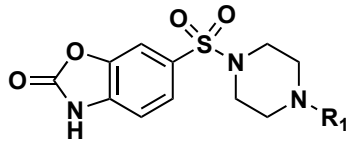
Supplementary Table 1. Small molecule screening data

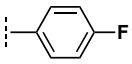
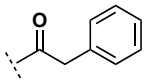
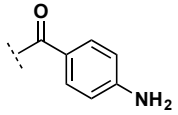
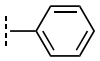
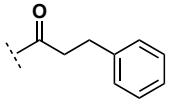
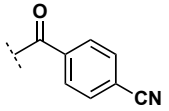
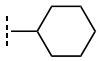
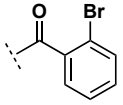
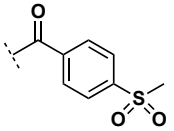
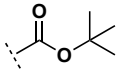
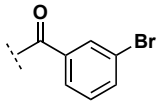
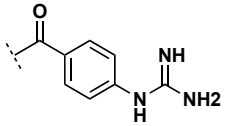
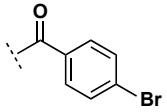
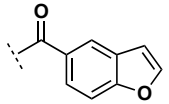
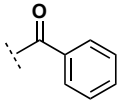
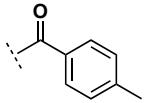
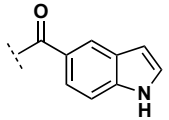
Category	Parameter	Description
Assay	Type of assay	in vitro target based
	Target	NUDT15
	Primary measurement	Absorbance at 630 nm using a coupled enzymatic assay to detect inorganic phosphate (Pi) using the malachite green assay
	Key reagents	Human recombinant NUDT15 (In house produced in E. coli, see text for details) Inorganic pyrophosphatase Malachite green detection reagents (Malachite Green, ammonium molybdate and Tween-20)
	Assay protocol	See online method
	Additional comments	Protocol according to: Baykov, A. A., Evtushenko, O. & Avaeva, S. M. A malachite green procedure for orthophosphate determination and its use in alkaline phosphatase-based enzyme immunoassay. Anal. Biochem. 171, 266–270 (1988). Take care to ensure phosphate contamination in preparation of all reagents
Library	Library size	17,946
	Library composition	The library consists of a chemically diverse collection of compounds containing both commercial (Enamine, TimTec, Maybridge and ChemDiv) and internal compounds (donation from Biovitrum). The library includes a small fraction of compounds with known bioactivities, e.g. the Prestwick set, and a set of nucleosides from Barry Associates.
	Source	The screen was done based on plating of 10 mM DMSO solutions from Labcyte 384 LDV plates using an Echo 550
	Additional comments	See online method for further details on the composition of the Biovitrum derived compounds
Screen	Format	384-well format Assay plate: 384-well PS plate, Nunc 242757
	Concentration(s) tested	Compound concentration at 10 µM, DMSO concentration at 0.1%
	Plate controls	Positive control: buffer only representing fully inhibited NUDT15 enzyme (16 on each plate) Negative control: uninhibited NUDT15 enzyme (16 on each plate)
	Reagent/ compound dispensing system	Compound dispensing system: Echo 550 from Labcyte Reagent dispensing system: FlexDrop IV from PerkinElmer Multidrop from Thermo Scientific
	Detection instrument and software	Victor3 plate reader from PerkinElmer
	Assay validation/QC	Positive control: average absorbance 0.30, standard deviation 0.03. Negative control: average absorbance 1.03, standard deviation 0.04. Average Z´ factor/plate: 0.87. QC also included monitoring of plate edge effects and distribution of the hits, with no corrections necessary
	Correction factors	Not applicable
	Normalization	Data are normalized to the positive (100% inhibition) and negative controls (0% inhibition) on each plate and are expressed as % inhibition
	Additional comments	The screen was performed at Chemical Biology Consortium Sweden at Karolinska Institutet, Sweden
Post-HTS analysis	Hit criteria	Hit threshold = Average “% inhibition” of all test samples (-0,39%) + 3 times standard deviation of all test samples (3*5.00%) = 14,60%
	Hit rate	0.5%

Additional assay(s)	Retesting of hits in 3 concentration hit confirmation experiment followed by a full concentration response experiments at 11 concentrations
Confirmation of hit purity and structure	ID and purity analysis with LC-UV/MS detection
Additional comments	

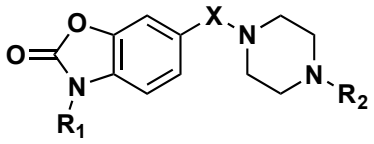
1
2
3

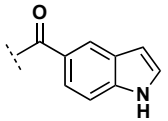
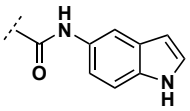
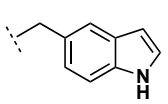
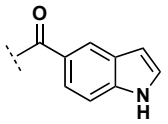
Supplementary Table 2



Compound	R_1	IC_{50}	Compound	R_1	IC_{50}	Compound	R_1	IC_{50}
1 (TH884)		7.0 μM	7 (TH1318)		11.1 μM	13 (TH7444)		361 nM
2 (TH1092)		4.0 μM	8 (TH1320)		6.1 μM	14 (TH1743)		512 nM
3 (TH5724)		9.1 μM	9 (TH7044)		174 nM	15 (TH7512)		1.69 μM
4 (TH1322)		1.3 μM	10 (TH1741)		107 nM	16 (TH7365)		1.80 μM
5 (TH1191)		> 100 μM	11 (TH1740)		46 nM	17 (TH3544)		77 nM
6 (TH1319)		192 nM	12 (TH1754)		146 nM	**18 (TH1760)**		**25 nM**

Supplementary Table 3

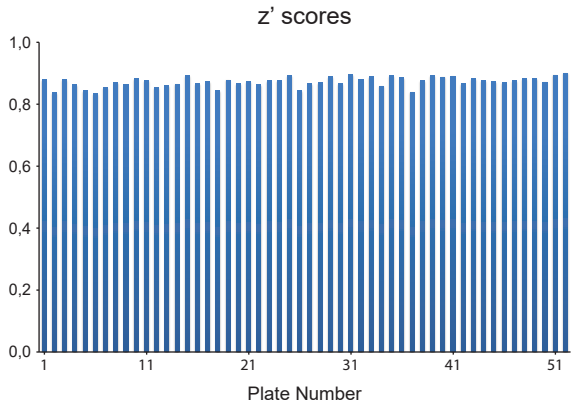


Compound	Modifications			IC_{50}
	R_1	X	R_2	
19 (TH7108)	H			> 100 μM
20 (TH7252)	H			3.5 μM
21 (TH7257T)	H			219 nM
22 (TH7285)	CH_3			> 100 μM

