Supporting Information for

Structure-based design of selective, orally available, salt-inducible kinase inhibitors that stimulate bone formation in mice

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SI Materials and Methods

Animals
The following genetically modified strains were used: *Sik2* floxed mice (RRID:MGI:5905012) [1], and *Sik3*^{tm1a}(EUCOMM)Hmgu mice (RRID:MGI:5085429) bred to PGK1 FLPo mice (JAX #011065) in order to generate *Sik3* floxed mice [2]. Ubiquitin-Cre^{ERt2} mice (JAX #007001) [3] and C57BL/6J and CD1 male mice were obtained from the Jackson Laboratory (Bar Harbor, ME). All animals were housed in the Center for Comparative Medicine at the Massachusetts General Hospital (21.9 ± 0.8°c, 45 ± 15% humidity and 12-hour light cycle 7 am-7 pm), and all experiments were approved by the hospital's Subcommittee on Research Animal Care (IACUC protocol number 2020N000151). For studies with Ubiquitin-Cre^{ERt2} mice, tamoxifen (Sigma-Aldrich) was injected (1 mg, IP, Q48 hours, 3 injections total) in all animals including Cre-negative control littermates housed in cages with Pure-o'Cel bedding at 12 weeks of age. Male C57B6 mice were treated with three different doses SK-124, and vehicle (15% hydroxy-propyl beta-cyclodextrin dissolved in sterile water) by oral gavage for three weeks and compared their phenotype to mice daily sc. injected with PTH (1-34).

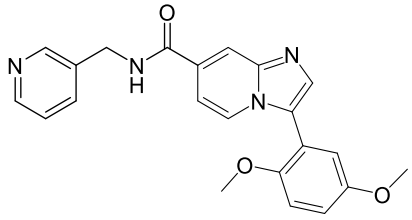
Generation of refined SIK2 homology model and in silico *screening*
The crystal structure of human salt-inducible kinase 2 has not been determined. The human SIK2 sequence (Q9H0K1 UniProtKB) was submitted to SWISS-MODEL [4]. 50 kinase templates were considered and two models generated. The top homology model is based on the 2QNJ crystal structure of microtubule affinity-regulating kinase3 [5]. The proteins share 55.8% sequence identity. The model retains key features of the active site especially the situation of the residues previously modeled to interact with known SIK2 inhibitors [6]. 2QNJ is an apo structure and the ATP binding site appeared conformationally closed. To open the ATP binding site, we aligned the ATP and 2 Mg^{+2} ions from 3-phosphoinositide-dependent protein kinase 1 (4AW0). Then the SIK2 homology model, with aligned ATP and 2 Mg^{+2} ions, was energy minimized with GROMACS. This conformation of the protein was used for virtual screening.

An in house compound library (>100,000 compounds), a collection of Asinex, ChemBridge, ChemDiv, Hitfinder, NIH Clinical Collections (NCC) and NIH Diversity 5 (Div5), was docked to the SIK2 homology model using Autodock Vina [7]. The compound library was designed for chemical diversity using hole filling calculations and there is some built in SAR so similar compounds can be tested. The exhaustiveness level was set at 8. All other parameters were set at the default value. The grid was centered around the ATP molecule and set at 20x20x20 Å. We selected 70 compounds for experimental testing after visual inspection of the binding poses from the top 100 compounds sorted according to the docking scores.

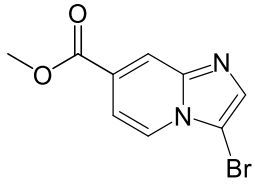
We also selected 30 compounds for experimental testing based on selectivity to SIK2 and not p38α or SRC. We performed a selectivity analysis on the 30 compounds from above, that were the best binders to SIK2, by docking them to off-target kinases, as determined by the kinome experiment. To determine selective binding to SIK2, we docked the in-house library to p38α (5XYY, X-ray crystallographic structure with a triazol inhibitor of a resolution 1.7 Å) and to SRC (4MXO, X-ray crystallographic structure with inhibitor bosutinib of a resolution 2.1 Å). We calculated the difference in the docking scores between the SIK2 homology model docking and p38α and between the SIK2 homology model docking and SRC were calculated. We sorted it such that only compounds with docking scores that were favorable were retained. Finally, we selected 10 compounds for experimental testing from similarity search based on three known SIK2 inhibitors (AZD07762, YKL-05-099, and crenolanib) on the whole in-house library using Ligand Similar using Clique Algorithm (LiSiCA) [8].

Synthetic chemistry

Compound 6. 3-(2,5-dimethoxyphenyl)-N-(3-pyridylmethyl)imidazo[1,2-a] pyridine-7-carboxamide

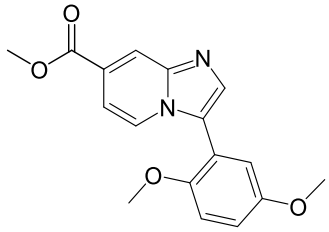


Step 1 Methyl 3-bromoimidazo[1,2-a]pyridine-7-carboxylate



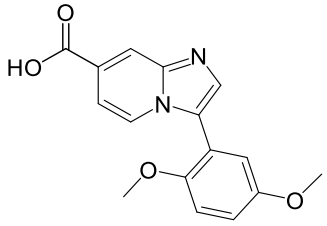
To a mixture of methyl imidazo[1,2-a]pyridine-7-carboxylate (300 mg, 1.70 mmol, 1 eq) in DMF (5 mL) was added NBS (333.39 mg, 1.87 mmol, 1.1 eq) at 20°C, then the reaction mixture was stirred at 20°C for 1 h. The mixture was added to water (30 mL) and extracted with EtOAc (2 mL, 3x). The organic layer was dried over Na_2SO_4 and concentrated to afford the title compound (450 mg, crude) as a yellow solid.

Step 2. Methyl 3-(2,5-dimethoxyphenyl)imidazo[1,2-a]pyridine-7-carboxylate



To a mixture of methyl 3-bromoimidazo[1,2-a]pyridine-7-carboxylate (450 mg, 1.76 mmol, 1 eq), (2,5-dimethoxyphenyl)boronic acid (321.06 mg, 1.76 mmol, 1 eq) in dioxane (15 mL), and H_2O (1.5 mL) was added Na_2CO_3 (373.98 mg, 3.53 mmol, 2 eq) and $Pd(dppf)Cl_2$ (129.09 mg, 176.42 µmol, 0.1 eq) at 20°C, and the mixture was heated at 100°C for 12 h under N_2. The reaction mixture was then concentrated to give the crude product, which was purified by column chromatography on silica gel (Petroleum ether: Ethyl acetate = 10:1-1:1) to afford the title compound (240 mg, 768.44 µmol, 43.56% yield) as a yellow solid.

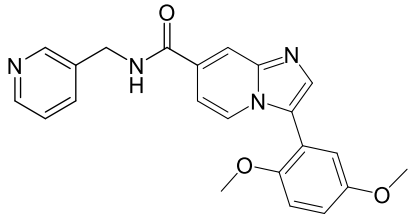
Step 3. 3-(2,5-Dimethoxyphenyl)imidazo[1,2-a]pyridine-7-carboxylic acid



To a mixture of methyl 3-(2,5-dimethoxyphenyl)imidazo[1,2-a]pyridine-7-carboxylate (240 mg, 768.44 µmol, 1 eq) in MeOH (2 mL)/H_2O (7 mL) was added NaOH (61.47 mg, 1.54 mmol, 2 eq) at 20°C, then the reaction mixture was stirred at 20°C for 12 h. The mixture was concentrated to

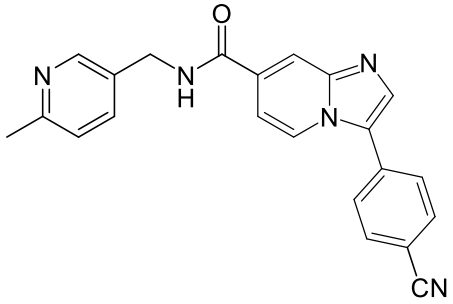
remove MeOH, then the water layer was acidified with HCl (6M) to pH = 3, filtered, and the filter cake was dried under vacuum. The title compound (180 mg, crude) was obtained a yellow solid.

Step 4. 3-(2,5-Dimethoxyphenyl)-N-(3-pyridylmethyl)imidazo[1,2-a]pyridine-7-carboxamide

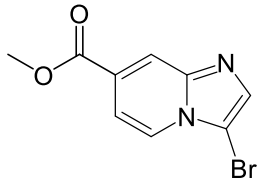


To a mixture of 3-(2,5-dimethoxyphenyl)imidazo[1,2-a]pyridine-7-carboxylic acid (80 mg, 268.19 μmol, 1 eq) in DMF (2 mL) was added DIEA (69.32 mg, 536.39 μmol, 93.43 μL, 2 eq), 3-pyridylmethanamine (29.00 mg, 268.19 μmol, 27.11 μL, 1 eq), and HATU (122.37 mg, 321.83 μmol, 1.2 eq), and the reaction mixture was stirred at 40°C for 12 h. The reaction mixture was then concentrated to give the crude product, which was purified by prep-HPLC (basic condition, column: Waters Xbridge Prep OBD C18 150*40mm*10um;mobile phase: [water(0.04%NH3H2O+10mM NH4HCO3)-ACN];B%: 15%-45%,10min) to afford the title compound (39.5 mg, 100.21 μmol, 37.37% yield, 98.544% purity) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.74 (s, 3 H) 3.78 (s, 3 H) 4.54 (d, J=5.75 Hz, 2 H) 7.04 (d, J=3.13 Hz, 1 H) 7.07 - 7.12 (m, 1 H) 7.16 - 7.20 (m, 1 H) 7.36 - 7.41 (m, 2 H) 7.77 (br d, J=7.88 Hz, 1 H) 7.81 (s, 1 H) 8.03 (d, J=7.25 Hz, 1 H) 8.24 (s, 1 H) 8.48 (d, J=3.50 Hz, 1 H) 8.59 (d, J=1.63 Hz, 1 H) 9.30 - 9.37 (m, 1 H) 9.33 (br t, J=5.82 Hz, 1 H).

Compound 10. 3-(4-cyanophenyl)-N-[(6-methyl-3-pyridyl)methyl]imidazo[1,2-a]pyridine-7-carboxamide

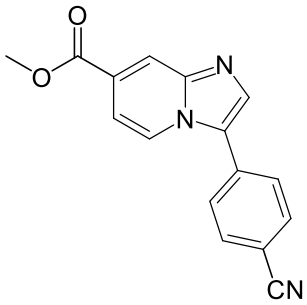


Step 1. Methyl 3-bromoimidazo[1,2-a]pyridine-7-carboxylate



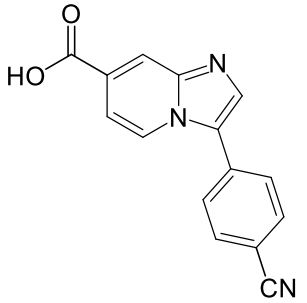
Methyl imidazo[1,2-a]pyridine-7-carboxylate (760 mg, 4.31 mmol, 1 eq) was mixed with NBS (844.60 mg, 4.75 mmol, 1.1 eq) in DMF (10 mL), and then the mixture was stirred at 20°C for 1 h. The reaction mixture was then quenched by addition water (30 mL) and extracted with EtOAc (150 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the title compound (1.1 g, crude) as a yellow solid.

