EXPERIMENTAL SECTION

Discovery of Isoxazole Amides as Potent and Selective SMYD3 Inhibitors

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All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.

General synthetic experimental procedures

Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra were recorded on a Bruker UltraShield™ 400 spectrometer operating at 400.132 MHz using a 5mm QNP probe unless otherwise specified. The 2D NMR spectra were recorded on a Bruker Avance 600 spectrometer operating at 600.13 MHz using a 5mm TXI cryoprobe. Spectra were taken in the indicated solvent at ambient temperature, and the chemical shifts are reported in parts per million (ppm (δ)) relative to the lock of the solvent used. Resonance patterns are recorded with the following notations: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Second order spectra in which coupling cannot be obtained by inspection are reported as multiplets, with the center of the signal indicated by the δ value given.

Starting reagents were purchased from commercial suppliers and were used without further purification unless otherwise specified. Microwave reactions were performed in a Biotage™ Initiator Sixty reactor using the manufacturer supplied vials, stir bars, and caps. Microwave reaction times are for time held at the specified temperature.

Normal phase column chromatography was carried out in the indicated solvent system (in the percentage of volume) using pre-packed silica gel cartridges (Reveleris®, Analogix® or Isco Redisep Gold) on the Analogix Intelliflash280® , Varian Intelliflash 971-FP, or Combiflash Companion purification systems. Analytical thin layer chromatography (TLC) visualization was

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performed using 254 nm wavelength ultraviolet light. Preparative HPLC purifications (reversed-phase) were performed using a Gilson 215 Liquid Handler with Trilution software, typically with a SunFire(TM) Prep C18 OBD(TM) 5 μM, 30 mm x 50 mm column. A 7 min gradient followed by a 3 min 100% organic hold time (47 mL/min, 30% gradient of ACN/H_2O, 0.1% TFA) with UV detection at 254 nm was typically used.

All novel compounds were characterized by LCMS, and gave satisfactory results in agreement with the proposed structure. LCMS M+H signals were consistent with expected molecular weight for all reported products. LCMS data were acquired from a Waters Acquity Ultra Performance LC system with UV detector (Waters Acquity PDA) set to 210-350 nm. Samples were injected (0.2 μL) onto a Thermo Hypersil Gold column (C18, 20x2.1 mm, 1.9 u particle diam.) maintained at 55 °C. A linear gradient (1.9 min) from 2% to 95% B (MeCN/H_2O, 0.02% TFA) with a flow rate of 1.6 mL/min was used. The mass spectra (MS) data were acquired in positive ion mode using a Water Acquity SQD (single quadrupole) mass spectrometer with a positive electrospray ionization source.

Biochemical Studies

Recombinant proteins were prepared as described previously.[14] Scintillation proximity assay (SPA) technology was utilized to detect SMYD3 activity. Formation of the ^{3}H-MEKK2 product was followed via incorporation of the methyl group from ^{3}H-SAM to MEKK2 which is captured by a SPA bead (PerkinElmer). Increased proximity of the tritium labeled methyl group from ^{3}H-MEKK2 to the SPA bead produces a luminescent signal.

For IC_{50} profiling, final assay concentrations were 1.5 nM SMYD3, 60 nM ^{3}H-SAM and 25 nM MEKK2 in 25 mM Tris pH 8.0, 4 mM DTT, 50 μM $ZnCl_2$, 0.0025% BSA, and 0.02% Pluronic Acid F127. After 40 minutes, an equal volume of quench (3 mM cold SAM and 1 mg/mL His tag capture SPA beads) was added to stop SMYD3 turnover. Plates were read on a Viewlux Imager (PerkinElmer).

For mode of inhibition studies (MOI), final assay concentrations were 1 nM SMYD3 in 25 mM Tris pH 8.0, 4 mM DTT, and 0.02% Pluronic Acid F127. Substrate concentrations of SAM and MEKK2 varied depending on the assay in addition to compound of interest. When the SAM concentration was varied (0.63-80 nM), MEKK2 was held at $K_{m\ app}$ (8.7 nM). Conversely, when MEKK2 was varied (0.78-100 nM), SAM was held at $K_{m\ app}$ (7.3 nM). After 60 minutes, an equal volume of quench (2 mM SAH) was added followed by the addition of 3x RNA binding SPA beads (4.5 mg/mL). Plates were read on a MicroBeta (PerkinElmer). For testing the inhibition of SMYD3 using a H4 substrate, final concentrations were adjusted to 100 nM SMYD3, 85 nM SAM and 1 μM recombinant histone H4. IC_{50} values were determined by fitting data to equation (1, fixed background) which assumes that V_i/V_o decreases with increasing

[I]. If assessment of inhibitor modality was based on the Cheng-Prusoff relation, data was fit to equation (2) for uncompetitive inhibition. Global fitting of the initial velocity data used equation (3) to determine the inhibition constant, K_i, and alpha values ($\alpha = K_i'/K_i$) while resulting double-reciprocal plots were utilized to determine mode of inhibition. All data was analyzed using GraFit software.

$$(1)\ V_i/V_0 = Background + \frac{Range - Background}{1 + (\frac{[I]}{IC_{50}})^s}$$

$$(2)\ K_i^{app} = \frac{IC_{50}}{1 + (K_m^{app}/[S])}$$

$$(3)\ v = \frac{V_{max} * [S]}{K_m\left(1 + \frac{[I]}{K_i}\right) + [S]\left(1 + \frac{[I]}{K_i'}\right)}$$

Cell culture

HEK293 cells (American Type Culture Collection, CRL-1573) were cultured in RPMI (Life Technologies, A10491) supplemented with 10% fetal bovine serum (Sigma-Aldrich, 12176C).