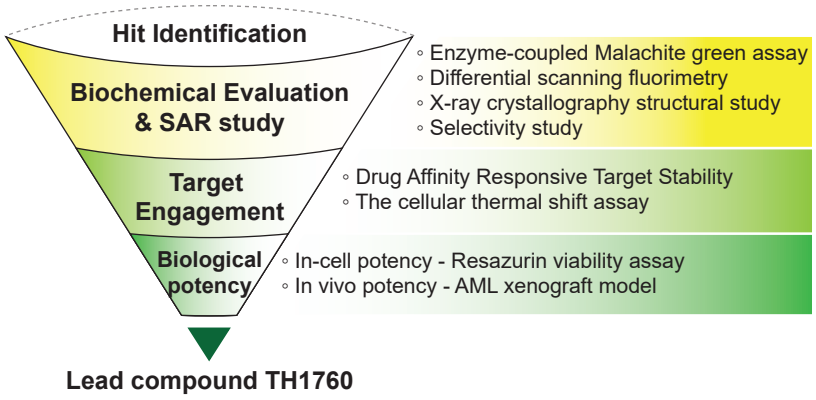
Supplementary Table 4 Data collection and refinement statistics (molecular replacement)

	PDB ID: 6T5J
Data collection	
Space group	*P* 2_1 2_1 2_1
Cell dimensions	
a, *b*, *c* (Å)	46.8, 46.8, 137.6
α, β, γ (°)	90, 90, 90
Resolution (Å)	46.81-1.60 (1.63-1.60)*
R_{merge}	13.1 (55)
$I / \sigma I$	11.3 (4.2)
Completeness (%)	98.6 (97.6)
Redundancy	11.9 (12.4)
Refinement	
Resolution (Å)	44.31-1.60
No. reflections	40061
R_{work} / R_{free}	16.1/19.5
No. atoms	
Protein	2513
Ligand/ion	66
Water	454
B-factors	
Protein	18.9
Ligand/ion	19.4
Water	31.6
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	0.79

*Values in parentheses are for highest-resolution shell. A single crystal was used for data collection and structure refinement.

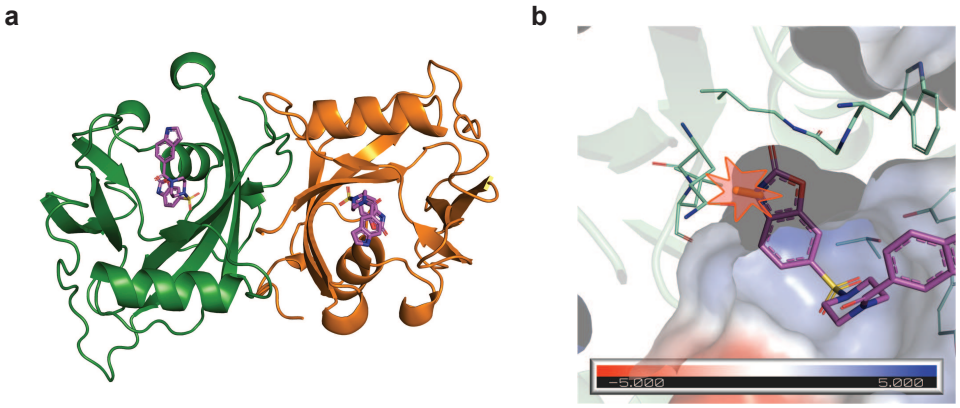


Supplementary Fig. 1 Plotted z' values of the screening campaign for putative NUDT15 inhibitors



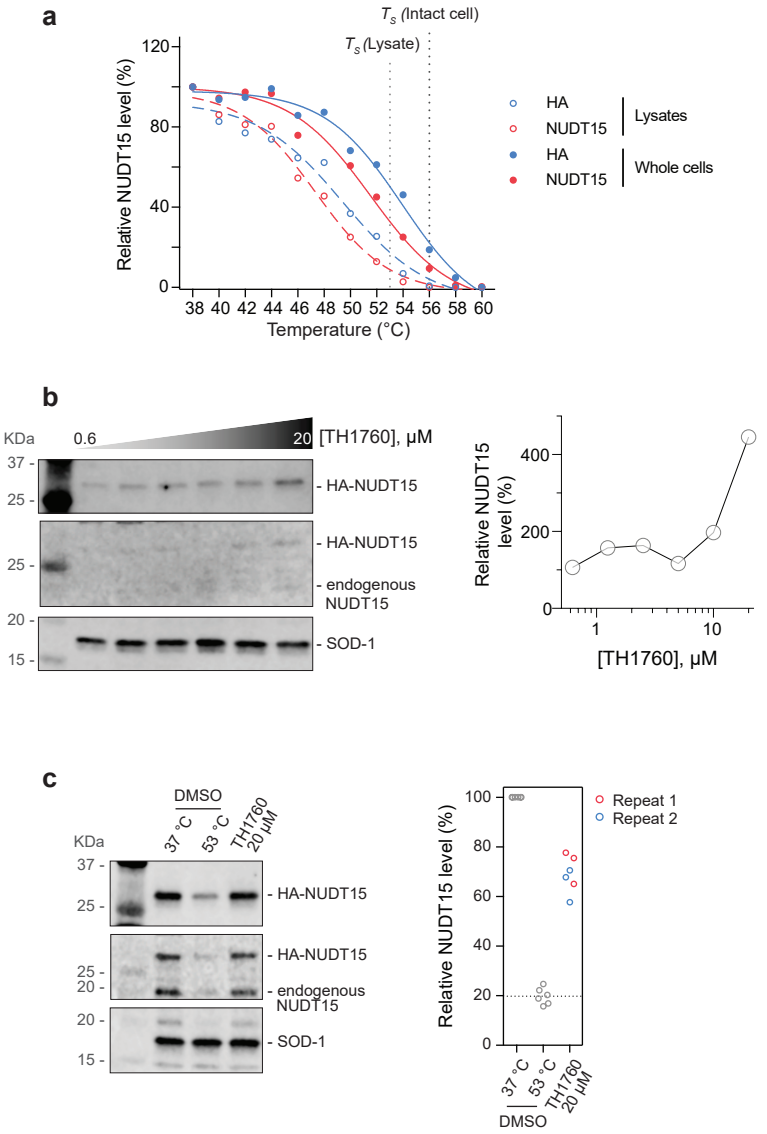
Supplementary Fig. 2 NUDT15 inhibitor screening and development funnel

Putative NUDT15 inhibitors were subjected to a three-stage screening and development funnel, comprised sequentially, 1) medicinal chemistry coupled with biochemical assay and X-ray crystallography, to determine the inhibitory potency and binding modality against recombinant NUDT15; 2) target engagement assays to assess the engagement of NUDT15 in a cellular context; and 3) biological potency evaluation *via* thiopurine potentiation *in vitro* and *in vivo*.



Supplementary Fig. 3 Additional views of NUDT15-TH1760 co-crystal structure

a. Structure of NUDT15 dimer in complex with TH1760, in cartoon representation (NUDT15 monomers colored green and orange, and TH1760 in magenta). **b**. Design of the negative control compound TH7285 was guided by the NUDT15-TH1760 structure. Close-up view of the NUDT15 binding pocket occupied by TH1760 (purple), with solvent accessible surface area colored by electrostatic potential. According to the structure, the benzoxazolone group of TH1760 fits tightly in the binding pocket of NUDT15. We therefore reason that addition of a methyl group (shown in orange) would disallow the binding of the resulting compound, TH7285, due to steric hindrance.