Step 2: Methyl 3-(4-cyanophenyl)imidazo[1,2-a]pyridine-7-carboxylate



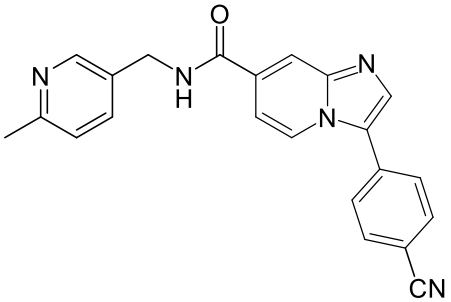
A mixture of methyl 3-bromoimidazo[1,2-a]pyridine-7-carboxylate (750 mg, 2.94 mmol, 1 eq), (4-cyanophenyl)boronic acid (518.47 mg, 3.53 mmol, 1.2 eq), Na_2CO_3 (623.30 mg, 5.88 mmol, 2 eq), $Pd(dppf)Cl_2$ (215.15 mg, 294.04 μmol, 0.1 eq) in dioxane (20 mL) and H_2O (2 mL) was degassed and purged with N_2 (3x), and then the mixture was stirred at 90°C for 12 h under N_2 atmosphere. The reaction mixture was then filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was triturated with MeOH (30 mL) at 25ºC for 30 min, filtered, and the filter cake was concentrated under reduced pressure to afford the title compound (300 mg, crude) as a gray solid.

Step 3. 3-(4-Cyanophenyl)imidazo[1,2-a]pyridine-7-carboxylic acid



A mixture of methyl 3-(4-cyanophenyl)imidazo[1,2-a]pyridine-7-carboxylate (280 mg, 1.01 mmol, 1 eq), NaOH (80.79 mg, 2.02 mmol, 2 eq) in MeOH (30 mL), THF (10 mL), and H_2O (5 mL) was prepared, and the mixture was stirred at 25°C for 24 h. The reaction mixture was concentrated under reduced pressure to remove MeOH and THF, then HCl (12 N; 1mL) was added. The resulting solution was filtered, and the filter cake was concentrated under reduced pressure to afford the title compound (300 mg, crude) as a brown solid.

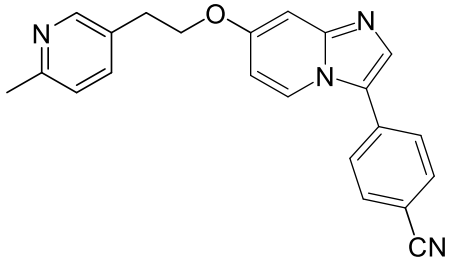
Step 4. 3-(4-Cyanophenyl)-N-[(6-methyl-3-pyridyl)methyl]imidazo[1,2-a]pyridine-7-carboxamide



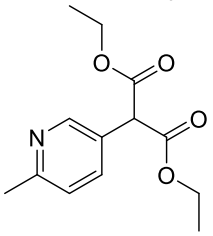
A mixture of 3-(4-cyanophenyl)imidazo[1,2-a]pyridine-7-carboxylic acid (70 mg, 265.91 μmol, 1 eq), (6-methyl-3-pyridyl)methanamine (35.73 mg, 292.50 μmol, 1.1 eq) and DIEA (68.73 mg, 531.81 μmol, 92.63 μL, 2 eq) in DMF (3 mL) was prepared, then HATU (151.66 mg, 398.86 μmol, 1.5 eq) was added and the resulting mixture was stirred at 40°C for 12 h. The reaction mixture was filtered, and the resulting residue was purified by prep-HPLC (basic condition;

column: Waters Xbridge Prep OBD C18 150*40mm*10um;mobile phase: [water(0.04%NH3H2O+10mM NH4HCO3)-ACN];B%: 15%-45%,10min) to afford the title compound (62.4 mg, 169.52 μmol, 63.75% yield, 99.810% purity) as a white solid. ^{1}H NMR (400MHz, DMSO-d_6) δ: 9.35 (t, J=5.8 Hz, 1H), 8.77 (d, J=7.1 Hz, 1H), 8.44 (d, J=1.9 Hz, 1H), 8.28 (s, 1H), 8.13 (s, 1H), 8.05 - 7.99 (m, 2H), 7.97 - 7.93 (m, 2H), 7.65 (dd, J=2.2, 7.9 Hz, 1H), 7.46 (dd, J=1.6, 7.3 Hz, 1H), 7.23 (d, J=7.9 Hz, 1H), 4.49 (d, J=5.8 Hz, 2H), 2.45 (s, 3H).

Compound 13. 4-[7-[2-(6-Methyl-3-pyridyl)ethoxy]imidazo[1,2-a]pyridin-3-yl]benzonitrile

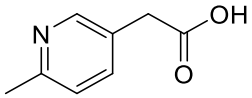


Step 1. Diethyl 2-(6-methyl-3-pyridyl)propanedioate



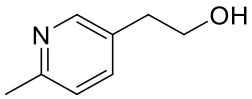
A mixture of 5-bromo-2-methylpyridine (5 g, 29.1 mmol, 1 eq), diethyl propanedioate (11.6 g, 72.7 mmol, 11.0 mL, 2.5 eq), $Pd(OAc)_2$ (653 mg, 2.91 mmol, 0.1 eq), K_3PO_4 (18.5 g, 87.2 mmol, 3eq), and ditert-butyl-(2-phenylphenyl)phosphane (867 mg, 2.91 mmol, 0.1 eq) in toluene (30 mL) was degassed and purged with N_2 (3x), and then the mixture was stirred at 100 °C for 48 h under N_2 atmosphere. The reaction mixture was filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=1/0 to 8/1) to afford the title compound (5 g, crude) as a yellow oil.

Step 2. 2-(6-Methyl-3-pyridyl)acetic acid



A mixture of diethyl 2-(6-methyl-3-pyridyl)propanedioate (5 g, 19.9 mmol, 1 eq) in HCl (6 N, 30 mL) was stirred at 100°C for 4 h. The mixture was concentrated to give a crude product, which was added into H_2O (20 mL), then extracted with EtOAc (30 mL, 2x), and the water phase was lyophilized to afford the title compound (3.7 g, crude) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.75 (s, 3 H) 3.90 (s, 2 H) 7.89 (d, J=8.25 Hz, 1 H) 8.39 (dd, J=8.25, 1.75 Hz, 1 H) 8.70 (d, J=1.38 Hz, 1 H).

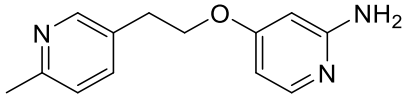
Step 3. 2-(6-Methyl-3-pyridyl) ethanol



To a solution of 2-(6-methyl-3-pyridyl)acetic acid (2 g, 13.2 mmol, 1 eq) in THF (30 mL) was added BH_3·THF (1 M, 46.3 mL, 3.5 eq) dropwise at 0°C, and the resulting mixture was stirred at 0°C for 30 min, followed by stirring at 25°C for 2 h. The reaction was quenched with methanol

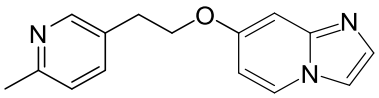
(50 mL) at 0°C, then the mixture was stirred at 25°C for 12 h. The mixture was filtered, and the filtrate was concentrated to give a crude product, which was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=1:0 to 1:4, 5%TEA) to afford the title compound (1.17 g, crude) as a yellow oil.

Step 4. 4-[2-(6-Methyl-3-pyridyl)ethoxy]pyridin-2-amine



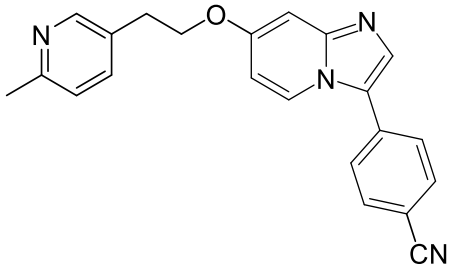
To a solution of 2-aminopyridin-4-ol (0.6 g, 5.45 mmol, 1 eq), 2-(6-methyl-3-pyridyl)ethanol (449 mg, 3.27 mmol, 0.6 eq) and PPh_3 (1.43 g, 5.45 mmol, 1 eq) in DCM (60 mL), was added DIAD (1.10 g, 5.45 mmol, 1.06 mL, 1 eq) at 0°C under N_2, and the resulting mixture was stirred at 25°C for 12 h under N_2. The mixture was filtered, and the filtrate was concentrated to give a crude product, which was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate/Methanol=1:0:0 to 0:10:1,5%TEA) to afford the title compound (390 mg, crude) as a yellow solid.

Step 5. 7-[2-(6-Methyl-3-pyridyl)ethoxy]imidazo[1,2-a]pyridine



To a mixture of 4-[2-(6-methyl-3-pyridyl)ethoxy]pyridin-2-amine (97 mg, 423 μmol, 1 eq) in EtOH (4 mL) was added $NaHCO_3$ (142 mg, 1.69 mmol, 65.8 μL, 4 eq) and 2-chloroacetaldehyde (415 mg, 2.12 mmol, 340 μL, 5 eq) in one portion at 25°C, and the resulting mixture was stirred at 70°C for 2 h. Four batches were worked together and concentrated under reduced pressure to remove EtOH, then extracted with EtOAc (50 mL, 2x). The combined organic phase was washed with saturated NH_4Cl (aq., 50 mL) and brine (50 mL), then dried over Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give a residue, which was purified by column (SiO_2, Petroleum ether/Ethyl acetate/Methanol=1:0:0 to 0:10:1, 5%TEA) to afford the title compound (380 mg, crude) as a yellow oil.

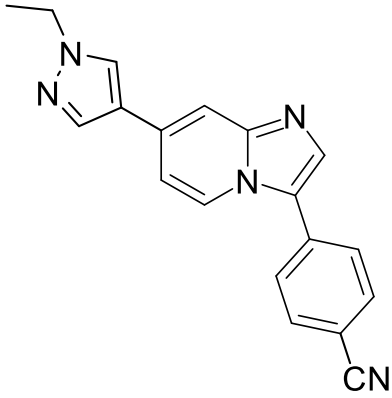
Step 6. 4-[7-[2-(6-Methyl-3-pyridyl)ethoxy]imidazo[1,2-a]pyridin-3-yl]benzonitrile



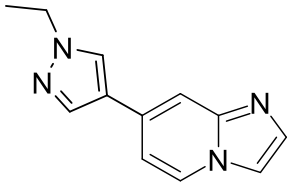
To a solution of 4-bromobenzonitrile (201 mg, 1.11 mmol, 1.4 eq), 7-[2-(6-methyl-3-pyridyl)ethoxy]imidazo[1,2-a]pyridine (200 mg, 790 μmol, 1 eq), and Cs_2CO_3 (772 mg, 2.37 mmol, 3 eq) in DMAC (5 mL) was added $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (32.2 mg, 39.5 μmol, 0.05 eq), in one portion at 25°C under N_2, and the resulting mixture was stirred at 100°C for 12 h. The reaction mixture was added into saturated NH_4Cl (aq., 50 mL), then extracted with EtOAc (50 mL, 2x). The combined organic phase was washed with brine (50 mL), then dried over Na_2SO_4, filtered, and filtrate was concentrated under reduced pressure to give a residue, which was purified by prep-TLC (Ethyl acetate : Methanol:NH_3. H_2O=100:10:4) to give a crude product. The crude was purified by prep-HPLC (basic condition, column: Waters Xbridge Prep OBD C18 150*40mm*10um;mobile phase: [water(0.05%NH_3H_2O+10mM NH_4HCO_3)-ACN];B%: 30%-

60%,8min) to afford the title compound (73.8 mg, 207.72 μmol, 26.31% yield, 99.753% purity) as a gray solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.43 (s, 3 H) 3.06 (t, J=6.44 Hz, 2 H) 4.32 (t, J=6.50 Hz, 2 H) 6.68 (dd, J=7.63, 2.50 Hz, 1 H) 7.10 (d, J=2.50 Hz, 1 H) 7.19 (d, J=7.88 Hz, 1 H) 7.65 (dd, J=7.94, 2.31 Hz, 1 H) 7.78 - 7.87 (m, 3 H) 7.90 - 7.97 (m, 2 H) 8.41 (d, J=2.00 Hz, 1 H) 8.52 (d, J=7.63 Hz, 1 H).