MEKK2me cellular mechanistic assay

HEK293 cells were transfected in bulk by seeding 3 X 10^6 cells per T75 flask and incubated for 24 hr at 37 °C under 5% CO_2. Transfection reactions were prepared by combining 6 µg of HA-tagged MEKK2 plasmid DNA (pLEX/HA-MEKK2, GRITS:132173) and 0.3 µg SMYD3 plasmid DNA (pLenti-SMYD3) or empty plasmid (pcDNA3.1+, Invitrogen #V90-20) in 1.5 ml Opti-MEM reduced serum media (Thermo Fisher Scientific, 51985091). Sixty µL of Lipofectamine 2000 (Thermo Fisher Scientific, 11668019) was diluted in 1.5 ml Opti-MEM reduced serum media and combined at a 1:1 ratio with the diluted plasmid DNA. Lipo-DNA complexes were allowed to form for 10 minutes at room temperature before transferring drop-wise to the HEK293 flasks. Cells were incubated for 4-6 hr to allow absorption of the lipo-DNA complexes. Cell plates were prepared by plating transfected cells at 5,000 cells/48 µl per well in 384-well clear bottom plates (Greiner Bio-One, 781956) with MEKK2+SMYD3 transfected cells in 22 columns and MEKK2+empty vector in 2 columns. Compound plates were prepared by diluting compounds in DMSO over a 20-pt, 2-fold dilution scheme using the Hamilton Starlet liquid handing automation. An intermediate dilution was generated by stamping 4 µl of the compound plate into 105 µl of RPMI+10%FBS followed by stamping 2 µl of the intermediate dilution plate into the prepared cell plates (final DMSO concentration of 0.15%). Cells were

incubated at room temperature for 30 minutes to allow even distribution and settling prior to incubation for 24 hr at 37°C, 5% CO_2 and 95% relative humidity.

After 24 hr compound incubation, all media was aspirated, and cells were fixed and permeabilized by adding 50 µl of ice-cold 100% methanol and incubating at room temperature for 10 minutes. Plates were then washed 3 times with PBS using a Bioteck ELX405 plate washer. Assay plates were blocked using 50 µl of Odyssey blocking buffer (Li-Cor, 927-40000) and incubated at room temperature for 1 hr with gentle rocking. Primary antibodies were prepared together in Odyssey blocking buffer + 0.1% tween with mouse anti-HA (Covance: MSS-10 IR HA.11, 16B12) at 1:20,000, and rabbit anti-MEKK2-K260me (Custom Yenzym; YZ5047 1st cycle, 0.14mg/ml) at 1:4,000. The anti-MEKK2-K260me antibody recognizes mono, di and trimethylated MEKK2-K260. Twenty-five µl of antibody mixture was added per well and plates were sealed tightly and placed at 4°C overnight with gentle rocking. Following overnight incubation, assay plates were washed 5 times with PBS+0.1% tween. Secondary antibodies were prepared together (Goat anti-mouse 680LT; LI-COR, 926-68020 and goat anti-rabbit 800CW; LI-COR, 926-32211) at 1:800 in Odyssey blocking buffer + 0.1% tween. Plates were incubated for 1 hr at room temperature with gentle shaking followed by 5 washes with PBS+0.1% tween, a final PBS rinse, and complete drying.

Plates were imaged using the Odyssey Imager (LI-COR) such that both the 680 and 800 channels are within the dynamic range of the instrument. To quantify image intensity a 384-well grid was overlaid onto each plate and the raw values exported. Wells co-transfected with SMYD3+MEKK2 and dosed with DMSO served as the positive controls. Any compound treated-well with less than 70% of the positive control HA signal was omitted from analysis due to cell loss. The ratio of MEKK2-K260me to MEKK2-HA was plotted versus compound concentration and fit to a standard 4-parameter logistic model to calculate EC_{50} and IC_{50}. All data represent a minimum of 2 separate biological replicas with an EC_{50} range of less than 3-fold.

ERK1/2 phosphorylation

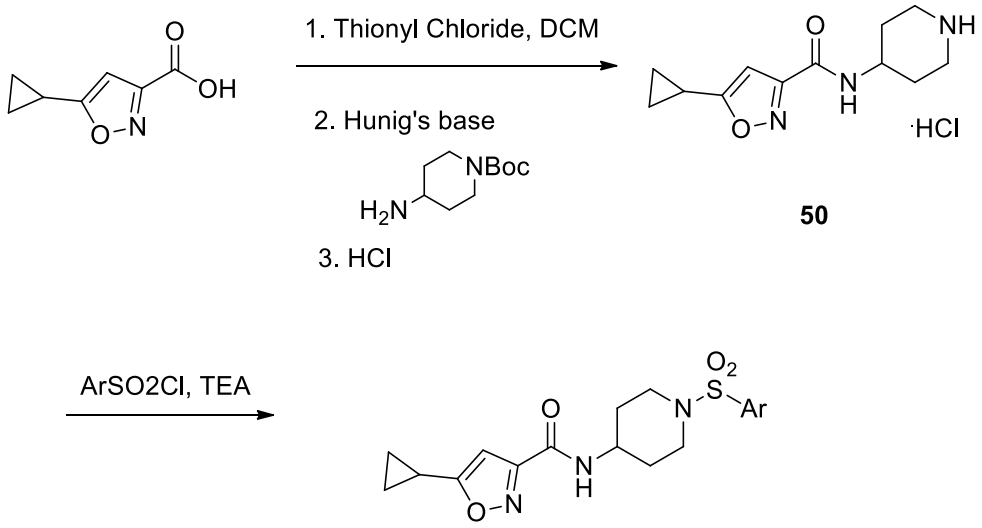
A549 cells were seeded in 6-well plates in RPMI-1640 with 10% fetal bovine serum. After 48 hours, cells were washed with 1X D-PBS and low serum media (0.5% fetal bovine serum) was added. Duplicate wells were treated with 30 µM SMYD3 inhibitors, 250 nM Trametinib/GSK1120212 (ThermoFisher #NC0370092), or DMSO for 24 hrs. Immediately prior to cell harvest, one well of each duplicate treatment was stimulated with 25 ng/µl Epidermal Growth Factor (ThermoFisher #PHG0311L) for 15 min. Cells were washed in cold 1X D-PBS and scraped in cold 1X RIPA buffer containing protease and phosphatase inhibitors. Lysates were sonicated and centrifuged for 10 min at maximum speed, and supernatants were collected. Five micrograms of each lysate were subjected to SDS-PAGE and Western blot analysis with antibodies