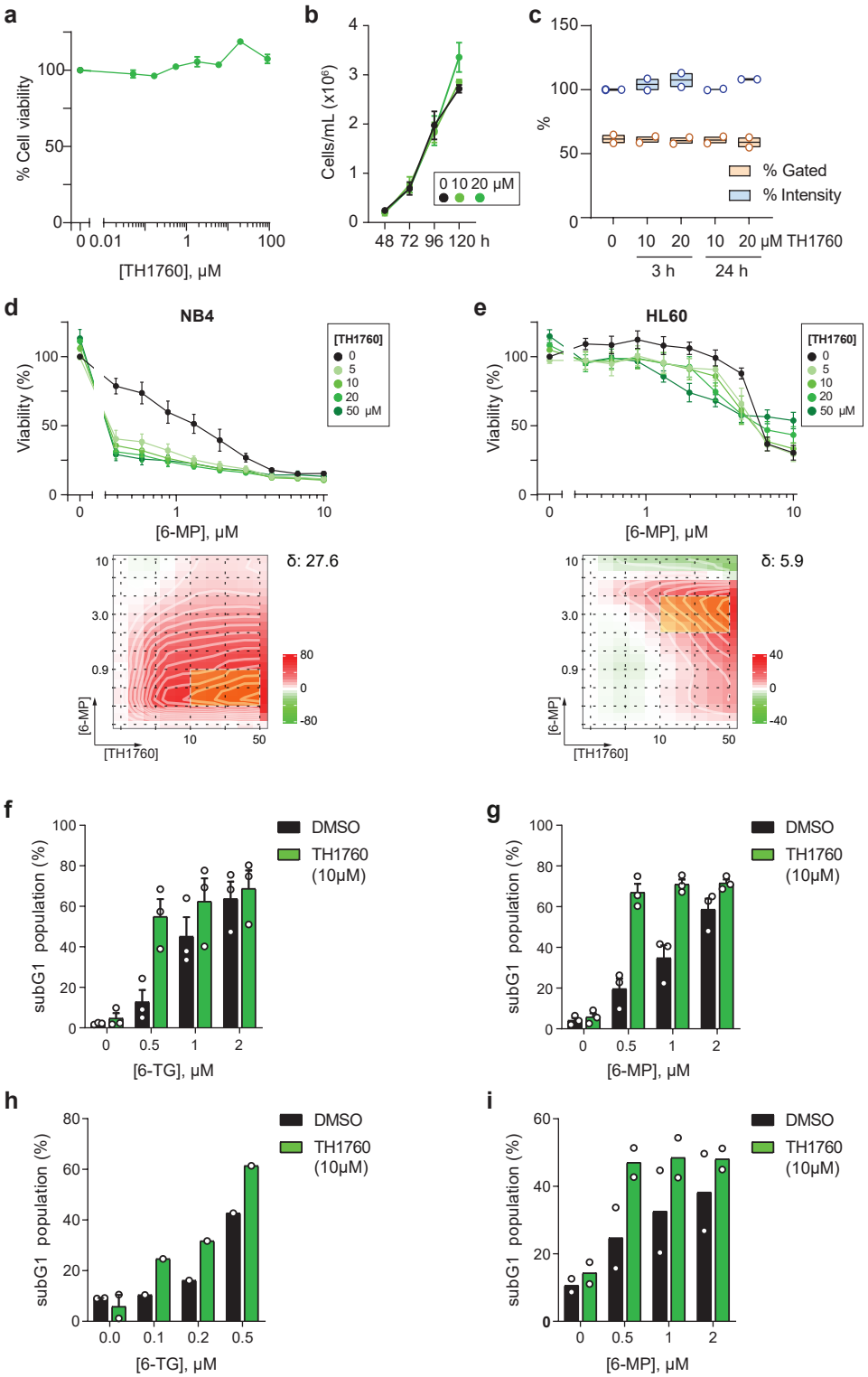


Supplementary Fig. 4 TH1760 substantially stabilized intracellular NUDT15 from 10 µM, demonstrated using ITDRF.

a. Determination of screening temperature (Ts) for intact cell and lysates, using melting profile of both untagged and HA-tagged NUDT15. Mean of n=2 experiments shown.

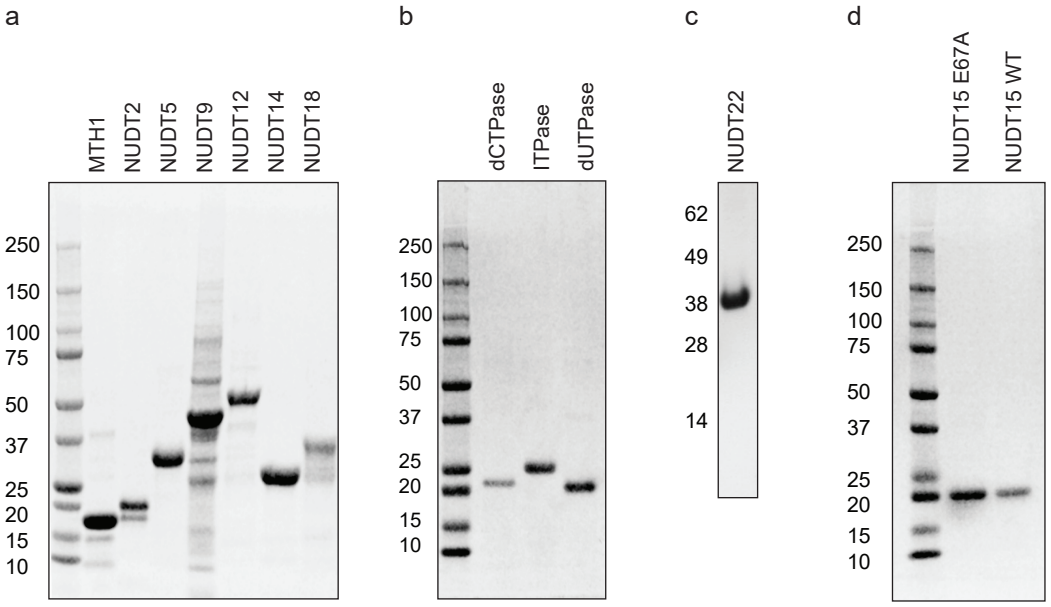
b. TH1760, tested from 0.6 to 20 µM, dose-dependently stabilized intracellular NUDT15 protein in intact NB4 cells overexpressing HA-tagged NUDT15, assayed at Ts (Intact cell).

c. TH1760 tested at 20 µM substantially stabilized NUDT15 in cell lysate of NB4 cells overexpressing HA-tagged NUDT15, assayed at Ts (Lysate). Individual repeats of n=2 experiments performed in triplicate shown.