Compound 14. 4-[7-(1-Ethylpyrazol-4-yl) imidazo[1,2-a]pyridin-3-yl]benzonitrile

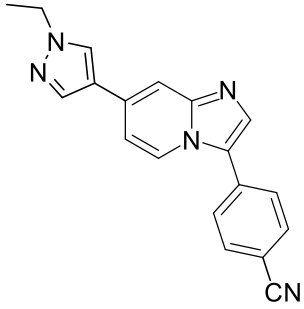


Step 1. 7-(1-Ethylpyrazol-4-yl)imidazo[1,2-a]pyridine



A mixture of 7-bromoimidazo[1,2-a]pyridine (150 mg, 761.3 μmol, 1 eq), 1-ethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazole (169.1 mg, 761.3 μmol, 1 eq), Na_2CO_3 (161.4 mg, 1.5 mmol, 2 eq), Pd(dppf)Cl_2 (55.7 mg, 76.1 μmol, 0.1 eq) in dioxane (5 mL) and H_2O (0.5 mL) was degassed and purged with N_2 (3x), then the mixture was stirred at 90°C for 12 h under N_2 atmosphere. The reaction mixture was diluted with water (30 mL) and extracted with EtOAc (150 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the title compound (160 mg, crude) as a brown oil.

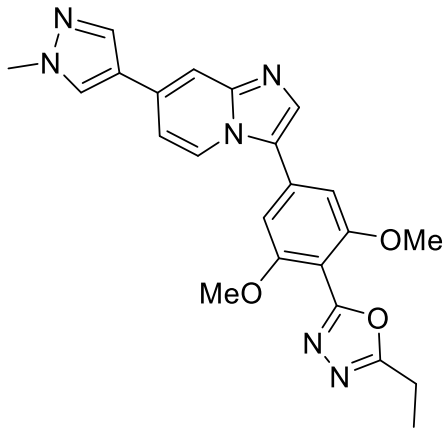
Step 2. 4-[7-(1-Ethylpyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl]benzonitrile



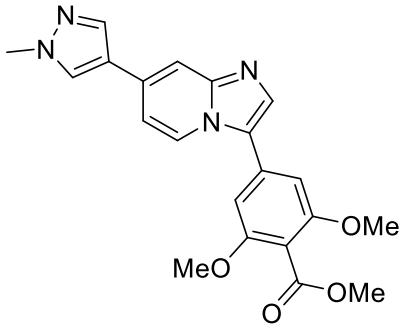
To a solution of 7-(1-ethylpyrazol-4-yl)imidazo[1,2-a]pyridine (160 mg, 753.8 μmol, 1 eq), 4-bromobenzonitrile (164.7 mg, 904.6 μmol, 1.2 eq), and Cs_2CO_3 (736.8 mg, 2.3 mmol, 3 eq) in DMA (5 mL) was added Pd(dppf)Cl_2·CH_2Cl_2 (30.8 mg, 37.7 μmol, 0.05 eq), and the resulting mixture was stirred at 100°C for 12 h. The reaction mixture was filtered and concentrated under reduced pressure to give a residue, which was triturated with EtOAc (15 mL) at 25°C for 20 min

to afford the title compound (60.6 mg, 191.7 μmol, 25.4% yield, 99.1% purity) as a yellow solid. ^{1}H NMR (400MHz, DMSO-d_6) δ: 8.63 (d, J =7.3 Hz, 1H), 8.41 (s, 1H), 8.07 (s, 1H), 7.97 - 7.85 (m, 6H), 7.25 (dd, J =1.5, 7.1 Hz, 1H), 4.14 (q, J =7.3 Hz, 2H), 1.39 (t, J =7.3 Hz, 3H).

Compound 16. 2-[2,6-Dimethoxy-4-[7-(1-methylpyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl]phenyl]-5-ethyl-1,3,4-oxadiazole

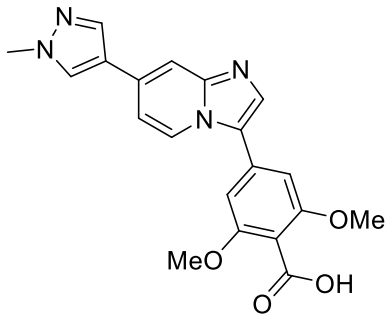


Step 1. Methyl 2,6-dimethoxy-4-[7-(1-methylpyrazol-4-yl)imidazo[1,2-a]pyridine-3-yl]benzoate



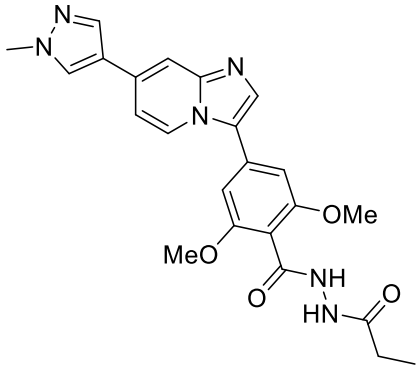
To a solution of 7-(1-methylpyrazol-4-yl)imidazo[1,2-a]pyridine (920 mg, 4.6 mmol, 1 eq), methyl 4-bromo-2,6-dimethoxy-benzoate (1.5 g, 5.6 mmol, 1.2 eq), and Cs_2CO_3 (4.5 g, 13.9 mmol, 3 eq) in DMA (30 mL) was added Pd(dppf)Cl_2·CH_2Cl_2 (189.5 mg, 232.1 μmol, 0.05 eq), in one portion at 25°C under N_2, and the resulting mixture was stirred at 100°C for 12 h. The reaction mixture was added into saturated NH_4Cl (aq. 50 mL), then extracted with EtOAc (50 mL, 3x). The combined organic phase was washed with brine (50 mL), then dried over Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate: Methanol=10:1:0 to 0:10:1) to afford the title compound (1.4 g, crude) as a brown solid.

Step 2. 2,6-Dimethoxy-4-(7-(1-methyl-1H-pyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl)benzoic acid



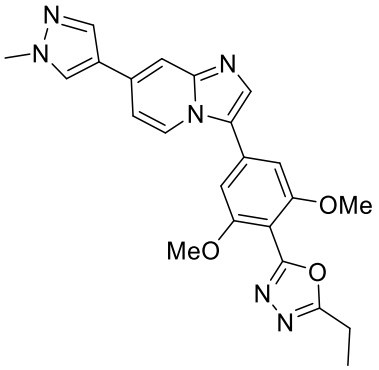
A mixture of methyl 2,6-dimethoxy-4-[7-(1-methylpyrazol-4-yl)imidazo[1,2-a]pyridine-3-yl]benzoate (380 mg, 968.4 μmol, 1 *eq*), NaOH (2 M, 2.9 mL, 6 *eq*) in MeOH (4 mL) was stirred at 70°C for 12 h. The reaction mixture was concentrated under reduced pressure to remove MeOH, then HCl (3 N) was added to the solution to pH = 5. The mixture was filtered, and the filter cake was concentrated under reduced pressure to afford the title compound (360 mg, crude) as a yellow solid.

Step 3. 2,6-dimethoxy-4-[7-(1-methylpyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl]-N'-propanoyl-benzohydrazide



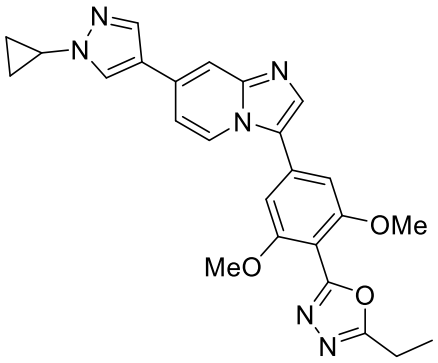
To a solution of 2,6-dimethoxy-4-[7-(1-methylpyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl]benzoic acid (200 mg, 528.6 μmol, 1 eq), propanehydrazide (186.3 mg, 2.1 mmol, 4 eq), and DIEA (204.9 mg, 1.6 mmol, 276.2 μL, 3 eq) in DMF (5 mL), was added HATU (301.5 mg, 792.8 μmol, 1.5 eq) in one portion at 20°C, and the resulting mixture was stirred at 40°C for 12 h. The reaction mixture was added into saturated NH_4Cl (aq. 50 mL), then extracted with EtOAc (50 mL, 3x). The combined organic phase was washed with brine (50 mL), then dried over Na_2SO_4, filtered, and filtrate was concentrated under reduced pressure to afford the title compound (180 mg, crude)as a yellow oil.

Step 4. 2-[2,6-dimethoxy-4-[7-(1-methylpyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl]phenyl]-5-ethyl-1,3,4-oxadiazole

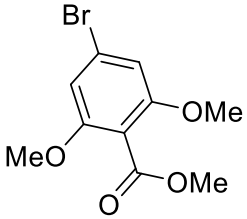


A mixture of 2,6-dimethoxy-4-[7-(1-methylpyrazol-4-yl)imidazo[1,2-a]pyridine-3-yl]-N'-propanoylbenzohydrazide (100 mg, 222.9 μmol, 1 eq) and 1-methoxy-N-triethylammoniosulfonylmethanimidate (79.7 mg, 334.5 μmol, 1.5 eq) in THF (4 mL) was stirred at 70°C for 2 h. The mixture was concentrated to give a crude product, which was purified by prep-HPLC (basic condition column: Waters X bridge Prep OBD C18 150*40mm*10um;mobile phase: [water(0.05%NH_3H_2O+10mM NH_4HCO_3)-ACN]; B%: 25%-38%,8min) to afford the title compound (10.6 mg, 24.6 μmol, 11.0% yield, 100% purity) as a white solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ: 1.42 (t, J =7.57 Hz, 3 H) 2.98 (d, J =7.51 Hz, 2 H) 3.90 (s, 6 H) 3.97 (s, 3 H) 6.92 - 7.09 (m, 2 H) 7.23 - 7.36 (m, 1 H) 7.70 - 7.94 (m, 1 H) 7.97 - 8.10 (m, 1 H) 8.19 (br s, 1 H) 8.52 - 9.06 (m, 1 H).