against phospho ERK1/2 (Cell Signaling #4370S), total ERK1/2 (Cell Signaling #4695S), SMYD3 (Santa Cruz #sc-67210), and Tubulin (Sigma #T9026).

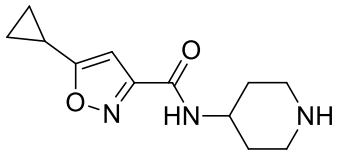
CHEMISTRY

The compounds in Table 1 were prepared straightforwardly according to the route depicted in Scheme 1. Amide coupling following up by the deprotection of Boc group offered the key intermediate **50**. Simply, sulfonamide formation with either the commercially available or readily accessible sulfonyl chlorides delivered the desired analogs.

Scheme 1. General Synthetic Route



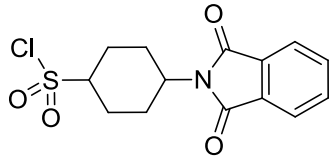
Preparation of tert-butyl 4-(5-cyclopropylisoxazole-3-carboxamido)piperidine-1-carboxylate (49)



Preparation of 5-cyclopropyl-N-(piperidin-4-yl)isoxazole-3-carboxamide, hydrochloride

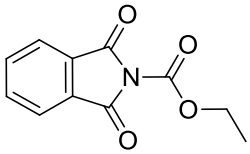
To a solution of 5-cyclopropylisoxazole-3-carboxylic acid (4 g, 26.1 mmol) in Dichloromethane (DCM) (40 mL) was added oxalyl chloride (2 M DCM solution) (39.2 mL, 78 mmol) followed by DMF (0.18 mL, 2.35 mmol). The mixture was stirred at room temperature for 15 min and concentrated. To the residue in Tetrahydrofuran (THF) (40.0 mL) was added DIEA (13.69 mL, 78 mmol) and tert-butyl 4-aminopiperidine-1-carboxylate (5.23 g, 26.1 mmol). The mixture was

stirred at room temperature for 16 h, then diluted with ethyl acetate. The mixture was washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried (sodium sulfate), filtered, and concentrated. To the residue in Methanol (60.0 mL) was added 4 M HCl in dioxane (26.1 mL, 104 mmol). The mixture was stirred at room temperature for 16 h, then concentrated to provide 5-cyclopropyl-N-(piperidin-4-yl)isoxazole-3-carboxamide, hydrochloride (7.1 g, 25.8 mmol, 99% yield): LCMS (ES) m/z = 236.0 $(M+H)^+$.



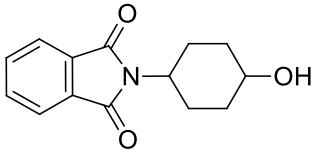
Preparation of 4-(1,3-dioxoisoindolin-2-yl)cyclohexane-1-sulfonyl chloride

a) ethyl 1,3-dioxoisoindoline-2-carboxylate,



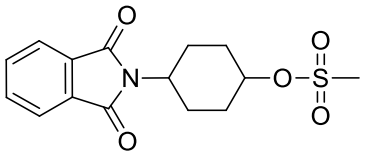
Ethyl carbonochloridate (1.95 mL, 20.39 mmol) was added slowly to a solution of isoindoline-1,3-dione (3000 mg, 20.39 mmol) and TEA (3.41 mL, 24.47 mmol) in N,N-Dimethylformamide (DMF) (80 mL) at 0 ºC under N_2. The reaction was warmed to RT gradually over 4 h, then water was added and the precipitate filtered. The collected solid was dried to give ethyl 1,3-dioxoisoindoline-2-carboxylate (4.2 g, 19.2 mmol, 94% yield): LC-MS (ES) m/z 220.0 $(M+H)^+$.

b) 2-(4-hydroxycyclohexyl)isoindoline-1,3-dione,



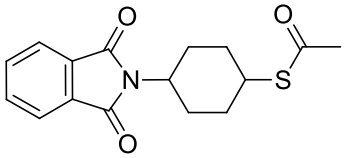
4-Aminocyclohexanol (1.31 g, 11.37 mmol) was added to a solution of ethyl 1,3-dioxoisoindoline-2-carboxylate (2.8 g, 12.77 mmol) and K_2CO_3 (1.59g, 11.50 mmol) in Water (30 mL) under N_2. The reaction was warmed to RT gradually and stirred for 4 h. The mixture was filtered and the solid was dried to give 2-(4-hydroxycyclohexyl)isoindoline-1,3-dione (2.5 g, 10.2 mmol, 90% yield): LC-MS (ES) m/z 246.1 $(M+H)^+$.