Supplementary Fig. 5 TH1760 treatment sensitized AML cells to thiopurine

a. TH1760 was not cytotoxic in HL-60 cells up to 100 μM. Viabilities of HL-60 cells treated with TH1760 for 96h were assessed by resazurin viability assay. Viability % was calculated by normalizing to DMSO-treated controls, and mean ± SEM of n=4 experiments performed in duplicates shown. **b**. TH1760 did not alter proliferation profile of HL-60 cells. The proliferation profiles of HL-60 cells treated with 0, 10, or 20 μM TH1760 were followed up to 5 days post-treatment. Mean cell number per mL ± SEM of n=3 experiments shown. **c.** TH1760 did not alter DNA replication, evidenced by EdU incorporation. EdU incorporation in HL-60 cells treated with 0, 10, or 20 μM TH1760 were examined by flow cytometry at 3 and 24 h post-treatment. Mean EdU+ve population percentage and mean relative fluorescence intensity of n=2 experiments shown with lower/upper limits. **d. e.** TH1760 synergized with 6-MP in killing AML cell lines NB4 (d) and HL-60 (e), assessed using resazurin viability assay. Cells treated with a dose-response concentration matrix of TH1760 and 6-MP were incubated for 96 h before viabilities were assayed. Viability % was calculated by normalizing to DMSO-treated controls and mean ± SEM of n = 3 experiments shown (left panels). Cell viabilities were further used to calculate drug synergy maps and scores (δ), using SynergyFinder (right panels). **f - i** TH1760-mediated thiopurine potentiation was confirmed by assaying subG1 population in HL-60 cells treated with 6-TG (f) or 6-MP (g), and also in NB4 cells treated with 6-TG (h) or 6-MP (i). SubG1 population was determined through propidium iodide staining followed by FACS. f. g., Mean ± SEM and individual values of n=3 experiments shown. h, Mean and individual values of n =2 experiments, and I, n= 1 experiment.



Supplementary Fig. 6 Purified proteins used in this study

Purified human MTH1, NUDT2, NUDT5, NUDT9, NDUT12, NUDT14, NUDT18 (a), dCTPase, ITPase, dUTPase (b), NUDT22 (c), NUDT15, and NUDT15 E67A (d) were analyzed by SDS-PAGE and Coomassie blue staining. Two independent experiments performed.

Conditions for developed enzymatic assays

Enzyme	Coupled enzyme	Substrate	Enzyme concentration
MTH1	PPase; 0.2 U/ml	dGTP; 100 μM	4.75 nM
NUDT15	PPase; 0.2 U/ml	dGTP; 100 μM	8 nM
NUDT2	BIP; 10 U/ml	AP4A; 16 μM	8 nM
NUDT5	BIP; 10 U/ml	ADPR; 50 μM	2 nM
NUDT9	BIP; 10 U/ml	ADPR; 50 μM	20 nM
NUDT12	BIP; 10 U/ml	NADH; 50 μM	20 nM
NUDT14	BIP; 10 U/ml	ADPR; 50 μM	2 nM
NUDT18	PPase;0.2 U/ml	8-oxo-dGTP; 50 μM	200 nM
ITPase	PPase; 0.2 U/ml	ITP; 25 μM	0.2 nM
dCTPase	PPase; 0.2 U/ml	dCTP; 35 μM	35 nM
dUTPase	PPase;0.2 U/ml	dUTP; 12.5 μM	1 nM
NUDT22	BIP; 10 U/ml	UDP galactose; 50 μM	30 nM

Supplementary Fig. 7 Conditions for biochemical enzymatic assays for all the proteins used to determine TH1760 selectivity

SUPPLEMENTARY NOTE – Synthetic Procedures

Abbreviations and Acronyms

aq: aqueous; Boc: *tert*-butyloxycarbonyl; br: broad; CH_2Cl_2: dichloromethane; d: doublet; dd: doublet of doublets; DIPEA: diisopropylethylamine; DMF: dimethylformamide; DMSO: dimethylsulfoxide; h: hour; HCl: hydrochloric acid; prep HPLC: preparative high-pressure liquid chromatography; Et_3N: trimethylamine; EtOAc: ethyl acetate; HATU: *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-b]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; LC-MS: liquid chromatography mass spectrometry; m/z: mass-to-charge ratio; min: minutes; m: multiplet; MeCN: acetonitrile; MeOH: methanol; MHz: megahertz; $MgSO_4$: magnesium sulfate; $NaHCO_3$: sodium bicarbonate; NH_4HCO_3: ammonium bicarbonate; NMR: nuclear magnetic resonance; RT: room temperature; s: singlet; T3P®: 1-propanephosphonic anhydride solution; THF: tetrahydrofuran; TFA: trifluoroacetic acid; t: triplet.

General Experiment Conditions

Solvents and reagents

All the reagents were commercially available and were used without further purification. Analytical thin-layer chromatography was performed on silica gel 60 F-254 plates (E. Merck) and visualized with UV light.

Flash chromatography

Purification of compounds by flash chromatography was achieved using a Biotage SP4 system using the stated cartridges.

NMR Spectroscopy

^{1}H NMR spectra were recorded at 400 MHz. The chemical shifts for ^{1}H are referenced *via* residual solvent signal (^{1}H, DMSO-d_6 at 2.51 ppm or ^{1}H, CDCl3 at 7.27 ppm).

^{13}C NMR spectra were recorded at 100 MHz. The chemical shifts for ^{13}C are referenced *via* residual solvent signal (^{13}C, DMSO-d_6 at 39.51 ppm or ^{13}C, CDCl3 at 77.00 ppm).

Liquid Chromatography Mass Spectrometry (LC-MS)

Liquid Chromatography Mass Spectrometry (LC-MS) experiments to determine retention times (R_T) and associated mass ions were performed on an Agilent MSD mass spectrometer connected to an Agilent 1100 system with:

System A: Column ACE 3 C8, 3 µm, 50 x 3.0 mm maintained at 40°C. 0.1% (v/v) TFA in water (A) and MeCN (B) were used as mobile phases at a flow rate of 1 mL/min, with a gradient time of 3.0 min.

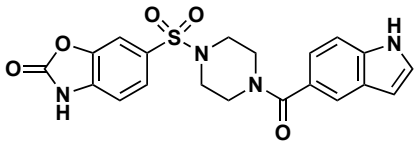
System B: Column Xterra MSC18, 3.5 µm, 50 x 3.0 mm maintained at 40°C. Water (containing 10 mM NH_4HCO_3; pH = 10, A) and MeCN (B) were used as mobile phases at a flow rate of 1 mL/min, with a gradient time of 3.0 min.

Preparative HPLC
Performed on a Gilson HPLC system.
System A: small quantities, column ACE C8, 5 µm (50 x 21.2 mm); large quantities, column ACE C8, 5 µm (150 x 30 mm); 0.1% TFA (v/v) in H_2O and MeCN were used as mobile phases at a flow rate of 30 or 38 mL/min (for small and large quantity respectively) with a gradient time of 7 min.
System B: small quantities, column XTerra Prep MS C18, 5 µm OBD (19 x 50 mm); large quantities, column Xbridge Prep C18, 5 µm CBD (30 x 75 mm); H_2O (containing 50 mM NH_4HCO_3; pH = 10) and MeCN were used as mobile phases at a flow rate of 45 mL/min, with a gradient time of 11 min.

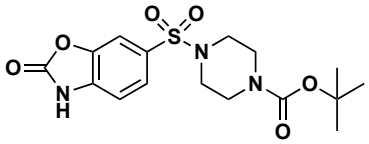
For LC-MS and preparative HPLC, detection was made by UV (254 or 214 nm) and MS (ESI^+).

Nomenclature
Unless otherwise indicated, the nomenclature of structures was determined using the "Import Name" function of MarvinSketch 17.24.