Compound 17. 2-[4-[7-(1-Cyclopropylpyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl]-2,6-dimethoxy-phenyl]-5-ethyl-1,3,4-oxadiazole



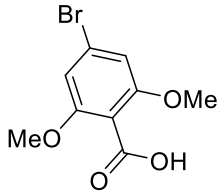
Step 1. Methyl 4-bromo-2,6-dimethoxybenzoate



To a solution of methyl 4-bromo-2,6-difluorobenzoate (2 g, 7.9 mmol, 1 eq) in MeOH (20 mL) was added sodium methanolate (3.2 g, 17.5 mmol, 30% purity, 2.2 eq), and the resulting mixture was stirred at 80°C for 12 h. The reaction mixture was concentrated under reduced pressure to remove MeOH, then the reaction mixture was added to water (20 mL) and extracted with EtOAc (10 mL, 3x). The combined organic layers were washed with brine (20 mL), dried

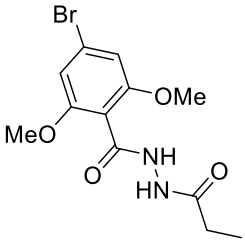
over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the title compound (1.5 g, crude) as a white solid.

Step 2. 4-Bromo-2,6-dimethoxybenzoic acid



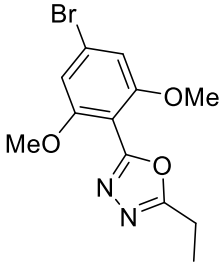
A mixture of methyl 4-bromo-2,6-dimethoxybenzoate (200 mg, 727.0 μmol, 1 eq), NaOH (58.2 mg, 1.5 mmol, 2 eq) in MeOH (20 mL), and H_2O (5 mL) was stirred at 70°C for 12 h. The reaction mixture was concentrated under reduced pressure to remove MeOH, then HCl (12 N, 0.5 mL) was added to the solution. The mixture was filtered and the filter cake was concentrated under reduced pressure to afford the title compound (100 mg, crude) as a white solid.

Step 3. 4-Bromo-2,6-dimethoxy-N'-propanoylbenzohydrazide



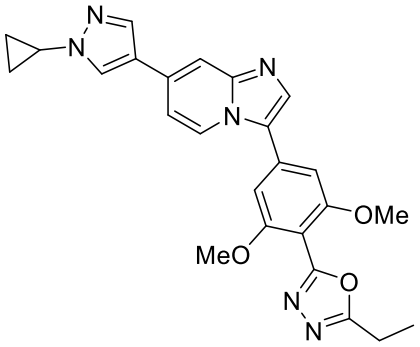
To a solution of 4-bromo-2,6-dimethoxybenzoic acid (1 g, 3.8 mmol, 1 eq) and propanehydrazide (371.2 mg, 4.2 mmol, 1.1 eq), and 4-bromo-2,6-dimethoxy-benzoic acid (1 g, 3.8 mmol, 1 eq) in DMF (20 mL), was added HATU (2.2 g, 5.8 mmol, 1.5 eq) and DIEA (990.1 mg, 7.7 mmol, 1.3 mL, 2 eq), and the resulting mixture was stirred at 40°C for 12 h. The reaction mixture was added to water (20 mL) and extracted with EtOAc (10 mL, 3x). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the title compound (280 mg, crude) as a white solid.

Step 4. 2-(4-Bromo-2,6-difluorophenyl)-5-ethyl-1,3,4-oxadiazole



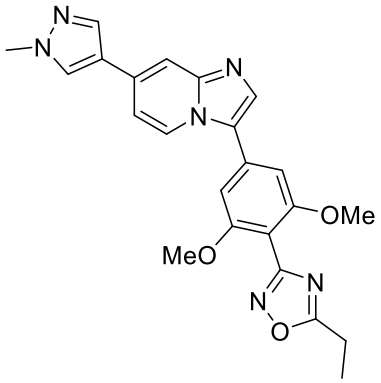
To a solution of 4-bromo-2,6-difluoro-N'-propanoyl-benzohydrazide (300 mg, 976.9 μmol, 1 eq) in THF (3 mL) was added methoxycarbonyl (triethylammonio)sulfonylazanide (Burgess reagent, 698.4 mg, 2.9 mmol, 3 eq), and the resulting mixture was stirred at 70°C for 12 h. The mixture was partitioned between water (5 mL) and ethyl acetate (5 mL, 3x). The organic phase was separated, washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the title compound (200 mg, crude) as a yellow oil.

Step 5. 2-[4-[7-(1-Cyclopropylpyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl]-2,6-dimethoxyphenyl]-5-ethyl-1,3,4-oxadiazole

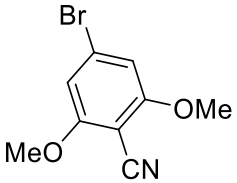


A mixture of 2-(4-bromo-2,6-dimethoxyphenyl)-5-ethyl-1,3,4-oxadiazole (70 mg, 223.5 μmol, 1 eq), 7-(1-cyclopropylpyrazol-4-yl)imidazo[1,2-a]pyridine (50.1 mg, 223.5 μmol, 1 eq), Cs_2CO_3 (218.5 mg, 670.6 μmol, 3 eq), and $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (18.3 mg, 22.4 μmol, 0.1 eq) in DMA (1 mL) was degassed and purged with N_2 (3x), and then the mixture was stirred at 100°C for 12 h under N_2 atmosphere. The resulting residue was purified by prep-HPLC (basic condition; column: Phenomenex Gemini-NX C18 75*30mm*3um;mobile phase: [water(0.05%NH_3H_2O+10mM NH_4HCO_3)-ACN];B%: 20%-50%,8min) to afford the title compound (16.2 mg, 35.5 μmol, 15.9% yield, 100% purity) as a pink solid. ^{1}H NMR (400 MHz, METHANOL-d_4) δ: 8.65 (br d, J = 7.0 Hz, 1H), 8.29 (s, 1H), 8.01 (s, 1H), 7.82 (br d, J = 16.8 Hz, 2H), 7.32 (br d, J = 7.3 Hz, 1H), 7.05 (s, 2H), 3.92 (s, 6H), 3.81 - 3.70 (m, 1H), 3.00 (q, J = 7.6 Hz, 2H), 1.44 (t, J = 7.6 Hz, 3H), 1.23 - 1.07 (m, 4H).

Compound 19. 3-[2,6-Dimethoxy-4-[7-(1-methylpyrazol-4-yl)imidazo[1, 2-a]pyridin-3-yl]phenyl]-5-ethyl-1,2,4-oxadiazole

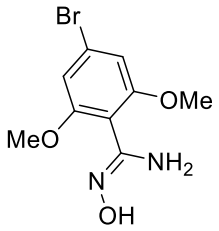


Step 1. 4-Bromo-2,6-dimethoxybenzonitrile



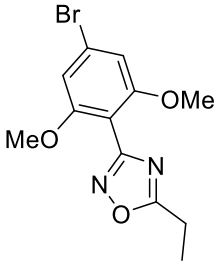
A mixture of 4-bromo-2,6-difluorobenzonitrile (1 g, 4.6 mmol, 1 eq) and NaOMe (1 g, 18.5 mmol, 4.0 eq) in MeOH (10 mL) was stirred at 25°C for 2 h. The reaction mixture was filtered and concentrated under reduced pressure to afford the title compound (1 g, crude) as a white solid.

Step 2. 4-Bromo-N'-hydroxy-2,6-dimethoxybenzamidine



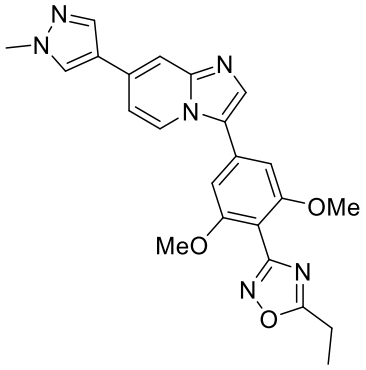
A mixture of 4-bromo-2,6-dimethoxybenzonitrile (0.8 g, 3.3 mmol, 1 eq), $NH_2OH{\cdot}HCl$ (298.6 mg, 4.3 mmol, 1.3 eq), and $NaHCO_3$ (416.4 mg, 4.9 mmol, 192.8 µL, 1.5 eq) in EtOH (3 mL), was stirred at 80°C for 12 h. The mixture was filtered and the filtrate was concentrated to afford the title compound (1 g, crude) as a white solid.

Step 3. 3-(4-Bromo-2,6-dimethoxyphenyl)-5-ethyl-1,2,4-oxadiazole



A mixture of 4-bromo-N'-hydroxy-2,6-dimethoxybenzamidine (900 mg, 3.3 mmol, 1 eq), ethyl propanoate (501.2 mg, 4.9 mmol, 563.1 µL, 1.5 eq), and NaOH (196.3 mg, 4.9 mmol, 1.5 eq) in DMSO (15 mL) was stirred at 30°C for 2 h. The mixture was filtered and the filter cake was dried to afford the title compound (900 mg, crude) as a white solid.

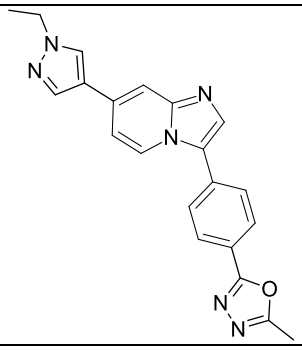
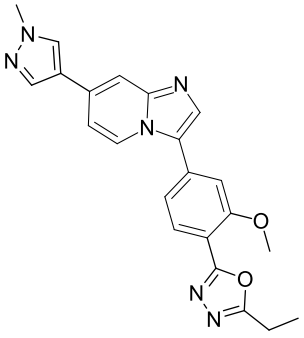
Step 4. 3-[2,6-Dimethoxy-4-[7-(1-methylpyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl]phenyl]-5-ethyl-1,2,4-oxadiazole



To a solution of 7-(1-methylpyrazol-4-yl)imidazo[1,2-a]pyridine (100 mg, 504.5 µmol, 1 eq), 3-(4-bromo-2,6-dimethoxyphenyl)-5-ethyl-1,2,4-oxadiazole (165.9 mg, 529.7 µmol, 1.0 eq) and $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (20.6 mg, 25.2 µmol, 0.05 eq) in DMA (5 mL) was added Cs_2CO_3 (493.1 mg, 1.5 mmol, 3 eq), in one portion at 20°C under N_2, and the resulting mixture was stirred at 100 °C for 12 h. The reaction mixture was added into saturated NH_4Cl (aq., 50 mL) then extracted with EtOAc (50 mL, 3 mL). The combined organic phase was washed with brine (50 mL), then dried over Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give a residue, which was purified by prep-HPLC (basic condition, column: Phenomenex Gemini-NX C18 75*30mm*3um;mobile phase: [water (0.04%NH_3H_2O+10mM NH_4HCO_3)-ACN];B%: 15%-40%,10min) to afford the title compound (31.7 mg, 73.4 µmol, 14.6% yield, 99.6% purity) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.33 (t, J =7.61 Hz, 3 H) 3.02

(q, J =7.50 Hz, 2 H) 3.82 (s, 6 H) 3.89 (s, 3 H) 7.04 (s, 2 H) 7.25 (dd, J =7.17, 1.21 Hz, 1 H) 7.88 (s, 2 H) 8.09 (s, 1 H) 8.36 (s, 1 H) 8.73 (d, J =7.28 Hz, 1 H).

Compounds 15 and 20 were prepared according to the procedures described for Compounds 16, 17 and 19 using appropriately substituted starting materials. NMR data for Compounds 15 and 20 is shown below.

