	**AICDA Expression**	**EC_{50} (nM) Ex. 29**	**EC_{50} (nM) Ex. 31**	**EC_{50} (nM) Ex. 66A**	**EC_{50} (nM) Ex. 67A**
Daudi (Lymphoma)	High	43	20	18	311
WSU-FSCCL (Lymphoma)	Negative	67	<40	25	344
U-698-M (Lymphoma)	High	113	31	88	791
CCRF-SB (Leukemia)	High	1283	2164	183	932
KYSE-70 (Head and Neck)	Low	4660	4701	2639	2629
SNU-1 (Gastric)	Negative	n.d.	n.d.	609	2927
KG-1 (Leukemia)	Negative	>10000	8785	3067	2995
KYSE-510 (Head and Neck)	Negative	>10000	>10000	4516	3403
SNU-5 (Gastric)	Low	n.d.	n.d.	2941	3845
TOV-1120D (Ovary)	Negative	9172	n.d.	2377	4924
OV56 (Ovary)	Low	9086	n.d.	5944	7228
ARPE19/HPV16 (HPV Immortalized RPE)	Negative	>10000	>10000	>10000	>10000
WI-38 (Normal Human Lung Fibroblast)	Negative	>10000	>10000	>10000	>10000

n.d. not determined

Example 79. Pharmacokinetic (PK)

PK studies in mice were used to determine the fate of the compounds in a whole organism. Rats were treated with the compounds either orally of via IV at the indicated doses

and followed for up to 24 hours. Plasma samples were taken at different time points and analyzed by LC-MS.

Table 5

po @ 80 mg/kg (Formulation: 30% PEG400, 10% Vitamin E TPGS in water)		Ex. 29	Ex. 31	Ex. 66A	Ex. 67A
Rat Female	$T_{1/2}$ (hr)	4.66	4.75	2.59	11.5
	F (%) @ 5 mg/kg	8.39	2.77	3.31	86.5
Rat Male	$T_{1/2}$ (hr)	3.97	3.79	1.86	6.46
	F (%) @ 5 mg/kg	3.69	1.49	2.55	46.9

Example 80. Compound 67A Activity in Pancreatic Cancer Cell Lines

Cells were harvested during the logarithmic growth period and cells were counted using Count-star. The cell concentrations were adjusted to 4.44×10^4 cells/mL with respective culture medium. 90 µL cell suspensions was added to two 96-well plates (plates A and B) with the final cell density of 4×10^3 cells/well (cell concentration were adjusted according to the data base or density optimization assay). One plate was used for the day 0 reading, the second plate was treated with compound and incubated for seven days with medium replacement at 72 hours.

For the day 0 reading, 10 µL of culture medium was added to each well of plate A. The vehicle control was 0.25 % v/v DMSO. The plate was equilibrated and its content was at room temperature for approximately 30 minutes. 50 µL CellTiter-Glo reagent was added to each well. The contents were mixed for 5 minutes on an orbital shaker to induce cell lysis. The plate was allowed to incubate at room temperature for 20 minutes to stabilize luminescent signal. Luminescence (for day 0) was recorded using EnVision Multi Label Reader.

For the day 7 reading, a 10×solution of test article was prepared (100 µM of test article in media with 3.16-fold serial dilutions was used to achieve 9 dose levels). 10 µL (10×) of Compound 67A was dispensed into each well (triplicate for each drug concentration) of plate B. The DMSO final concentration in culture medium was 0.25% [v/v]).

The test plate B was incubated for 72 hours in the humidified incubator at 37°C with 5% CO_2. 80 µL of media containing Compound 67A was aspirated with liquid handling Biomek FXP. 80 µL fresh culture media containing 1× Compound 67A solutions was added to each well. The mixture was incubated for an additional 96 hours in a humidified incubator at 37°C with 5% CO_2 (168 hours total), and then measured by CTG assay. The plate was equilibrated and its content brought to room temperature for approximately 30 minutes. 50 µL of CellTiter-Glo reagent was added to each well. The contents were mixed for 5 minutes

on an orbital shaker to induce cell lysis. The plates were allowed to incubate at room temperature for 20 minutes to stabilize the luminescent signal. Luminescence was then recorded.

The data was then displayed graphically using GraphPad Prism 5.0. Absolute IC_{50} (EC_{50}), was calculated by fitting a dose-response curve using a nonlinear regression model with a sigmoidal dose response. The formula for calculating surviving rate is shown below and the absolute IC_{50} (EC_{50}) was then calculated according to the dose-response curve generated by GraphPad Prism 5.0.

$$\text{The surviving rate (\%)} = \frac{(\text{LumTest article} - \text{LumMedium control})}{(\text{LumNontreated} - \text{LumMedium control})}\ x\ 100\%$$

For IC_{50} values shown in Table 6, "A" means IC_{50} < 10 μM; "B" means IC_{50} ranging between 10 μM and 25 μM; "C" means IC_{50} ranging between 25 μM and 35 μM; "D" means IC_{50} ranging between 35 μM and 50 μM; "E" means IC_{50} > 50 μM; "Nd" means Not determined.