<u>COMPOUND 18 (TH1760):</u> *6-{[4-(1H-indole-5-carbonyl)piperazin-1-yl]sulfonyl}-2,3-dihydro-1,3-benzoxazol-2-one*

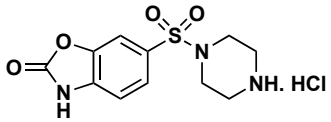


Step 1: **COMPOUND 4 (TH1322):** *tert-butyl 4-[(2-oxo-2,3-dihydro-1,3-benzoxazol-6-yl)sulfonyl]piperazine-1-carboxylate*



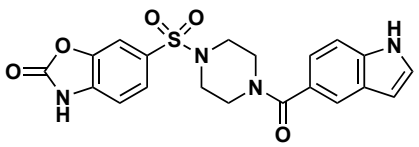
tert-butyl piperazine-1-carboxylate (399 mg, 2.14 mmol) is added to a solution of 2-oxo-2,3-dihydro-1,3-benzoxazole-6-sulfonyl chloride (500 mg, 2.14 mmol) in CH_2Cl_2 (5 mL). The reaction mixture is stirred at room temperature overnight, then diluted with CH_2Cl_2, and successively washed with HCl 2N, a saturated solution of $NaHCO_3$ and brine. The organic layer is dried over $MgSO_4$ and concentrated under reduced pressure to afford 662 mg (81% yield) of the title compound as a yellow powder.
LC-MS (system A): *m/z* = 284 $[M\text{-}Boc+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 12.19 (br s, 1H), 7.62 (d, *J*=1.4 Hz, 1H), 7.53 (dd, *J*=8.21, 1.7 Hz, 1H), 7.29 (d, *J*=8.2 Hz, 1H), 3.36 - 3.42 (m, 4H), 2.78 - 2.89 (m, 4H), 1.33 (s, 9H). m/z calculated for $[C_{16}H_{21}N_3O_6S]$ = 383.1151, found 383.1106.

Step 2: **COMPOUND 5 (TH1191):** *6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride*



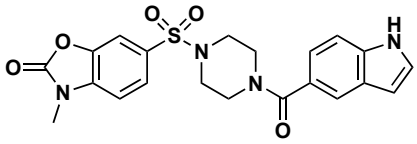
HCl 4N in dioxane (199 µL, 5.74 mmol) is added to the solution of *tert*-butyl 4-[(2-oxo-2,3-dihydro-1,3-benzoxazol-6-yl)sulfonyl]piperazine-1-carboxylate (220 mg, 0.574 mmol) in 1,4-dioxane (500 µL). The reaction mixture is stirred at room temperature for 16 h and the white precipitate formed is dried off to afford 158 mg (86% yield) of the title compound.
LC-MS (system A): *m/z* = 284 $[M+H]^+$. m/z calculated for $[C_{11}H_{13}N_3O_4S]$ = 283.0626, found 283.0677.

Step 3: **<u>COMPOUND 18 (TH1760):</u>** *6-{[4-(1H-indole-5-carbonyl)piperazin-1-yl]sulfonyl}-2,3-dihydro-1,3-benzoxazol-2-one*



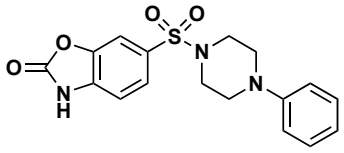
T3P® (50% in EtOAc, 56 µL, 0.188 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol), 1*H*-indazole-5-carboxylic acid hydrochloride (37.3 mg, 0.188 mmol) and Et_3N (87 µL, 0.625 mmol) in CH_2Cl_2 (1 mL) at 0 °C. The reaction mixture is then stirred at room for 16 h and then purified by column chromatography on silica gel (Biotage SP4, eluent gradient CH_2Cl_2/MeOH, 1 to 10% of MeOH) to give the title compound (13 mg, 19% yield) as a white solid.
LC-MS (system A): *m/z* = 427 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.29 (br s, 1H), 7.63 (d, *J*=1.4 Hz, 1H), 7.54 - 7.57 (m, 1H), 7.53 (d, *J*=1.7 Hz, 1H), 7.36 - 7.42 (m, 2H), 7.30 (d, *J*=8.2 Hz, 1H), 7.06 (dd, *J*=8.4, 1.6 Hz, 1H), 6.45 - 6.48 (m, 1H), 3.55 - 3.66 (m, 4H), 2.90 - 2.99 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 171.2, 155.0, 143.8, 136.9, 136.0, 128.3, 127.4, 127.1, 126.1, 124.8, 121.0, 120.2, 111.6, 110.5, 109.2, 102.2, 46.4. m/z calculated for [C20H18N4O5S] = 426.0998, found 426.1078.

<u>COMPOUND 22 (TH7285):</u> *6-{[4-(1H-indole-5-carbonyl)piperazin-1-yl]sulfonyl}-3-methyl-2,3-dihydro-1,3-benzoxazol-2-one*



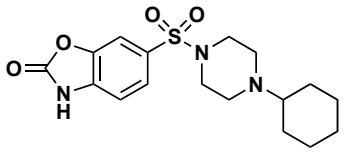
A mixture of 3-methyl-2-oxo-2,3-dihydro-1,3-benzoxazole-6-sulfonyl chloride (14.9 mg, 0.06 mmol), 5-(piperazine-1-carbonyl)-1*H*-indole (11 mg, 0.048 mmol), and DIPEA (13 µL, 0.0746 mmol) is stirred in CH_2Cl_2 at reflux for 2 h. The mixture is then concentrated under reduced pressure and purified by preparative HPLC (system B) to give 6 mg (28%) of the title compound. LC-MS (system A): *m/z* = 441 $[M+H]^+$.
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.29 (br s, 1H), 7.71 (d, *J*=1.4 Hz, 1H), 7.65 (dd, *J*= 8.2, 1.6 Hz, 1H), 7.56 (br s, 1H), 7.51 (d, *J*=8.2 Hz, 1H), 7.41 (m, 1H), 7.40 (d, *J*=8.4 Hz, 1H), 7.06 (dd, *J*= 8.3, 1.3 Hz, 1H), 6.47 (br s, 1H), 3.61 (br s, 4H), 3.41 (s, 3H), 2.96 (br s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 171.2, 154.4, 142.3, 136.9, 136.7, 128.8, 127.4, 127.1, 126.0, 124.8, 121.0, 120.3, 111.6, 109.8, 109.3, 102.2, 46.5, 28.9. m/z calculated for [C21H20N4O5S] = 440.1154, found 440.1242.

<u>COMPOUND 2 (TH1092)</u>*: 6-[(4-phenylpiperazin-1-yl)sulfonyl]-2,3-dihydro-1,3-benzoxazol-2-one*



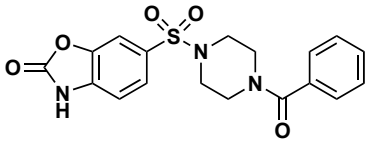
1-phenylpiperazine (33 µL, 0.214 mmol) is added to the solution of 2-oxo-2,3-dihydro-1,3-benzoxazole-6-sulfonyl chloride (50 mg, 0.214 mmol) in CH_2Cl_2 (1 mL) and Et_3N (45 µL, 0.321 mmol). The reaction mixture is stirred at room temperature for 16 h and purified by column chromatography on silica gel (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 1 to 3% of MeOH) to give 5 mg (6% yield) of the title compound.
LC-MS (system A): *m/z* = 360 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.68 (d, *J*=1.4 Hz, 1H), 7.57 (dd, *J*=8.2, 1.7 Hz, 1H), 7.32 (d, *J*=8.2 Hz, 1H), 7.15 - 7.22 (m, 2H), 6.86 - 6.94 (m, 2H), 6.76 - 6.84 (m, 1H), 3.17 - 3.24 (m, 4H), 2.96 - 3.04 (m, 4H). m/z calculated for [C17H17N3O4S] = 359.0940, found 359.1046.