Compound	Compound Name	Structure	NMR
15	2-(4-(7-(1-ethyl-1H-pyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl)phenyl)-5-methyl-1,3,4-oxadiazole		^{1}H NMR (400MHz, DMSO-d_6) δ: 8.75 (d, *J* =7.3 Hz, 1H), 8.55 (s, 1H), 8.23 - 8.08 (m, 4H), 8.03 - 7.89 (m, 3H), 7.50 - 7.42 (m, 1H), 4.21 (q, *J* =7.3 Hz, 2H), 2.64 (s, 3H), 1.45 (t, *J* =7.3 Hz, 3H)
20	2-ethyl-5-(2-methoxy-4-(7-(1-methyl-1H-pyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl)phenyl)-1,3,4-oxadiazole		^{1}H NMR (400 MHz, MeOD-d_4) δ: 8.64 (d, *J* = 7.3 Hz, 1H), 8.19 (s, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 8.01 (s, 1H), 7.85 (s, 1H), 7.79 (s, 1H), 7.50 - 7.40 (m, 2H), 7.31 (br d, *J* = 6.9 Hz, 1H), 4.07 (s, 3H), 3.98 (s, 3H), 3.03 (q, *J* = 7.6 Hz, 2H), 1.47 (t, *J* = 7.6 Hz, 3H)

Pharmacology studies
Full length recombinant SIK proteins were used for radioisotope kinase assays with AMARAASAAALARRR (20 μM) peptide substrate. Assays were done using 1 μM ATP at Reaction Biology (Malvern, PA). The Km of SIK isoforms for ATP ranges from 0.5-1 μM ATP. 10-point dose responses (maximum dose 10 μM, 4-fold serial dilutions) were performed to calculate IC_{50} values. NanoBRET assays for SIK1, SIK2, and SIK3 were performed at Reaction Biology (Malvern, PA) in HEK293 cells transiently expressing Nano-Luc SIK fusion vectors in 384 well plates. Cells were pre-treated with the K-4 traced and then treated for 1 hour with compounds of interest in 10-point dose response. Dasatinib was used as a positive control. BRET signal was measured on an Envision 2104 Multilabel reader. The IC50 value was calculated using a sigmoidal dose response equation. Kinome profiling was performed using a panel of 300 recombinant human kinases in a microfluidic mobility shift platform at Nanosyn (Santa Clara, CA) testing compounds in duplicate at a dose of 0.5 μM. Assays for kinetic solubility, microsome stability, CYP inhibition, plasma protein binding, Caco2 cell permeability, and mouse pharmacokinetics were performed at WuXi (Shanghai, China). SafetyScreen44 profiling was performed for SK-124 (10 μM) at EuroFins (Cerep, France).

Cell culture and cell viability assay
Singe-cell subcloned Ocy454 cells [9] were used for all experiments. Saos2 cells were purchased from ATCC (HTB-85). Ocy454 cells were passaged in alpha-MEM supplemented with 10% Fetal Bovine Serum Qualified One Shot™ (Gibco) and 1% Anti-Anti (antibiotic-antimycotic, Gibco) at 33°C with 5% CO_2. Cells were seeded at 50,000 cells/mL and allowed to reach confluency at 33°C in 2-3 days. Then, cells were transferred from 33°C to 37°C to inactivate the temperature-sensitive T antigen for the acceleration of osteocytic differentiation. For protein and gene expression analyses, cells were analyzed after culture at 37°C for 14-21 days. Cells were routinely assessed by *Sost* and *Dmp1* expression at 37°C and examined for osteocytic morphology. Saos2 cells were passaged in McCoy's 5A medium supplemented as above. Cell viability assays in 96 well plates were performed using PrestoBlue (Invitrogen) following the manufacturer's instructions.

Immunoblotting
Immunoblotting was performed as previously described [10, 11]. Ocy454 and Saos2 were prepared using TNT (Tris-NaCl-Tween buffer, 20 mM Tris-HCl pH 8, 200 mM NaCl, 0.5% Triton X-100 containing PI (protease inhibitor cocktail, bimake.com B14001), 1 mM NaF, 1 mM vanadate and1 mM DTT). The cells were washed with ice-cold PBS, then scraped into TNT buffer on ice. Then the cell materials were transferred into 1.5 ml tubes kept on ice, vortexed at top speed for 30 seconds, then centrifuged at 14000g for 6 minutes at 4ºC. For immunoblotting, lysates were separated by SDS-PAGE, and proteins were transferred to nitrocellulose membranes. Membranes were blocked with 5% milk in TBST (tris-buffered saline plus 0.05% Tween-20) and incubated with the primary antibody in 5% BSA (bovine serum albumin)/TBST overnight at 4C. The next day, membranes were washed and incubated with appropriate HRP-coupled secondary antibodies. Moreover, signals were detected with ECL Western Blotting Substrate (Pierce), ECL Plus Western Blotting Substrate (Pierce), or SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher scientific). The primary antibodies were phospho-HDAC4/5/7 (S246/S259/S155) (Cell Signaling Technology, 3443), total HDAC5 (Assay Biotech, C0225), CRTC2 pS275 (Henriksson et al. 2015), total CRTC2 (TORC2, EMD Millipore Corp. ST1099), β-actin (Cell Signaling Technology, 4970), SIK2 (Cell Signaling Technology, 6919), and SP-1 (EMD Millipore Corp. 07-645), and β-tubulin (Cell Signaling Technology, 2128).

Subcellular fractionation

Ocy454 cells were resuspended in hypotonic lysis buffer (20 mM HEPES, 10 mM KCl, 1 mM MgCl2, 0.1% Triton X-100, 5% glycerol supplemented with PI (bimake.com B14001), 1 mM NaF, 1 mM vanadate and1 mM DTT) for 5 minutes on ice. Suspended buffers were spun down at 2,300g for 5 minutes, and the supernatant was saved as the cytoplasmic lysate. Thereafter, the nuclear pellets were washed twice in 1 mL hypotonic lysis buffer. The pellets were resuspended in hypertonic lysis buffer (20 mM HEPES, 400 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 5% glycerol supplemented with protease inhibitor, 1 mM NaF, 1 mM DTT and 1 mM vanadate), followed by vortexing twice for 30 seconds. Debris was spun down at 16,100g for 5 minutes, and the supernatants were saved as the nuclear lysate.

Cellular Thermal Shift Assay (CETSA)

CETSA assays were modified from the protocol described in [12]. Ocy454 cells were grown in 15 cm dishes at 37°C for 7 days. Ocy454 cells at a density of $4.8x10^7$ to $1x10^8$ were treated with vehicle (DMSO) or SK-124 for 1 hour and harvested with cold PBS. Then, the cell/PBS mixture was spun down at 300g for 3 minutes and washed with cold PBS twice. After washing, $3x10^6$ cells were resuspended in 1 mL PBS supplemented with protease inhibitors. Next, the cell/PBS mixture was transferred into 0.2 mL PCR tubes in 100 µL aliquots. The tubes were heated at 40°C - 67°C for 3 minutes, followed by three successive freeze-thaw cycles by snap freezing in liquid nitrogen and then thawing by heating at 25 °C in a PCR machine. Finally, materials were centrifuged at 16,100g for 20 minutes at 4°C and the supernatant was used for SIK2 and ß-actin immunoblotting.

Gene expression analysis

Total RNA was collected from cultured cells using QIAshredder (QIAGEN) and PureLink RNA mini kit (Thermo Fisher scientific) following the manufacturer's instructions. Lysis buffer with β-mercaptoethanol was added to cold PBS washed cells and collected into QIAshredder, then centrifuged at 15,000g for 3 minutes. The flow-through was collected into a new tube and RNA isolation was carried out with PureLink RNA mini kit. For qRT-PCR, cDNA was prepared with 750ng RNA using the Primescript RT kit (Takara Inc.) and analyzed with PerfeCTa® SYBR® Green FastMix® ROX (Quanta bio) in the StepOnePlusTM Real-time PCR System (Applied Biosystems) using specific primers designed for each targeted gene. Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method by normalizing to β-actin housekeeping gene expression, and presented as fold increase relative to β-actin. Primers used were β -actin (CCTCTATGCCAACACAGTGC and ACATCTGCTGGAAGGTGGAC), SOST (GCCTCATCTGCCTACTTGTG and CTGTGGCATCATTCCTGAAG), RANKL (GCTGGGCCAAGATCTCTAAC and GTAGGTACGCTTCCCGATGT), SPP1 (AGAATCTCCTTGCGCCACAG and TGTGGTCATGGCTTTCATTGGA), CTSK (CCGAATAAATCTAGCACCCTTAGT and GAAACTTGAACACCCACATCC), ACP5 (CCATTGTTAGCCACATACGG and ACTCAGCACATAGCCCACAC), and FGF23 (CCCCCATCAGACCATCTACA and CGTGGAGATCCATACAAAGGA).

Bone turnover markers and other serum parameters

The general hematology and blood chemistry analysis was performed by MGH Clinical Pathology Laboratory using HemaTrue and Dri-Chem 7000 (Heska, Loveland, CO). Bone turnover markers such as P1NP (N-terminal propeptide of type I procollagen) and CTX (C-Terminal Telopeptide of Type I Collagen) were measured by Rat/Mouse PINP EIA Immunoassay (Immunodiagnostic systems, MD) and RatLaps (CTX-I) EIA (Immunodiagnostic systems, MD), respectively. For mineral metabolism-related parameters were measured by Calcium (Arsenazo) LiquiColor® test (Stanbio Laboratory, Boerne TX), Phosphorus Liqui-UV® test (Stanbio Laboratory, Boerne TX), mouse PTH (1-84) ELISA (Quidel, San Diego, CA), 1,25-Dihydroxy Vitamin D EIA Immunoassay (Immunodiagnostic systems, MD), and Mouse/Rat FGF-

23 (Intact) ELISA (Quidel, San Diego, CA) according to the manufacturers' instructions. Mouse TNFa ELISA was from BD (catalog 560478), and Mouse IL-10 ELISA was from Invitrogen (catalog BMS614).

Immunohistochemistry, safranin-O, and TRAP (tartrate-resistant acid phosphatase) staining
Formalin-fixed paraffin-embedded decalcified tibia sections were obtained from SK-124 (2.5 mg/kg, 10 mg/kg, 60 mg/kg, PO) orally-treated mice and PTH (1-34) subcutaneously injected mice with their control groups by the oral gavage treatment. For anti-sclerostin immunohistochemistry, antigen retrieval was performed using proteinase K (20 µg/ml) for 15 minutes at room temperature. Endogenous peroxidases were quenched and slides were blocked in TNB buffer (Perkin-Elmer), then stained with anti-sclerostin biotinylated antibody at a concentration of 1:50 overnight at 4°C. Sections were washed with TBST 3 times, and incubated with HRP-coupled secondary antibodies, signals amplified using tyramide sinal amplification and HRP detection was performed using 3,3'-diaminobenzidine (DAB, Vector) for 5~10 minutes. Slides were counterstained with 0.02% fast green staining solution or hematoxylin. Quantification of sclerostin positive osteocytes was performed Image J imaging software on the blind-test manner. Here, hematoxylin counterstaining was used to identify periosteal cells, defined as flat cells present on periosteal surfaces. The primary antibody was goat anti-mSOST biotinylated (1:50, R&D systems, BAF1589). For safranin-O staining, mouse tibia paraffin section was performed with 0.02% fast green solution and 0.1% safranin-O. For TRAP staining, the mouse tibia paraffin section was stained with 0.2 M Acetate buffer (0.2 M sodium acetate and 0.05 M sodium tartrate, pH 5.0) and staining solution (naphthol AS-MX phosphate, and fast red TR salt, Sigma-Aldrich, MO) at 37 ºC for 30 minutes. Fast green counterstaining was performed for the TRAP staining.