c) 4-(1,3-dioxoisoindolin-2-yl)cyclohexyl methanesulfonate,



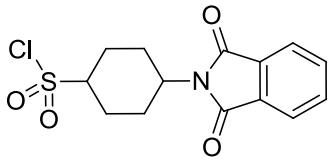
TEA (0.909 mL, 6.52 mmol) was added to a solution of 2-(4-hydroxycyclohexyl)isoindoline-1,3-dione (800 mg, 3.26 mmol) in Tetrahydrofuran (THF) (50 mL), followed by the addition of methanesulfonyl chloride (0.25 mL, 3.26 mmol) under 0 ºC. The mixture was warmed to RT gradually and stirred for 4 h. The reaction mixture was diluted with EtOAc and was washed with brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The crude was purified by silica gel chromatography (Biotage SNAP 25g, 0-30% ethyl acetate/hexanes) to give 4-(1,3-dioxoisoindolin-2-yl)cyclohexyl methanesulfonate (0.96 g, 2.97 mmol, 91% yield): LC-MS (ES) m/z 324.1 $(M+H)^+$.

d) S-(4-(1,3-dioxoisoindolin-2-yl)cyclohexyl) ethanethioate,



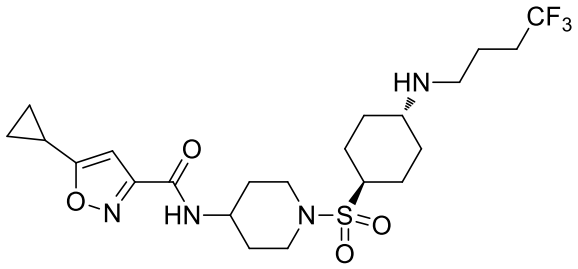
Ethanethioic S-acid (0.32 mL, 4.45 mmol) was added to a solution of 4-(1,3-dioxoisoindolin-2-yl)cyclohexyl methanesulfonate (960 mg, 2.97 mmol) in N,N-Dimethylformamide (DMF) (10 mL), followed by the addition of potassium carbonate (821 mg, 5.94 mmol) . The mixture was microwave irridiated at 120 °C for 30 min. The reaction mixture was diluted with EtOAc and was washed with water and brine. The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude was purified by silica gel chromatography (Biotage SNAP 25g, 0-30% ethyl acetate/hexanes) to provide S-(4-(1,3-dioxoisoindolin-2-yl)cyclohexyl) ethanethioate (585 mg, 1.93 mmol, 65% yield): LC-MS (ES) m/z 304.1 $(M+H)^+$.

e) 4-(1,3-dioxoisoindolin-2-yl)cyclohexane-1-sulfonyl chloride,



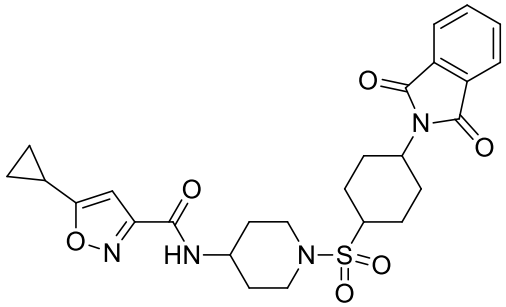
NCS (669 mg, 5.01 mmol) was added to a mixture of 2M HCl (0.4 mL) and Acetonitrile (2 mL), then cooled in an ice-bath. A solution of S-(4-(1,3-dioxoisoindolin-2-yl)cyclohexyl) ethanethioate (380 mg, 1.25 mmol) in Acetonitrile (8 mL) was added dropwise. The ice-bath cooled mixture was stirred for 1 h, then diluted with ethyl acetate. The organic layer was washed with brine (3x), then dried (sodium sulfate), filtered, and concentrated to provide 4-(1,3-dioxoisoindolin-2-

yl)cyclohexane-1-sulfonyl chloride (450 mg, 1.37 mmol, 110% yield): LC-MS (ES) m/z 328.1 $(M+H)^+$.



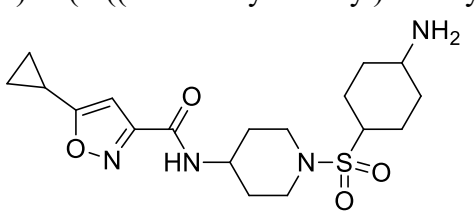
Preparation of 5-cyclopropyl-N-(1-(((1r,4r)-4-((4,4,4-trifluorobutyl)amino)cyclohexyl)sulfonyl)piperidin-4-yl)isoxazole-3-carboxamide

a) 5-cyclopropyl-N-(1-((4-(1,3-dioxoisoindolin-2-yl)cyclohexyl)sulfonyl)piperidin-4-yl)isoxazole-3-carboxamide,



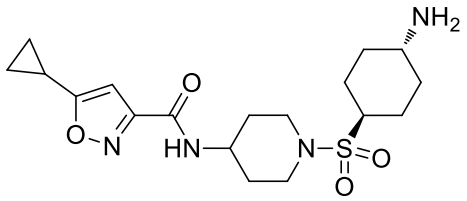
To a ice-bath cooled solution of 5-cyclopropyl-N-(piperidin-4-yl)isoxazole-3-carboxamide, Hydrochloride (300 mg, 1.10 mmol) and DIEA (0.39 mL, 2.21 mmol) in Dichloromethane (DCM) (10 mL) was added 4-(1,3-dioxoisoindolin-2-yl)cyclohexane-1-sulfonyl chloride (434 mg, 1.33 mmol). After stirring for 30 min, the ice-bath was removed and stirring was continued for 16 h. After concentration, the residue was purified by silica gel chromatography (Biotage SNAP ultra 50g, 0-6% methanol/dichloromethane) to provide 5-cyclopropyl-N-(1-((4-(1,3-dioxoisoindolin-2-yl)cyclohexyl)sulfonyl)piperidin-4-yl)isoxazole-3-carboxamide (360 mg, 0.68 mmol, 62% yield) as a white solid: LC-MS (ES) m/z 527.3 $(M+H)^+$.

b) N-(1-((4-aminocyclohexyl)sulfonyl)piperidin-4-yl)-5-cyclopropylisoxazole-3-carboxamide,