COMPOUND 3 (TH5724): *6-[(4-cyclohexylpiperazin-1-yl)sulfonyl]-2,3-dihydro-1,3-benzoxazol-2-one*



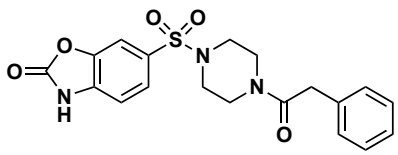
1-cyclohexylpiperazine (39.6 mg, 0.235 mmol) is added to the solution of 2-oxo-2,3-dihydro-1,3-benzoxazole-6-sulfonyl chloride (50 mg, 0.214 mmol) in CH_2Cl_2 (2 mL) and Et_3N (45 µL, 0.321 mmol). The reaction mixture is stirred overnight at room temperature and then purified by column chromatography on silica gel, (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 1 to 10% of MeOH) to give 34 mg (43% yield) of the title compound.
LC-MS (system A): *m/z* = 366 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.60 (d, *J*=1.7 Hz, 1H), 7.51 (dd, *J*=8.1, 1.7 Hz, 1H), 7.29 (d, *J*=8.2 Hz, 1H), 2.77 - 2.88 (m, 4H), 2.52 - 2.58 (m, 4H), 2.15 - 2.27 (m, 1H), 1.60 - 1.73 (m, 4H), 1.47 - 1.57 (m, 1H), 0.94 - 1.23 (m, 5H). m/z calculated for [C17H23N3O4S] = 365.1409, found 365.1515.

COMPOUND 6 (TH1319)*: 6-[(4-benzoylpiperazin-1-yl)sulfonyl]-2,3-dihydro-1,3-benzoxazol-2-one*



T3P® (50% in EtOAc, 56 µL, 0.188 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol), benzoic acid (22.9 mg, 0.188 mmol) and Et_3N (66 µL, 0.469 mmol) in CH_2Cl_2 (1 mL) at 0 °C. The reaction mixture is then stirred at room temperature overnight and purified by column chromatography on silica gel (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 1 to 10% of MeOH) to give 33 mg (54% yield) of the title compound as a white powder.
LC-MS (system A): *m/z* = 388 $[M+H]^+$. m/z calculated for [C18H17N3O5S] = 387.0889, found 387.0978.

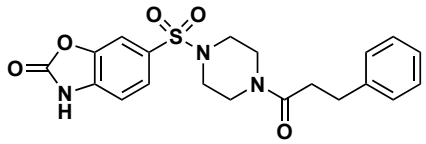
COMPOUND 7 (TH1318): *6-{[4-(2-phenylacetyl)piperazin-1-yl]sulfonyl}-2,3-dihydro-1,3-benzoxazol-2-one*



T3P® (50% in EtOAc, 56 µL, 0.188 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol), 2-phenylacetic acid and Et_3N (65 µL, 0.469 mmol) in CH_2Cl_2 (1 mL) at 0 °C. The reaction mixture is then stirred at room temperature overnight and purified by column chromatography on silica gel

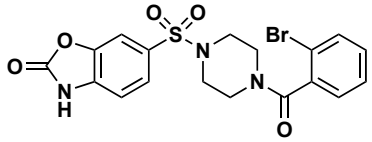
(Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 1 to 10% of MeOH) to give 23 mg (36% yield) of the title compound as a white powder.
LC-MS (system A): *m/z* = 402 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 12.20 (br s, 1H), 7.59 (d, *J*=1.4 Hz, 1H), 7.50 (dd, *J*=8.1, 1.7 Hz, 1H), 7.30 (d, *J*=8.2 Hz, 1H), 7.09 - 7.25 (m, 5H), 3.64 (s, 2H), 3.49 - 3.59 (m, 4H), 2.70 - 2.87 (m, 4H). m/z calculated for [C19H19N3O5S] = 401.1045, found 401.1128.

COMPOUND 8 (TH1320): *6-{[4-(3-phenylpropanoyl)piperazin-1-yl]sulfonyl}-2,3-dihydro-1,3-benzoxazol-2-one*



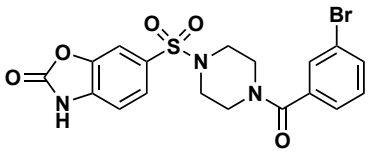
T3P® (50% in EtOAc, 56 µL, 0.188 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol), 3-phenylpropanoic acid (28.2 mg, 0.188 mmol) and Et_3N (65 µL, 0.469 mmol) in CH_2Cl_2 (1 mL) at 0 °C. The reaction mixture is then stirred at room temperature overnight and purified by column chromatography on silica gel (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 1 to 10% of MeOH) to give 28 mg (43% yield) of the title compound as a white powder.
LC/MS (system A): *m/z* = 416 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 12.19 (br s, 1H), 7.61 (s, 1H), 7.51 (d, *J*=8.2 Hz, 1H), 7.31 (d, *J*=8.2 Hz, 1H), 7.04 - 7.22 (m, 5H), 3.43 - 3.57 (m, 4H), 2.67 - 2.87 (m, 6H), 2.52 - 2.59 (m, 2H). m/z calculated for [C20H21N3O5S] = 415.1202, found 415.1285.

COMPOUND 9 (TH7044): *6-{[4-(2-bromobenzoyl)piperazin-1-yl]sulfonyl}-2,3-dihydro-1,3-benzoxazol-2-one*



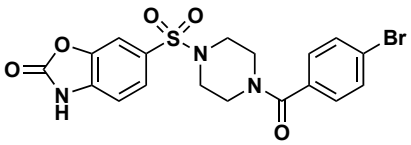
T3P® (50% in EtOAc, 56 µL, 0.188 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol), 2-bromobenzoic acid (31.4 mg, 0.156 mmol) and Et_3N (65 µL, 0.469 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The reaction mixture is then stirred at room temperature overnight and purified by column chromatography on silica gel (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 0 to 10% of MeOH) to give 12 mg (16% yield) of the title compound as a white solid.
LC-MS (system A): *m/z* = 466 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 12.24 (br s, 1H), 7.59 - 7.68 (m, 2H), 7.49 - 7.57 (m, 1H), 7.39 - 7.46 (m, 1H), 7.25 - 7.37 (m, 3H), 3.58 - 3.84 (m, 2H), 3.15 - 3.23 (m, 2H), 2.77 - 3.12 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.9, 154.6, 143.7, 137.7, 135.5, 133.0, 131.3, 128.5, 128.5, 124.8, 118.7, 110.5, 109.3, 46.3, 46.1, 46.0. m/z calculated for [C18H16BrN3O5S] = 464.9994, found 465.0080.