Histomorphometry of dynamic and static bone parameters
Static and dynamic histomorphometric analyses were performed according to criteria established by the American Society of Bone and Mineral Research [13]. In addition to traditional parameters, osteoclasts were evaluated in TRAP-stained sections in the primary and secondary spongiosa (the 800 micron region subjacent to the growth plate). Calcein and demeclocycline were administered at 2 and 7 days prior to sacrifice. Femurs were fixed, dehydrated, embedded in poly methyl methacrylate. Sections were stained with Goldner's Trichrome for evaluation of static histomorphometric parameters and evaluated with TRAP staining. Measurements were performed in the distal femoral metaphysis using an Osteomeasure image analyzer. Mineral apposition rate was determined by dividing the distance between the calcein and demeclocycline labels by the time interval between the two labeling solution injections and expressed in µm/day.

Femur micro-CT
Micro-CT imaging was performed according to criteria established by the American Society of Bone and Mineral Research [14]. Micro-CT imaging was performed on a bench-top scanner (µCT 40, Scanco Medical AG, Brutisellen, Switzerland) to measure mineral densities and bone architecture of the femoral mid-diaphysis and the primary spongiosa region of the distal metaphysis (Scanning parameters: 10 µm isotropic voxel, 70 kVp, 114 mA, 200 ms integration time). Analyses were performed using the Scanco Evaluation Program. Cortical architecture and mineral density were measured in a 500 µm long region (50 transverse slices) at the mid-diaphysis. Cortical bone at the mid-diaphysis was segmented from surrounding tissue using a threshold of 700 mgHA/cm^3 and the architecture and mineral density of the cortex was analyzed using the Scanco Mid-shaft evaluation script. In the distal metaphysis, a primary spongiosa region was selected by manually contouring the metaphyseal portion of the bone in a region of interest beginning at the peak of the distal growth plate and extending proximally 500 µm (50

transverse slices). The bone in the primary spongiosa region of interest was segmented using a threshold of 375 mgHA/cm^3 and then analyzed for bone volume fraction (BV/TV,%), bone mineral density (BMD, mgHA/cm^3), and tissue mineral density (TMD, mgHA/cm^3). Immediately superior to the primary spongiosa region of interest, a secondary spongiosa region of interest was selected by manually contouring the endocortical region of the bone beginning at the top of the primary spongiosa region and extending proximally 1.5 mm (150 transverse slices). The trabecular bone in the secondary spongiosa region of interest was segmented using a threshold of 375 mgHA/cm^3 and analyzed for trabecular bone volume fraction (Tb.BV/TV, %), number (Tb.N, 1/mm), thickness (Tb.Th, mm), separation (Tb.Sp, mm), connectivity density (Conn.D, 1/mm^3), and mineral density (Tb.BMD, mgHA/cm^3).

Data collection and analysis
Data were collected using Windows 10, Microsoft Excel for Office 365, StepOne Software v2.3, Epson scan 3.9.4.7US, and Azure biosystems cSeries capture software 2.1.4.0731. The data were analyzed by Windows 10, Microsoft Excel for Office 365, Microsoft Word for Office 365, GraphPad Prism 9.2, StepOne Software v2.3, NIH ImageJ 1.52a.

Statistical analyses
All statistical analyses were performed by GraphPad Prism 9.2 for Windows (GraphPad Software Inc, US). Variables were tested by either student's two-tailed t-test (for comparison of 2 groups) or one-way ANOVA followed by posthoc Tukey test (for comparison of 3 or more groups). Values were expressed as mean ± standard deviation unless otherwise stated. A p value less than 0.05 was considered significant.

Supplementary Figure 1. Tables showing serum chemistries (top) and blood counts (bottom) from control and SIK2/SIK3 inducible double knockout (DKO) mice at the indicated times post tamoxifen treatment. Student's t-tests were performed to compare control and DKO mice at each time point, analytes with p values less than 0.05 are shown in bold/yellow.

Supplemental Figure 2. Structures of inhibitors 17, 18, 19, and 20 with corresponding SIK IC_{50} values.

Supplemental Figure 3. Single dose mouse pharmacokinetic data for SK-124 upon oral (PO) and intravenous (IV) administration. N=3 CD-1 male mice were used.

Supplemental Figure 4. (a) Ocy454 (top) and Saos2 (bottom) cells were treated with the indicated compounds (20 μM) for the indicated times. Active compounds reduce phosphorylation levels of the SIK substrates HDAC5 and CRTC in a time-dependent manner. (b) Ocy454 cells were treated for 60 minutes with the indicated compounds followed by cytosol/nuclear fractionation and then immunoblotting. (c) Ocy454 cells were treated with SK-124 (**16**, 10 μM) for the indicated times followed by RT-qPCR. SK-124 reduces *SOST* and increases *RANKL* mRNA in a time-dependent manner. (d) Ocy454 cells were treated with the indicated compounds/doses for 18 hours followed by resavurin-based (PrestoBlue) viability assays. Significant cytotoxicity was only observed in response to YKL-05-099.

Supplemental Figure 5. (a) Mice were treated with the indicated agents and weighed twice per week. No differences in mouse weight were noted across the 5 treatment groups. (b) BUN levels were measured two hours after the final treatment dose and were unchanged across groups. (c) Cortical bone RNA was isolated followed by RT-qPCR for the indicated genes. One-way ANOVA was performed followed by Tukey's posthoc analysis to compare treatment groups to vehicle. P values less than 0.05 are shown on the graphs.

Supplemental Figure 6. Bone marrow macrophages were seeded in 96 well plates, and osteoclast differentiation was induced with RANKL (50 ng/mL) and M-CSF (30 ng/mL) plus the indicated compounds. Medium was changed every three days, and TRAP staining was performed after 9 days of differentiation. Top images are shown from cells treated continuously with compounds at the 78.1 nM dose. Bottom, quantification of secreted TRAP levels comparing YKL-05-099 (black) and SK-124 (blue).

Supplemental Figure 7. Male CD1 mice were treated with vehicle (15% hydroxypropyl-beta-cyclodextrin in sterile water) or SK-124 (**16**, 40 mg/kg) by IP injection. Thirty minute later, all mice were treated with lipopolysaccharide (0.5 mg/kg) by IP injections. Serum was collected 2 hours following LPS injection (2.5 hours following vehicle or SK-124 injection) for cytokine ELISAs as indicated. Student's t-tests were used to compare cytokine levels in the two treatment groups.

Dataset 1: MicroCT results from SIK2/SIK3 DKO study
Dataset 2: DMPK features of compounds **10** and **16**
Dataset 3: SafetyScreen44 results
Dataset 4: Nanosyn kinome profiling (0.5 μM) results for compounds **16**, YKL-05-099, **17**, **18**, **20**, **13**, and **14**
Dataset 5: MicroCT results from SK-124 treatment study
Dataset 6: Static and dynamic histomorphometry data

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Supplemental Figure 1

Chemistries

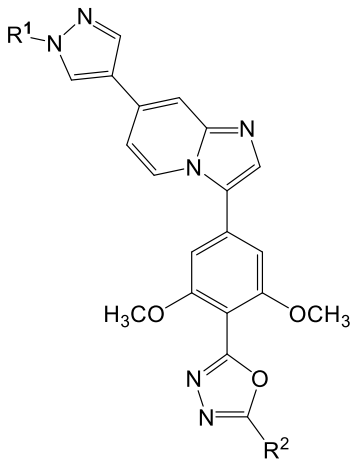
	2 weeks post tamoxifen			4 weeks post tamoxifen			8 weeks post tamoxifen			16 weeks post tamoxifen		
	Control	DKO	p value	Control	DKO	p value	Control	DKO	p value	Control	DKO	p value
Albumin (2-4 g/dl)	2.525 ± 0.22	2.716 ± 0.12	0.1083	3.167 ± 0.42	2.80 ± 0	0.32250251	2.73 ± 0.3	3.03 ± 0.31	0.20335	2.65 ± 0.07	n/d	n/d
ALP (0-260 U/l)	**136 ± 19**	**257.83 ± 74.9**	**0.0031**	**167.5 ± 37**	**448.3 ± 211**	**0.042526**	**127.5 ± 42.6**	**1274 ± 381**	**0.00004**	**70.67 ± 13.5**	**2641.6 ± 928**	**7.3992E-05**
ALT (GPT) (10-190 U/l)	27 ± 6.4	24.5 ± 4.04	0.4513	26.25 ± 4.6	22 ± 3.5	0.23915149	23.33 ± 2.73	33.33 ± 29.3	0.40080	50.25 ± 14.9	n/d	n/d
AST (GOT) (10-380 U/l)	52.25 ± 6.40	86.83 ± 50.3	0.2165	78 ± 31.1	64 ± 8.5	0.60178391	77.33 ± 27.7	109.33 ± 79.7	0.38265	62 ± 1.4	n/d	n/d
BUN (20-36 mg/dl)	27.78 ± 3.73	29.35 ± 2.25	0.3994	19.93 ± 4.8	25.84 ± 3.7	0.13614072	32.53 ± 5.7	26.24 ± 6.67	0.12444	36.83 ± 12.6	29.58 ± 2.1	0.19592283
Calcium (7.9-11.0 mg/dl)	10.48 ± 0.5	10.883 ± 0.66	0.2540	9.43 ± 0.63	9.67 ± 0.61	0.63428529	10.15 ± 0.5	10.66 ± 0.72	0.19687	10.65 ± 0.76	11.1 ± 0.5	0.26489902
Chloride (108-118 mEq/l)	114.16 ± 2.8	110.5 ± 3.62	0.0776	109.8 ± 2.1	97.7 ± 14.5	0.14926034	**113.33 ± 1.51**	**106.6 ± 4.5**	**0.00707**	113.17 ± 1.6	110 ± 5.7	0.20575048
Cholesterol (28-110 mg/dl)	75.83 ± 7.9	82.83 ± 5.98	0.1145	72 ± 14	72.5 ± 10.6	0.96729197	89.16 ± 18.3	116 ± 22.8	0.07261	119.8 ± 36.4	205 ± 33.9	**0.03639904**
CK	1024 ± 1072	2205 ± 2219	0.2677	464 ± 206	413 ± 139	0.78380923	597.83 ± 163.8	815 ± 371	0.23382	212 ± 204	232.5 ± 122	0.86518304
Glucose (120-220 mg/dl)	155 ± 23	176.83 ± 35.69	0.2371	118 ± 23.8	151 ± 41.0	0.23302426	135 ± 14.4	128.4 ± 34.7	0.67908	192.83 ± 52.7	146.83 ± 26.6	0.08545831
Magnesium (1.7-3 mg/dl)	2.475 ± 0.38	2.883 ± 0.48	0.1919	2.1 ± 0	2.75 ± 0.1	n/a	2.82 ± 0.25	2.95 ± 0.64	0.65252	2.3 ± 0	n/d	n/d
Phosphorus (5.6-12.2 mg/dl)	11.033 ± 1.7	11.26 ± 1.7	0.8193	8.75 ± 1.8	9.97 ± 1.5	0.38787575	8.95 ± 1.89	9.38 ± 1.77	0.70820	9.24 ± 2.0	10.85 ± 1.46	0.22455163
Potassium (5.3-6.3 mEq/l)	8.35 ± 1.51	8 ± 1.53	0.6983	8.03 ± 1.0	7.65 ± 0.1	0.64506286	7.1 ± 1.13	6.5 ± 0.5	0.30412	7.13 ± 0.98	5.8 ± 0	#DIV/0!
Sodium (138-186 mEq/l)	151.83 ± 2.32	149.5 ± 2.17	0.1018	147.3 ± 2.6	146 ± 0	0.56068859	152.67 ± 1.03	151.8 ± 3.1	0.53420	149.17 ± 1.7	150 ± 0	#DIV/0!
Total Bilirubin (0.2-0.8 mg/dl)	0.125 ± 0.05	0.35 ± 0.21	0.0700	1.5 ± 0	1.15 ± 0.2	n/a	0.52 ± 0.23	0.65 ± 0.7	0.47323	0.3 ± 0	n/d	n/d
Triglycerides (30-149 mg/dl)	104 ± 19.96	102.33 ± 19.76	0.8928	109.3 ± 19.5	103.3 ± 23.5	0.72999308	81.83 ± 20.5	75 ± 8.7	0.55205	113.8 ± 36.5	n/d	n/d
	12 week old mice at time of tamoxifen treatment											
	n=5-6 mice/group											