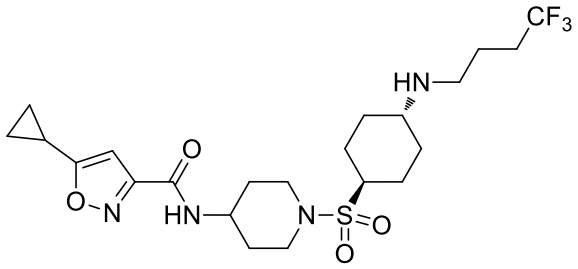
To a suspension of 5-cyclopropyl-N-(1-((4-(1,3-dioxoisoindolin-2-yl)cyclohexyl)sulfonyl)piperidin-4-yl)isoxazole-3-carboxamide (360 mg, 0.68 mmol) in Ethanol (7000 µl) and Tetrahydrofuran (THF) (7000 µl) was added hydrazine monohydrate (200 µl, 4.12 mmol). The mixture was stirred at 60 °C for 7 h, then diluted with dichloromethane and poured into 1N NaOH solution. The layers were separated and the organic layer was washed with brine, then dried (sodium sulfate), filtered, and concentrated to provide N-(1-((4-aminocyclohexyl)sulfonyl)piperidin-4-yl)-5-cyclopropylisoxazole-3-carboxamide (250 mg, 0.54 mmol, 78 % yield) as a white solid: LC-MS (ES) m/z 397.2 $(M+H)^+$.

c)N-(1-(((1R,4R)-4-aminocyclohexyl)sulfonyl)piperidin-4-yl)-5-cyclopropylisoxazole-3-carboxamide,



N-(1-((4-aminocyclohexyl)sulfonyl)piperidin-4-yl)-5-cyclopropylisoxazole-3-carboxamide (250 mg, 0.54 mmol) was purified by preparative chiral HPLC (Chiralpak AD-H, 5 microns (30 mm x 250 mm), 240 nm UV, 45 ml/min, 70:30:0.1 acetonitrile:methanol:isopropylamine) to provide N-(1-(((1R,4R)-4-aminocyclohexyl)sulfonyl)piperidin-4-yl)-5-cyclopropylisoxazole-3-carboxamide (116 mg, 0.29 mmol) as a white solid: LC-MS (ES) m/z 397.4 $(M+H)^+$.

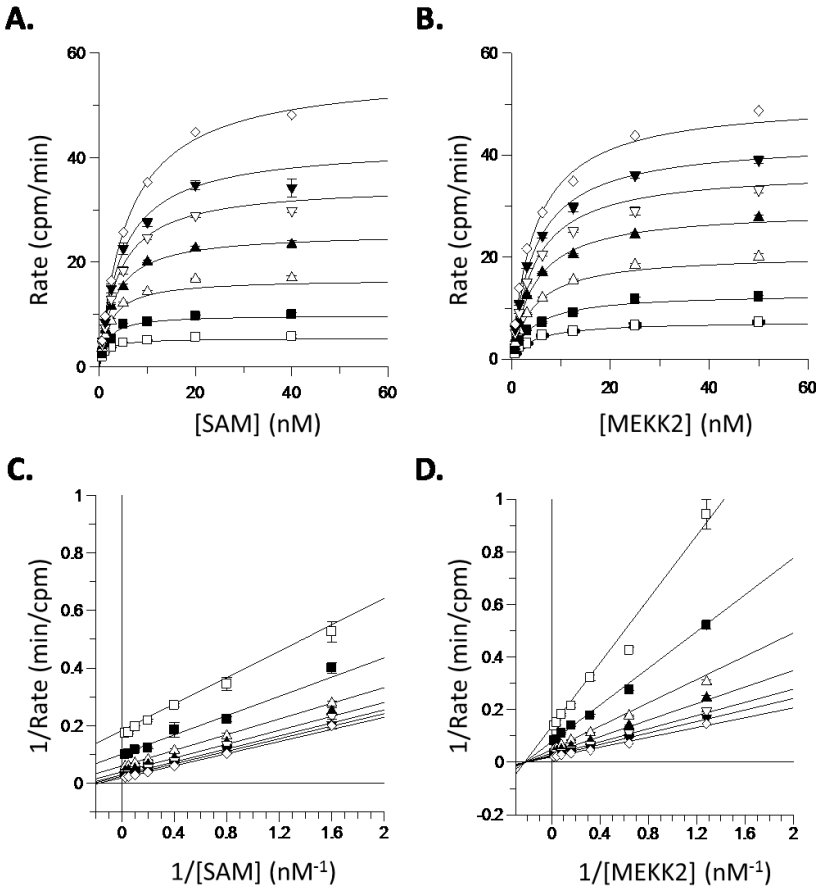
d) 5-cyclopropyl-N-(1-(((1R,4R)-4-((4,4,4-trifluorobutyl)amino)cyclohexyl)sulfonyl)piperidin-4-yl)isoxazole-3-carboxamide,



To a solution of N-(1-(((1r,4r)-4-aminocyclohexyl)sulfonyl)piperidin-4-yl)-5-cyclopropylisoxazole-3-carboxamide (75 mg, 0.19 mmol) and 4,4,4-trifluorobutanal (47.7 mg, 0.38 mmol) in Methanol (2 mL) and was added sodium cyanoborohydride (35.7 mg, 0.57 mmol). The mixture was stirred at room temperature for 16 h, then concentrated. The residue was purified by reversed-phase HPLC [Waters Sunfire 5 µm C18, 75 X 30 mm, 5-65% acetonitrile (0.1% TFA)/water (0.1% TFA)]. The fractions containing the desired product were combined and passed through a PL-HCO3 MP SPE cartridge. The eluate was concentrated to provide 5-cyclopropyl-N-(1-(((1R,4R)-4-((4,4,4-trifluorobutyl)amino)cyclohexyl)sulfonyl)piperidin-4-yl)isoxazole-3-carboxamide (37 mg, 0.07 mmol, 39 % yield) as a white solid: LC-MS (ES) m/z 507.4 $(M+H)^+$, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.89 - 0.95 (m, 2 H) 0.96 - 1.06 (m, 2 H) 1.07 - 1.12 (m, 2

H) 1.35 - 1.47 (m, 2 H) 1.49 - 1.63 (m, 5 H) 1.74 - 1.82 (m, 2 H) 1.90 - 2.04 (m, 4 H) 2.16 - 2.33 (m, 4 H) 2.56 (t, *J*=6.59 Hz, 2 H) 2.96 - 3.09 (m, 3 H) 3.63 (d, *J*=12.67 Hz, 2 H) 3.87 - 4.00 (m, 1 H) 6.49 (s, 1 H) 8.68 (d, *J*=8.11 Hz, 1 H).