COMPOUND 10 (TH1741): *6-{[4-(3-bromobenzoyl)piperazin-1-yl]sulfonyl}-2,3-dihydro-1,3-benzoxazol-2-one*



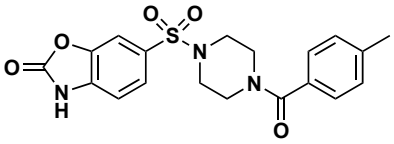
The 3-bromobenzoyl chloride (34.3 mg, 0.156 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol), and Et_3N (65 µL, 0.469 mmol) in CH_3CN (1 mL). The reaction mixture is then stirred at room temperature overnight then purified by column chromatography on silica gel (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 1 to 10% of MeOH) to afford 21 mg (29% yield) of the title compound.
LC-MS (system A): *m/z* = 466 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 12.23 (br s, 1H), 7.61 - 7.69 (m, 2H), 7.51 - 7.58 (m, 2H), 7.29 - 7.41 (m, 3H), 3.57 - 3.79 (m, 2H), 3.35 - 3.47 (m, 2H, obscured by water peak), 2.85 - 3.07 (m, 4H). m/z calculated for [C18H16BrN3O5S] = 464.9994, found 465.0068.

COMPOUND 11 (TH1740): *6-{[4-(4-bromobenzoyl)piperazin-1-yl]sulfonyl}-2,3-dihydro-1,3-benzoxazol-2-one*



4-bromobenzoyl chloride (34 mg, 0.156 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol) and Et_3N (65.4 µL, 0.469 mmol) in CH_3CN (1 mL). The reaction mixture is then stirred at room temperature overnight then purified by column chromatography in silica gel (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 1 to 10% of MeOH) to give 25 mg (34% yield) of the title compound.
LC-MS (system A): *m/z* = 466 $[M+H]^+$. m/z calculated for [C18H16BrN3O5S] = 464.9994, found 465.0066.

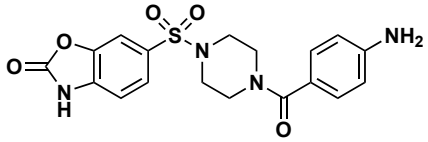
COMPOUND 12 (TH1754): *6-{[4-(4-methylbenzoyl)piperazin-1-yl]sulfonyl}-2,3-dihydro-1,3-benzoxazol-2-one*



T3P® (50% in EtOAc, 56 µL, 0.188 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol), 4-methylbenzoic acid (25.5 mg, 0.188 mmol) and Et_3N (65 µL, 0.469 mmol) in CH_2Cl_2 (1 mL). The reaction mixture is then stirred at room temperature overnight and purified by column

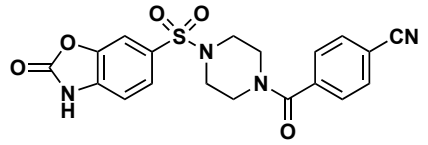
chromatography on silica gel (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 1 to 10% of MeOH) to give 18 mg (29% yield) of the title compound.
LC-MS (system A): *m/z* = 402 $[M+H]^+$. m/z calculated for [C19H19N3O5S] = 401.1045, found 401.1132.

COMPOUND 13 (TH7444): *6-{[4-(4-aminobenzoyl)piperazin-1-yl]sulfonyl}-2,3-dihydro-1,3-benzoxazol-2-one*



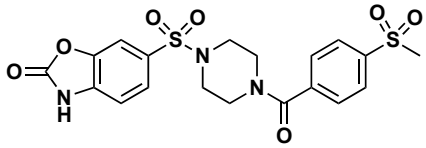
T3P® (50% in EtOAc, 88.3 µL, 0.297 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (70 mg, 0.247 mmol), 4-aminobenzoic acid (33.9 mg, 0.247 mmol) and Et_3N (103 µL, 0.741 mmol) in CH_2Cl_2 (5 mL). The reaction mixture is then stirred at room temperature overnight and purified by column chromatography on silica gel (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 0 to 20% of MeOH) to give 45 mg (45% yield) of the title compound as a white powder.
LC-MS (system A): *m/z* = 403 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.22 (br s, 1H), 7.63 (d, *J*=1.4 Hz, 1H), 7.54 (dd, *J*=8.2, 1.7 Hz, 1H), 7.30 (d, *J*=8.2 Hz, 1H), 7.03 - 7.07 (m, 2H), 6.47 - 6.52 (m, 2H), 5.54 (s, 2H), 3.50 - 3.59 (m, 4H), 2.86 - 2.97 (m, 4H). m/z calculated for [C18H18N4O5S] = 402.0998, found 402.1079.

COMPOUND 14 (TH1743): *4-{4-[(2-oxo-2,3-dihydro-1,3-benzoxazol-6-yl)sulfonyl]piperazine-1-carbonyl}benzonitrile*



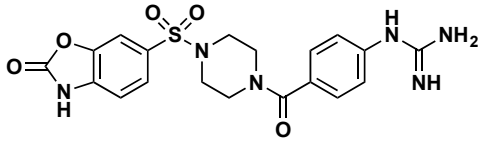
4-cyanobenzoyl chloride (25.9 mg, 0.156 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol) and Et_3N (66 µL, 0.469 mmol) in CH_3CN (1 mL). The reaction mixture is then stirred at room temperature overnight and purified by column chromatography in silica gel (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 1 to 10% of MeOH) to give 19 mg (29% yield) of the title compound.
LC-MS (system A): *m/z* = 413 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.86 - 7.92 (m, 2H), 7.64 (d, *J*=1.4 Hz, 1H), 7.49 - 7.57 (m, 3H), 7.32 (d, *J*=8.2 Hz, 1H), 3.60 - 3.76 (m, 2H), (m, 2H, obscured by water peak), 2.85 - 3.05 (m, 4H). m/z calculated for [C19H16N4O5S] = 412.0841, found 412.0913.

COMPOUND 15 (TH7512): *6-{[4-(4-methanesulfonylbenzoyl)piperazin-1-yl]sulfonyl}-2,3-dihydro-1,3-benzoxazol-2-one*



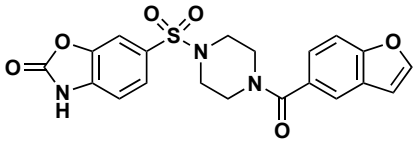
T3P® (50% in EtOAc, 90 µL, 0.151 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one; trifluoroacetic acid (19.9 mg, 0.050 mmol), 4-methanesulfonylbenzoic acid (15 mg, 0.075 mmol) and DIPEA (50 µL, 0.287 mmol) in CH_2Cl_2 (1 mL). The reaction mixture is then stirred at reflux overnight and purified by column chromatography on silica gel (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 1 to 10% of MeOH) to give 6 mg (26% yield) of the title compound.
LC-MS (system A): *m/z* = 466 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.92 - 7.97 (m, 2H), 7.63 (d, *J*=1.6 Hz, 1H), 7.58 - 7.62 (m, 2H), 7.54 (dd, *J*=8.2, 1.7 Hz, 1H), 7.31 (d, *J*=8.2 Hz, 1H), 3.61 - 3.80 (m, 2H), 3.41 - 3.47 (m, 2H, obscured by water peak), 3.24 (s, 3H), 2.80 - 3.09 (m, 4H). m/z calculated for [C19H19N3O7S2] = 465.0664, found 465.0791.