Complete Blood Count

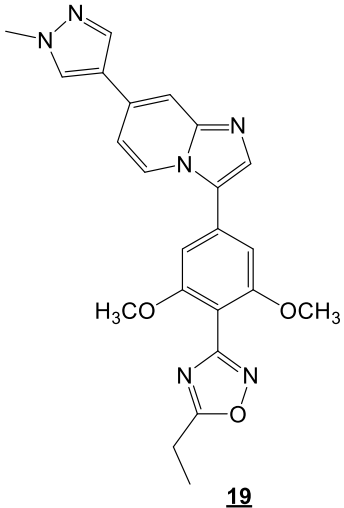
	2 weeks post tamoxifen				8 weeks post tamoxifen		
	Control	DKO	p value		Control	DKO	p value
WBC (10^3/µl, 2.6-12)	8.15 ± 2.55	8.66 ± 2.13	0.7529		9.8 ± 2.14	7.7 ± 3.44	0.2461
LYM (10^3/µl, 1.3-9)	5.85 ± 1.58	5.96 ± 1.15	0.9068		**7.92 ± 1.63**	**5.38 ± 1.90**	**0.0411**
MONO (10^3/ul, 0.1-0.5)	0.575 ± 0.22	0.6 ± 0.21	0.8681		0.53 ± 0.14	0.62 ± 0.54	0.7089
GRAN (10^3/µl, 0.4-2.5)	1.725 ± 0.81	2.1 ± 0.8	0.5103		1.35 ± 0.49	1.7 ± 1.8	0.6622
LYM %	72.475 ± 4.51	69.72 ± 4.81	0.4096		81.15 ± 3.09	74.6 ± 19.9	0.4434
MONO %	6.35 ± 0.83	6.28 ± 0.67	0.8927		4.83 ± 0.48	6.54 ± 3.86	0.3057
GRAN %	21.175 ± 4.5	24 ± 4.2	0.3630		14.02 ± 2.69	18.86 ± 16.25	0.4862
HCT (%, 32-58)	55.725 ± 1.60	54.66 ± 3.36	0.5816		56.75 ± 2.21	58.98 ± 5.69	0.3966
MCV (fl, 42-55)	55.15 ± 1.01	54.6 ± 1.13	0.4716		**55.43 ± 0.84**	**50.3 ± 3.37**	**0.0055**
RDWa (fl, 0-99)	39.25 ± 0.70	39.14 ± 1.38	0.8897		**39.38 ± 0.75**	**35.88 ± 2.86**	**0.0173**
RDW % (0-99)	16.375 ± 0.15	16.7 ± 0.48	0.2423		**16.35 ± 0.23**	**17.06 ± 0.59**	**0.0222**
HGB (g/dl, 10.1-16.1)	15.725 ± 0.78	15.58 ± 1.19	0.8401		**15.75 ± 0.63**	**16.8 ± 0.5**	**0.0158**
MCHC (g/dl, 29-35)	28.175 ± 0.61	28.5 ± 0.57	0.4378		27.83 ± 0.39	28.66 ± 2.18	0.3819
MCH (pg, 13-18.1)	15.525 ± 0.19	15.54 ± 0.47	0.9540		**15.42 ± 0.40**	**14.36 ± 0.59**	**0.0064**
RBC (10^6/µl, 6.5-10.1)	10.1075 ± 0.40	10.004 ± 0.52	0.7555		**10.24 ± 0.48**	**11.71 ± 0.66**	**0.0020**
PLT (10^3/µl, 300-1500)	**334.75 ± 32.76**	**440.8 ± 68.74**	**0.0261**		363.83 ± 35.1	322.4 ± 75.89	0.2603
MPV (fl, 0-99.9)	5.775 ± 0.31	6.02 ± 0.24	0.2203		5.7 ± 0.2	5.68 ± 0.29	0.8945
	n=5-6 mice/group						

Supplementary Figure 1. Tables showing serum chemistries (top) and blood counts (bottom) from control and SIK2/SIK3 inducible double knockout (DKO) mice at the indicated times post tamoxifen treatment. Student's t-tests were performed to compare control and DKO mice at each time point, analytes with p values less than 0.05 are shown in bold/yellow.

Supplemental Figure 2

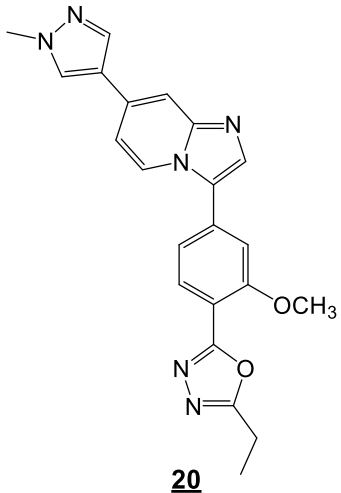


17, **18**



19

Inhibitor	R^1	R^2	SIK1 IC_{50} (nM)	SIK2 IC_{50} (nM)	SIK3 IC_{50} (nM)
17	Cyclopropyl	-Et	16	1.5	2.7
18	$-CH_3$	Cyclopropyl	4.5	1.5	1.6
19	-	-	38	3.1	8.3

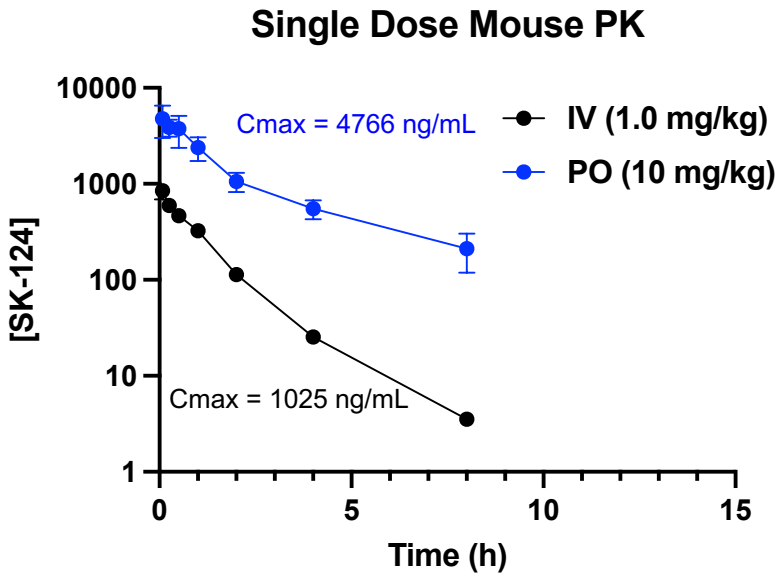


20

SIK1 IC_{50} = 91 nM
SIK2 IC_{50} = 8.8 nM
SIK3 IC_{50} = 11 nM

Supplemental Figure 2. Structures of inhibitors **17**, **18**, **19**, and **20** with corresponding SIK IC_{50} values.

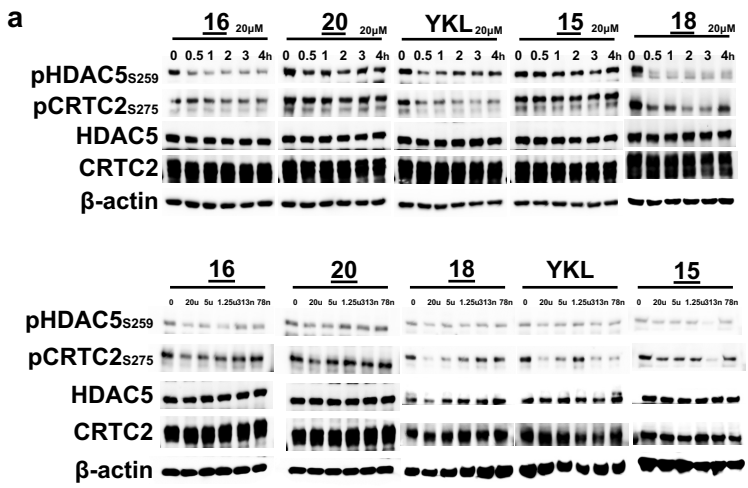
Supplemental Figure 3

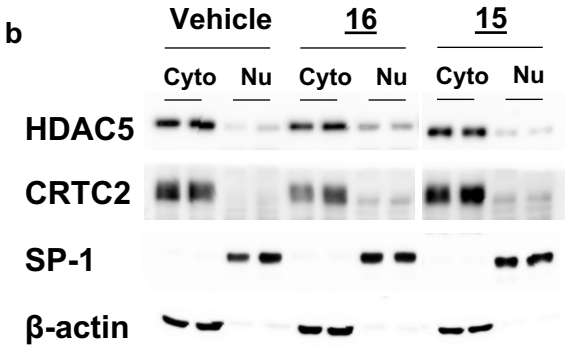


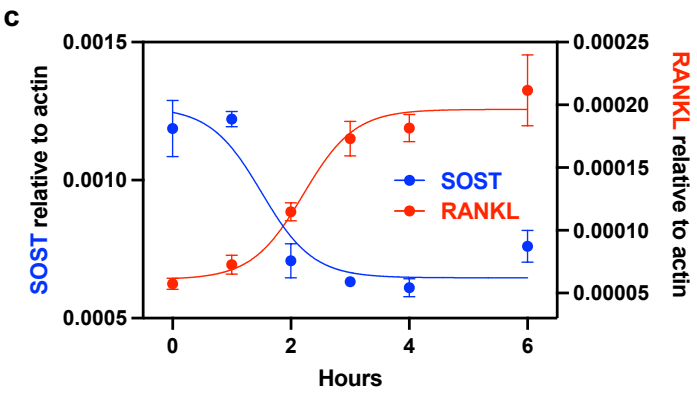
IV (1 mg/Kg)	
Half-life (hr)	1.1
Cl (mL/min/Kg)	18.8
Vdss (L/Kg)	1.4
AUC (ng.hr/mL)	892
PO (10 mg/Kg)	
Half-life (hr)	1.7
AUC (ng.h/mL)	12,700
F%	142

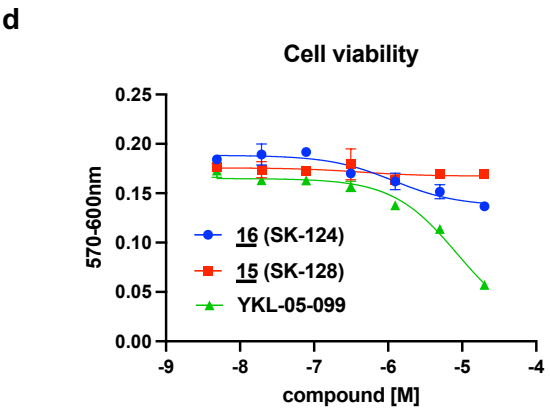
Supplemental Figure 3. Single dose mouse pharmacokinetic data for SK-124 upon oral (PO) and intravenous (IV) administration. N=3 CD-1 male mice were used.