Supplemental Data



Analysis	Substrate SAM	Substrate MEKK2
MoI	UC	NC
calculated α	0.13	0.93
line pattern	parallel	intersecting
F statistic	7.93E-06	5.00E-04
Probability	1.00	0.98

Supplemental Figure 1. Mode of inhibition against SMYD3 by compound **49**. Data were fit globally to an equation for mixed inhibition ($v = (V_{max} * [S]) / ((K_m * (1+[I]/K_i)) + ([S] * (1+[I]/K')))$) to determine kinetic parameters. Each symbol represents an inhibitor concentration, ranging from 3.13 nM (▼) to 100 nM (□), 2-fold serial dilution or DMSO (◊). **Top Panel**. Averaged results (n=4) showing initial velocity data for inhibition of SMYD3 with respect to substrate SAM (A) or MEKK2 (B). **Bottom Panel**. Lineweaver-Burk plots with respect to substrate SAM (C) or MEKK2 (D). **Table**. Mode of inhibition for compound **49** was deduced as SAM uncompetitive and MEKK2 noncompetitive based on the calculated alpha value (α = Ki'/Ki), characteristic line pattern of the Lineweaver-Burk plots and through statistical analysis.

Protein preparation for crystallization and structure solution

SMYD3 protein expression, purification, crystallization and structure solution as previously described.[14] Crystallizations with cofactors SAH or SAM were carried out under the same protocols. Data were collected at Argonne National Labs at the APS, LS-CAT, 21idD and 21idF.

Table S1. Crystallograpic Data

	Comp 28	**Comp 3**	**Comp 1**	**Comp 14**
Wavelength	0.9787	1.07805	1.0000	1.07805
Resolution range	56.61 -1.586 (1.643 -1.586)	31.52 - 1.55 (1.606 - 1.55)	31.62 - 1.19 (1.233 - 1.19)	31.52 - 1.221 (1.265 - 1.221)
Space group	P 21 21 21	P 21 21 21	P 21 21 21	P 21 21 21
Unit cell	61.4 66.6 107.3 90 90 90	60.7 66.0 105.8 90 90 90	61.1 66.2 107.3 90 90 90	61.0 66.0 106.5 90 90 90
Unique reflections	60111 (5707)	61111 (6001)	138729 (13635)	124464 (11677)
Completeness (%)	99.55 (96.11)	97.89 (97.26)	99.22 (98.48)	97.32 (92.24)
Wilson B-factor	18.40	16.39	9.29	11.29
R-merge	0.064	0.063	0.054	0.082
Reflections used in refinement	60106 (5707)	61092 (5993)	138725 (13635)	124455 (11676)
Reflections used for R-free	2963 (260)	3026 (287)	6967 (655)	6178 (528)
R-work	0.1723 (0.2725)	0.1786(0.2584)	0.1820 (0.2530)	0.1715 (0.2378)
R-free	0.2002 (0.3266)	0.2147(0.2799)	0.1992 (0.2737)	0.1914 (0.2515)
Number of non-hydrogen atoms	3833	3833	4048	3922
-macromolecules	3377	3393	3462	3370
-compound	37	23	21	28
-cofactor	SAH 26	SAM 27	SAM 27	SAM 27
-solvent	396	402	560	507
Protein residues	426	424	425	423
RMS (bonds)	0.006	0.006	0.005	0.011
RMS (angles)	0.86	0.90	0.85	1.29
Ramachandran favored (%)	99.06	99.28	99.05	99.28
Ramachandran allowed (%)	0.94	0.72	0.95	0.72
Ramachandran outliers (%)	0.00	0.00	0.00	0.00
Rotamer outliers (%)	0.55	0.82	0.26	0.83

Clashscore	0.59	2.37	1.88	1.78
Average B-factor ($Å^2$)	24	25	15	17
-macromolecules	23	25	14	16
-ligand	26	23	29	15
-solvent	31	30	24	26
-cofactor	16	13	6	8

Table S2. Kinase Profiling Results for Compound 49

Compound **49** was tested in single dose duplicate mode at a concentration of 10 μM at Reaction Biology Corporation (Malvern, PA, USA). Reactions were carried out at 10 μM ATP. Data reported as % Enzyme activity (relative to DMSO controls).