COMPOUND 16 (TH7365): *N-(4-{4-[(2-oxo-2,3-dihydro-1,3-benzoxazol-6-yl)sulfonyl]piperazine-1-carbonyl}phenyl)guanidine*



T3P® (50% in EtOAc, 55.8 µL, 0.188 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol), 4-carbamimidamidobenzoic acid (33.7 mg, 0.156 mmol) and Et_3N (65 µL, 0.469 mmol) in CH_2Cl_2 (4 mL). The reaction mixture is then stirred at room temperature overnight, concentrated under reduced pressure and purified by preparative HPLC (system A) to give 5 mg (6% yield) of the title compound as a TFA salt (white powder). LC-MS (system A): *m/z* = 445 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.88 (br s, 3H), 7.52 - 7.56 (m, 1H), 7.43 - 7.50 (m, 1H), 7.32 - 7.39 (m, 2H), 7.17 - 7.29 (m, 3H), 3.25 - 3.72 (m, 4H, obscured by water peak), 2.81 - 3.04 (m, 4H). m/z calculated for [C19H20N6O5S] = 444.1216, found 444.1304.

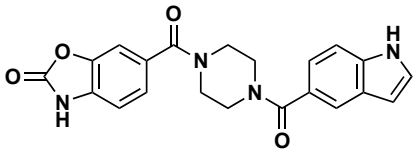
COMPOUND 17 (TH3544): *6-{[4-(1-benzofuran-5-carbonyl)piperazin-1-yl]sulfonyl}-2,3-dihydro-1,3-benzoxazol-2-one*



T3P® (50% in EtOAc, 55.8 µL, 0.188 mmol) is added to the mixture of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol), 1-benzofuran-5-carboxylic acid (30.4 mg, 0.188 mmol) and Et_3N (65 µL, 0.469 mmol) in CH_2Cl_2 (1 mL). The reaction mixture is then stirred at room temperature overnight and purified by column chromatography on silica gel (Biotage SP4, eluent CH_2Cl_2/MeOH, gradient 1 to 10% of MeOH). The impure product obtained is triturated in MeOH and the white precipitate formed is dried off to give 7 mg (11% yield) of the title compound. LC-MS (system A): *m/z* =

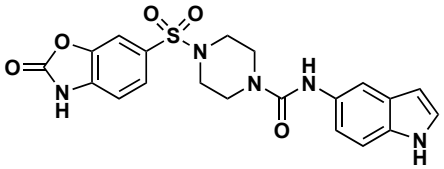
428 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.23 (br s, 1H), 8.07 (d, *J*=2.0 Hz, 1 H), 7.59 - 7.69 (m, 3H), 7.55 (dd, *J*=8.2, 1.4 Hz, 1H), 7.25 - 7.36 (m, 2H), 6.99 (d, *J*=1.4 Hz, 1H), 3.44 - 3.76 (m, 4H), 2.88 - 3.05 (m, 4H). m/z calculated for [C20H17N3O6S] = 427.0838, found 427.0928.

COMPOUND 19 (TH7108): *6-[4-(1H-indole-5-carbonyl)piperazine-1-carbonyl]-2,3-dihydro-1,3-benzoxazol-2-one*



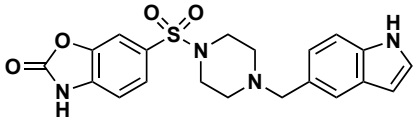
HATU (45.6 mg, 0.120 mmol) is added to the mixture of 2-oxo-2,3-dihydro-1,3-benzoxazole-6-carboxylic acid (17.9 mg, 0.100 mmol), 5-(piperazine-1-carbonyl)-1*H*-indole (22.9 mg, 0.100 mmol) and Et_3N (40 µL, 0.293 mmol) in DMF (1.5 mL) were stirred overnight The reaction mixture is then concentrated under reduced pressure and purified by preparative HPLC (system B) to afford 12 mg (31% yield) of the title compound. LC-MS (system A): *m/z* = 391 [M+H]$^+$. m/z calculated for [C21H18N4O4] = 390.1328, found 390.1398.

COMPOUND 20 (TH7252): *N-(1H-indol-5-yl)-4-[(2-oxo-2,3-dihydro-1,3-benzoxazol-6-yl)sulfonyl]piperazine-1-carboxamide*



Diphosgene (155 mg, 0.0782 mmol) is added to a stirred solution of 1*H*-indol-6-amine (20.7 mg, 0.156 mmol), DIPEA (14 µL, 0.156 mmol) in THF (1 mL). The reaction mixture is stirred for 15 min. before being added to a stirred solution of 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol) and DIPEA (14 µL, 0.156 mmol) in CH_2Cl_2 (3 mL). The mixture is then stirred for 24 h at room temperature. The reaction mixture is then concentrated under reduced pressure and purified by preparative HPLC (system B) to give 8 mg (12% yield) of the title compound as a white solid.
LC-MS (system A): *m/z* = 442 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.20 (br s, 1H), 10.89 (br s, 1H), 8.35 (s, 1H), 7.67 (d, *J*=1.6 Hz, 1H), 7.56 (dd, *J*=8.1, 1.8 Hz, 1H), 7.49 (d, *J*=2.0 Hz, 1H), 7.30 (d, *J*=8.2 Hz, 1H), 7.19 - 7.26 (m, 2H), 7.00 (dd, *J*=8.7, 2.0 Hz, 1H), 6.26 - 6.31 (m, 1H), 3.48 - 3.58 (m, 4H), 2.86 - 2.94 (m, 4H). m/z calculated for [C20H19N5O5S] = 441.1107, found 441.1193.

COMPOUND 21 (TH7257T): *6-({4-[(1H-indol-5-yl)methyl]piperazin-1-yl}sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one*



A mixture of 1*H*-indole-5-carbaldehyde (22.7 mg, 0.156 mmol) and 6-(piperazine-1-sulfonyl)-2,3-dihydro-1,3-benzoxazol-2-one hydrochloride (50 mg, 0.156 mmol) is stirred for 10 min. at RT. Sodium cyanoborohydride (11.8 mg, 0.188 mmol) is then added and the reaction mixture is stirred at room temperature. The reaction mixture is filtered and purified by preparative HPLC (system A) to give 17 mg (21% yield) of the title compound as a TFA salt (pale yellow solid). LC-MS (system A): *m/z* = 413 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.35 (br s, 1H), 11.31 (s, 1H), 7.69 (d, *J*=1.6 Hz, 1H), 7.61 (s, 1H), 7.55 (dd, *J*=8.2, 1.7 Hz, 1H), 7.40 - 7.46 (m, 2H), 7.34 (d, *J*=8.2 Hz, 1H), 7.11 (dd, *J*=8.4, 1.4 Hz, 1H), 6.43 - 6.50 (m, 1H), 4.37 (s, 2H), 3.63 - 3.82 (m, 2H), 3.30 - 3.45 (m, 2H), 3.08 - 3.25 (m, 2H), 2.51 - 2.62 (m, 2H). m/z calculated for [C20H20N4O4S] = 412.1205, found 412.1265.