Supplemental Figure 4



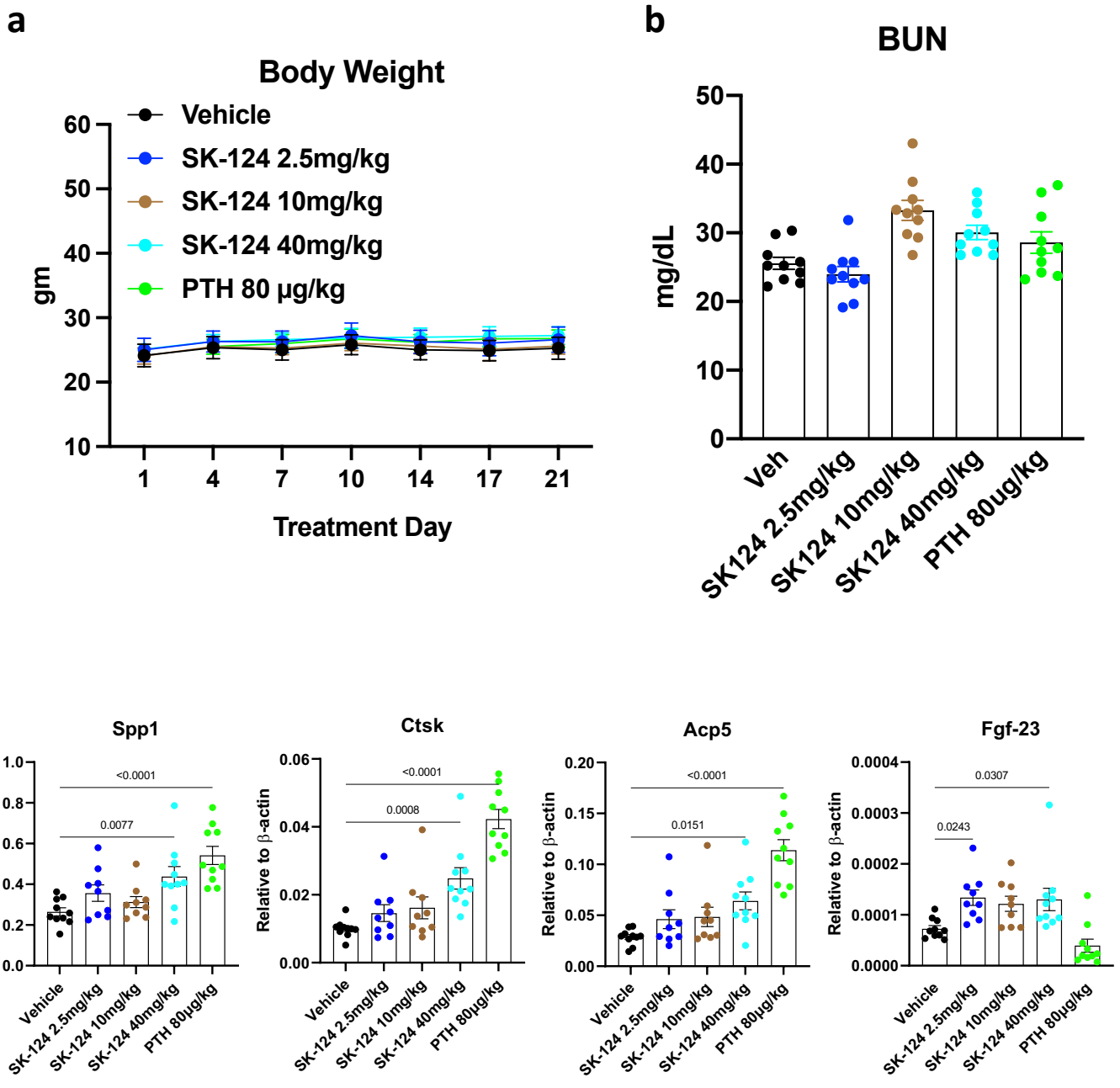






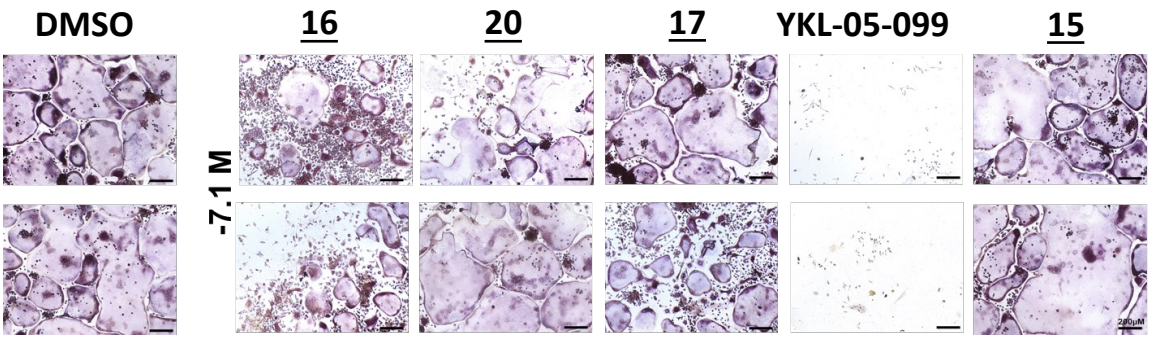
Supplemental Figure 4. (a) Ocy454 (top) and Saos2 (bottom) cells were treated with the indicated compounds (20 μM) for the indicated times. Active compounds reduce phosphorylation levels of the SIK substrates HDAC5 and CRTC in a time-dependent manner. (b) Ocy454 cells were treated for 60 minutes with the indicated compounds followed by cytosol/nuclear fractionation and then immunoblotting. (c) Ocy454 cells were treated with SK-124 (**16**, 10 μM) for the indicated times followed by RT-qPCR. SK-124 reduces *SOST* and increases *RANKL* mRNA in a time-dependent manner. (d) Ocy454 cells were treated with the indicated compounds/doses for 18 hours followed by resavurin-based (PrestoBlue) viability assays. Significant cytotoxicity was only observed in response to YKL-05-099.

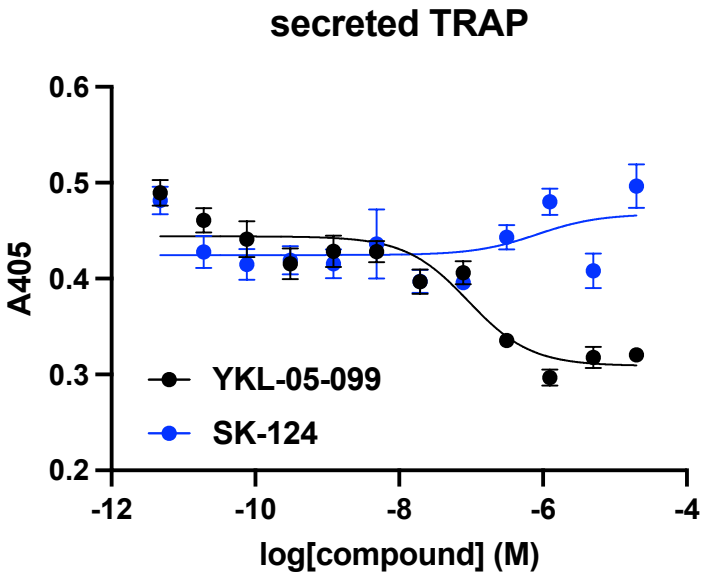
Supplemental Figure 5



Supplemental Figure 5. (a) Mice were treated with the indicated agents and weighed twice per week. No differences in mouse weight were noted across the 5 treatment groups. (b) BUN levels were measured two hours after the final treatment dose and were unchanged across groups. (c) Cortical bone RNA was isolated followed by RT-qPCR for the indicated genes. One-way ANOVA was performed followed by Tukey's posthoc analysis to compare treatment groups to vehicle. P values less than 0.05 are shown on the graphs.

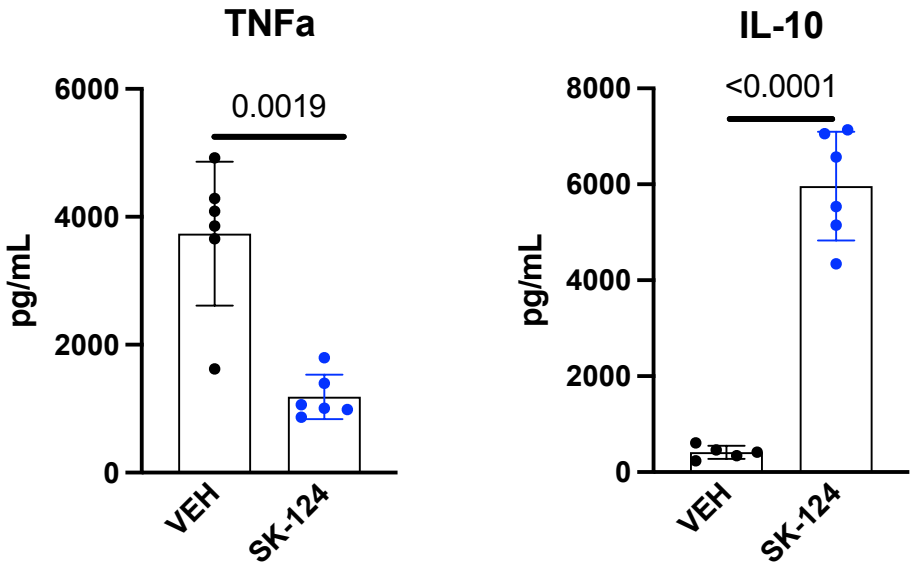
Supplemental Figure 6





Supplemental Figure 6. Bone marrow macrophages were seeded in 96 well plates, and osteoclast differentiation was induced with RANKL (50 ng/mL) and M-CSF (30 ng/mL) plus the indicated compounds. Medium was changed every three days, and TRAP staining was performed after 9 days of differentiation. Top images are shown from cells treated continuously with compounds at the 78.1 nM dose. Bottom, quantification of secreted TRAP levels comparing YKL-05-099 (black) and SK-124 (**16**, blue).

Supplemental Figure 7



Supplemental Figure 7. Male CD1 mice were treated with vehicle (15% hydroxypropyl-beta-cyclodextrin in sterile water) or SK-124 (**16**, 40 mg/kg) by IP injection. Thirty minute later, all mice were treated with lipopolysaccharide (0.5 mg/kg IP). Serum was collected 2 hours following LPS injection (2.5 hours following vehicle or SK-124 injection) for cytokine ELISAs as indicated. Student's t-tests were used to compare cytokine levels in the two treatment groups.