Kinase:	% Enzyme Activity (relative to DMSO controls)
ABL1	98.65
ABL2/ARG	93.30
ACK1	93.06
AKT1	99.79
AKT2	96.57
AKT3	98.86
ALK	94.41
ALK1/ACVRL1	103.35
ALK2/ACVR1	93.45
ALK3/BMPR1A	105.51
ALK4/ACVR1B	98.65
ALK5/TGFBR1	99.49
ALK6/BMPR1B	102.92
ARAF	94.49
ARK5/NUAK1	99.06
ASK1/MAP3K5	85.15
AURORA A	99.19
AURORA B	94.47
AURORA C	110.58
AXL	90.87
BLK	92.67
BMPR2	97.04
BMX/ETK	99.95
BRAF	95.66
BRK	94.20
BRSK1	93.93
BRSK2	97.04
BTK	105.96
CAMK1A	100.03
CAMK1B	81.59

CAMK1D	90.69
CAMK1G	96.64
CAMK2A	99.33
CAMK2B	104.41
CAMK2D	94.24
CAMK2G	99.61
CAMK4	89.66
CAMKK1	82.05
CAMKK2	84.08
CDC7/DBF4	94.04
CDK1/cyclin A	94.72
CDK1/cyclin B	83.36
CDK1/cyclin E	95.77
CDK14/cyclin Y (PFTK1)	100.45
CDK16/cyclin Y (PCTAIRE)	96.97
CDK17/cyclin Y (PCTK2)	100.37
CDK18/cyclin Y (PCTK3)	109.75
CDK2/cyclin A	93.61
CDK2/Cyclin A1	103.08
CDK2/cyclin E	96.23
CDK2/cyclin O	95.43
CDK3/cyclin E	95.80
CDK4/cyclin D1	105.56
CDK4/cyclin D3	95.96
CDK5/p25	102.67
CDK5/p35	90.70
CDK6/cyclin D1	95.95
CDK6/cyclin D3	98.54
CDK7/cyclin H	89.54
CDK9/cyclin K	107.37
CDK9/cyclin T1	105.44
CDK9/cyclin T2	90.74
CHK1	121.08
CHK2	102.58
CK1A1	96.99
CK1A1L	103.29
CK1D	101.41
CK1EPSILON	92.06
CK1G1	97.27
CK1G2	108.00
CK1G3	122.42

CK2A	94.23
CK2A2	97.36
C-KIT	103.32
CLK1	106.31
CLK2	94.72
CLK3	100.12
CLK4	87.73
C-MER	96.67
C-MET	109.56
COT1/MAP3K8	102.62
CSK	100.88
C-SRC	99.23
CTK/MATK	96.94
DAPK1	102.23
DAPK2	102.52
DCAMKL1	90.42
DCAMKL2	86.64
DDR1	96.27
DDR2	105.27
DLK/MAP3K12	89.97
DMPK	100.04
DMPK2	110.07
DRAK1/STK17A	87.26
DYRK1/DYRK1A	121.47
DYRK1B	98.32
DYRK2	98.21
DYRK3	101.66
DYRK4	100.40
EGFR	100.36
EPHA1	104.11
EPHA2	98.79
EPHA3	91.66
EPHA4	100.12
EPHA5	95.43
EPHA6	93.30
EPHA7	105.03
EPHA8	98.79
EPHB1	101.11
EPHB2	97.28
EPHB3	96.79
EPHB4	100.28

ERBB2/HER2	98.39
ERBB4/HER4	96.63
ERK1	94.86
ERK2/MAPK1	96.19
ERK5/MAPK7	81.23
ERK7/MAPK15	101.46
ERN1/IRE1	99.59
ERN2/IRE2	94.60
FAK/PTK2	93.72
FER	99.63
FES/FPS	94.84
FGFR1	100.58
FGFR2	102.54
FGFR3	100.61
FGFR4	98.89
FGR	101.98
FLT1/VEGFR1	94.93
FLT3	94.30
FLT4/VEGFR3	107.42
FMS	105.72
FRK/PTK5	99.49
FYN	93.05
GCK/MAP4K2	103.04
GLK/MAP4K3	105.03
GRK1	84.33
GRK2	102.07
GRK3	96.02
GRK4	100.50
GRK5	102.55
GRK6	104.30
GRK7	97.35
GSK3A	97.89
GSK3B	91.06
HASPIN	100.53
HCK	90.34
HGK/MAP4K4	99.29
HIPK1	106.59
HIPK2	102.89
HIPK3	100.65
HIPK4	97.63
HPK1/MAP4K1	104.01

IGF1R	96.51
IKKa/CHUK	100.13
IKKb/IKBKB	100.38
IKKe/IKBKE	99.61
IR	95.40
IRAK1	106.62
IRAK4	109.58
IRR/INSRR	93.00
ITK	100.01
JAK1	83.80
JAK2	101.16
JAK3	104.58
JNK1	87.58
JNK2	97.69
JNK3	102.58
KDR/VEGFR2	97.95
KHS/MAP4K5	99.20
KSR1	95.34
KSR2	97.01
LATS1	98.43
LATS2	99.80
LCK	92.84
LCK2/ICK	88.09
LIMK1	97.44
LIMK2	97.81
LKB1	87.61
LOK/STK10	96.85
LRRK2	91.14
LYN	98.97
LYN B	101.05
MAK	99.40
MAPKAPK2	100.14
MAPKAPK3	93.34
MAPKAPK5/PRAK	82.09
MARK1	103.69
MARK2/PAR-1Ba	103.98
MARK3	149.02
MARK4	90.22
MEK1	99.26
MEK2	80.84
MEK3	103.12

MEK5	104.33
MEKK1	104.78
MEKK2	103.12
MEKK3	102.52
MEKK6	119.25
MELK	96.49
MINK/MINK1	95.15
MKK4	101.67
MKK6	104.29
MKK7	99.54
MLCK/MYLK	90.50
MLCK2/MYLK2	86.89
MLK1/MAP3K9	96.91
MLK2/MAP3K10	101.71
MLK3/MAP3K11	80.55
MLK4	99.13
MNK1	94.26
MNK2	102.24
MRCKa/CDC42BPA	89.44
MRCKb/CDC42BPB	102.59
MSK1/RPS6KA5	102.36
MSK2/RPS6KA4	104.66
MSSK1/STK23	100.79
MST1/STK4	104.65
MST2/STK3	100.47
MST3/STK24	87.75
MST4	90.50
MUSK	93.35
MYLK3	99.54
MYLK4	97.13
MYO3A	91.74
MYO3B	87.44
NEK1	94.82
NEK11	82.91
NEK2	96.91
NEK3	95.27
NEK4	100.53
NEK5	99.96
NEK6	97.12
NEK7	100.26
NEK8	77.53