Table 6

Cell Line	IC50 (μM)
AsPC-1	C
BxPC-3	B
Capan-1	E
Capan-2	A
CFPAC-1	C
HPAF-II	B
HuP-T4	B
KP4	C
MIA PaCa-2	B
Panc 05.04	C
Panc 10.05	D
PANC-1	D
PL45	C
SU.86.86	Nd
SW1990	E

Example 81. PDX model 1

Activity of Compound 67A ("67A") was assessed in 3 different mouse PDX models with various levels of AID expression (low, medium, and high) (Figure 1). All models showed greater than 50% tumor growth inhibition with 67A treatment at 80 mg/kg QD (Low: 63%, Medium: 104%, high: 79%). For the medium expressing PDX model, the activity of

67A at two additional doses (1 mg/kg and 40 mg/kg) was examined. A response of 36% TGI at 1 mg/kg QD and a response of 96% TGI at 40 mg/kg QD was observed.

Next, a cross over experiment (Table 7) was performed to determine the activity of 67A in large, established tumors. On day 46 of treatment, vehicle treated and 1 mg/kg QD treated mice from the medium expressing PDX model were re-randomized into three treatment groups: vehicle, 1 mg/kg QD, and 80 mg/kg QD (Figure 2A). Tumor regression in the now 80 mg/kg treated cohort with a %TGI of 137 was observed.

The 40 and 80 mg/kg treatment groups were also re-randomized into three new groups: 80 mg/kg QD, 14 day dosing holiday followed by 80 mg/kg QD, and 21 day dosing holiday followed by 80 mg/kg QD (Figure 2B). During drug holiday, the tumors steadily began to grow at the same doubling rate as the vehicle control, and tumor growth was observed in the animals with tumors. Once treatment resumed with Compound 67A, the growth of the tumors was suppressed. The 80 mg/kg QD treatment group continued to show repressed tumor growth with a final %TGI of 96.

Table 7

Model	Tumor Characterization	Passage Number	AICDA Fold Gene Expression*
Model 1	Primary; Adenocarcinoma; Poorly Differentiated; Stage II	6	0.86
Model 2	Primary; Adenocarcinoma; Moderately Differentiated; Stage II	6	5.85
Model 3	Primary; Adenocarcinoma; Moderately Differentiated; Stage IV	5	9.85

*Fold change in expression determined by comparing the individual model RPKM score to the mean RPKM score of the entire PDX collection.

Compound 67A anti-tumor activity in Model 1

Engrafted mice were treated with either vehicle or Compound 67A at 80 mg/kg QD for 52 days. Tumor volumes were measured twice weekly and followed over the course of the experiment. The results demonstrated that Compound 67A treatment was successful (see

Figures 3A and 3B). Percent tumor growth inhibition was calculated for each Compound 67A treated animal on day 52.

Compound 67A anti-tumor activity in Model 2, Part 1

Engrafted mice were treated with either vehicle or Compound 67A at 1, 40, or 80 mg/kg QD for 45 days. Tumor volumes were measured twice weekly and followed over the course of the experiment. The results demonstrated that Compound 67A treatment was successful (see Figures 4A and 4B). Percent tumor growth inhibition was calculated for each Compound 67A treated animal on day 45.*Compound 67A anti-tumor activity in Model 2, Part 2*

Animals treated with vehicle and 1 mg/kg QD from Model 2, Part 1 were re-randomized and treated with either vehicle or Compound 67A at 1 mg/kg QD or 80 mg/kg QD (Figure 2A). Tumor volumes were measured twice weekly and followed for 49 days (Figure 6A). Change in tumor volume on day 49 as compared to day 0 was calculated for vehicle and Compound 67A at 80 mg/kg treated animals (Figure 6B). Progression free survival was measured as stasis of tumor volume and graphed for vehicle and Compound 67A at 80 mg/kg treated animals (Figure 6C).

Compound 67A anti-tumor activity after dosing holidays in Model 2, Part 3

Animals treated with Compound 67A at 40 and 80 mg/kg QD from Model 2, Part 1 were re-randomized and treated with either vehicle or Compound 67A at 80 mg/kg QD continuously, Compound 67A at 80 mg/kg after a 14 day dosing holiday, or Compound 67A at 80 mg/kg after a 21 day dosing holiday (Figure 2B). Tumor volumes were measured twice weekly and followed for 49 days. The results demonstrated that Compound 67A treatment was successful (see Figures 7A-7C).

Compound 67A anti-tumor activity in Model 3

Engrafted mice were treated with either vehicle or Compound 67A at 80 mg/kg QD for 33 days. Tumor volumes were measured twice weekly and followed over the course of the experiment. The results demonstrated that Compound 67A treatment was successful (see Figures 3A and 3B). Percent tumor growth inhibition was calculated for each Compound 67A treated animal on day 33.

Compound 67A showed anti-cancer activity in all Models (i.e. Model 1, Model 2, and Model 3) of pancreatic cancer tested with a mean % TGI ranging from 62% to 104%. Compound 67A is also active in large established tumors, and dosing holidays did not lead to the development of resistance. In Model 2 one partial responder and one tumor-free

responder was observed. The data demonstrate that 67A has anti-cancer activity in multiple preclinical models of pancreatic cancer.

Example 82. PDX model 2

Activity of Compound 67A ("67A") was assessed in 3 different mouse PDX models of pancreatic cancer engrafted onto the flanks of immunocompromised mice. The three models tested were Model A (CTG-0955), Model B (CTG-1462), and Model C (CTG-1295). Tumors were allowed to reach a mean tumor volume of 500 mm^3 prior to dosing. The dosing groups were as follows:

- Vehicle;
- Compound 67A at 80 mg/kg orally, once daily;
- Compound 67A at 80 mg/kg orally, once daily for four weeks, followed by a dosing holiday until tumors reach 1000 mm^3, at which point administration of Compound 67A at 80 mg/kg orally, once daily is reinitiated;
- Compound 67A at 80 mg/kg orally, daily every other week; and
- Compound 67A at 80 mg/kg orally, every other day.

All models were measured twice per week and showed anti-tumor activity in larger tumors, similar to the observations of Example 80. Dosing of Compound 67A daily resulted in greater anti-tumor activity in comparison to every other day dosing. Reinitiating treatment with Compound 67A after a dosing holiday resulted in slowed tumor growth or tumor shrinkage, depending on the initial sensitivity of the model.

Compound 67A anti-tumor activity in Model A

The dosing groups were analyzed to determining %TGI relative to continuous dosing in comparison to every other day dosages (FIG. 8A), as well as comparison of tumor volume (FIG. 8B). Tumor regression in the cohort treated with a continuous dosage of Compound 67A at 80 mg/kg with a %TGI of 52 was observed. Tumor regression in the cohort treated with an every other day dosage of Compound 67A at 80 mg/kg with a %TGI of 20 was observed. Tumor regression in the cohort with a 200mm^3 tumor treated daily with Compound 67A with a %TGI of 64 was observed. Tumor regression in the cohort with a 500mm^3 tumor treated with Compound 67A with a %TGI of 52 was observed.

Compound 67A anti-tumor activity in Model B

The dosing groups were analyzed to determing %TGI relative to continuous dosing in comparison to every other day dosages (FIG. 9A), comparison of tumor volume (FIG. 9B), and analysis of post holiday dosing (FIG. 9C). Tumor regression in the cohort treated with a

continuous dosage of Compound 67A at 80 mg/kg with a %TGI of 68 was observed. Tumor regression in the cohort treated with an every other day dosage of Compound 67A at 80 mg/kg with a %TGI of 54 was observed. Tumor regression in the cohort with a 200mm^3 tumor treated daily with Compound 67A with a %TGI of 79 was observed. Tumor regression in the cohort with a 500mm^3 tumor treated with Compound 67A with a %TGI of 68 was observed.

Treatment with oral Compound 67A administered daily for four weeks at 80 mg/kg showed a decrease in tumor growth. After four weeks, a dosing holiday was implemented until the tumor growth reached 1000 mm^3 (i.e., about 6 days for Model B). At this point, administration of Compound 67A daily at 80 mg/kg was resumed. Post dosing holiday treatment exhibited a decrease in tumor progression (FIG. 9D).

Compound 67A anti-tumor activity in Model C

The dosing groups were analyzed to determing %TGI relative to continuous dosing in comparison to every other day dosages (FIG. 10A), comparison of tumor volume (FIG. 10B), and analysis of post holiday dosing (FIG. 10C). Tumor regression in the cohort treated with a continuous dosage of Compound 67A at 80 mg/kg with a %TGI of 93 was observed. Tumor regression in the cohort treated with an every other day dosage of Compound 67A at 80 mg/kg with a %TGI of 65 was observed. Tumor regression in the cohort with a 200mm^3 tumor treated daily with Compound 67A with a %TGI of 104 was observed. Tumor regression in the cohort with a 500mm^3 tumor treated with Compound 67A with a %TGI of 93 was observed.

Treatment with oral Compound 67A administered daily for four weeks at 80 mg/kg showed a decrease in tumor growth. After four weeks, a dosing holiday was implemented until the tumor growth reached 1000 mm^3 (i.e., about 48 days for Model C). At this point, administration of Compound 67A daily at 80 mg/kg was resumed. Post dosing holiday treatment exhibited a decrease in tumor progression (FIG. 10D).

EQUIVALENTS

The details of one or more embodiments of the disclosure are set forth in the accompanying description above. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. Other features, objects, and advantages of the disclosure will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. All patents and publications cited in this specification are incorporated by reference.

The foregoing description has been presented only for the purposes of illustration and is not intended to limit the disclosure to the precise form disclosed, but by the claims appended hereto.