NEK9	96.49
NIM1	100.00
NLK	92.82
OSR1/OXSR1	95.11
P38a/MAPK14	101.99
P38b/MAPK11	113.21
P38d/MAPK13	99.69
P38G	98.66
p70S6K/RPS6KB1	98.49
p70S6Kb/RPS6KB2	102.96
PAK1	91.47
PAK2	92.52
PAK3	97.37
PAK4	108.55
PAK5	101.94
PAK6	102.03
PASK	91.09
PBK/TOPK	96.34
PDGFRA	96.90
PDGFRB	91.08
PDK1/PDPK1	88.20
PEAK1	91.97
PHKG1	97.00
PHKG2	102.93
PIM1	98.85
PIM2	96.40
PIM3	104.48
PKA	97.24
PKACB	107.76
PKACG	102.12
PKCA	90.33
PKCB1	84.78
PKCB2	99.22
PKCD	102.77
PKCEPSILON	101.20
PKCETA	102.77
PKCG	101.96
PKCIOTA	108.58
PKCmu/PRKD1	122.53
PKCnu/PRKD3	102.25
PKCTHETA	114.81

PKCZETA	103.25
PKD2/PRKD2	99.65
PKG1A	100.74
PKG1B	96.18
PKG2/PRKG2	97.16
PKN1/PRK1	92.79
PKN2/PRK2	100.16
PKN3/PRK3	92.89
PLK1	96.52
PLK2	102.66
PLK3	100.22
PLK4/SAK	109.30
PRKX	97.65
PYK2	106.16
RAF1	101.71
RET	96.28
RIPK2	103.10
RIPK3	107.25
RIPK5	101.41
ROCK1	96.85
ROCK2	99.80
RON/MST1R	98.63
ROS/ROS1	96.19
RSK1	87.79
RSK2	99.52
RSK3	113.93
RSK4	95.85
SBK1	100.83
SGK1	97.46
SGK2	100.17
SGK3/SGKL	85.85
SIK1	99.65
SIK2	94.50
SIK3	93.06
SLK/STK2	96.90
SNARK/NUAK2	92.80
SNRK	88.97
SRMS	96.44
SRPK1	92.59
SRPK2	96.97
SSTK/TSSK6	103.85

STK16	100.64
STK21/CIT	98.55
STK22D/TSSK1	94.52
STK25/YSK1	96.15
STK32B/YANK2	100.52
STK32C/YANK3	134.20
STK33	102.64
STK38/NDR1	94.80
STK38L/NDR2	98.25
STK39/STLK3	90.54
SYK	95.43
TAK1	100.60
TAOK1	96.21
TAOK2/TAO1	99.36
TAOK3/JIK	88.66
TBK1	98.12
TEC	102.75
TESK1	93.54
TGFBR2	124.69
TIE2/TEK	96.25
TLK1	102.61
TLK2	82.33
TNIK	93.36
TNK1	101.44
TRKA	96.96
TRKB	98.73
TRKC	103.58
TSSK2	102.40
TSSK3/STK22C	103.62
TTBK1	103.12
TTBK2	105.77
TXK	94.66
TYK1/LTK	99.77
TYK2	102.96
TYRO3/SKY	103.67
ULK1	104.29
ULK2	98.72
ULK3	93.11
VRK1	100.03
VRK2	94.58
WEE1	100.43

WNK1	80.75
WNK2	103.21
WNK3	90.77
YES/YES1	97.50
YSK4/MAP3K19	103.08
ZAK/MLTK	96.79
ZAP70	86.02
ZIPK/DAPK3	94.77

Table S3. Methyltransferase Profiling Results for Compound 49

Compound **49** was tested in single dose duplicate mode at a concentration of 10 μM at Reaction Biology Corporation (Malvern, PA, USA). Data reported as % Enzyme activity (no inhibition control as 100% activity).

Methyltransferase (GENE_NAME)	**% Enzyme Activity (relative to DMSO controls)**	**Substrate**	**SAM Concentration (μM)**
Dot1L	103.62	Nucleosomes	1
EZH1 Complex	101.33	Core Histone	1
EZH2 (Y641F) Complex	93.94	Core Histone	1
EZH2 Complex	101.54	Core Histone	1
G9a	101.30	Histone H3 (1-21)	1
G9a/GLP	98.16	Histone H3 (1-21)	1
GLP	104.66	Histone H3 (1-21)	1
MLL1 Complex	110.60	Nucleosomes	1
MLL2 Complex	98.88	Core Histone	1
MLL3 Complex	97.67	Core Histone	1
MLL4 Complex	89.20	Core Histone	1
NSD1	89.54	Nucleosomes	1
NSD2	93.01	Nucleosomes	1
NSD2 (E1099K)	98.26	Nucleosomes	1
PRDM9	99.09	Histone H3	1
PRMT1	91.60	Histone H4	1
PRMT3	98.22	Histone H4	1
PRMT4	102.08	Histone H3	1
PRMT5	100.92	Histone H2A	1
PRMT5/MEP50	107.45	Histone H2A	1

PRMT6	95.19	Histone H3	1
PRMT8	96.10	Histone H4	1
SET1B Complex	98.95	Core Histone	1
SET7	91.60	Core Histone	1
SET8	99.86	Nucleosomes	1
SETD2	100.07	Nucleosomes	1
SMYD2	100.79	Histone H4	1
SUV39H1	100.06	Histone H3	1
SUV39H2	96.31	Histone H3	1
SUV420H1-tv2	98.57	Nucleosomes	1