Supporting Information for:

Activators of the Tumor Cell Specific M2 Isoform of Pyruvate Kinase. Part 1: Substituted *N*,*N*'-Diarylsulfonamides.

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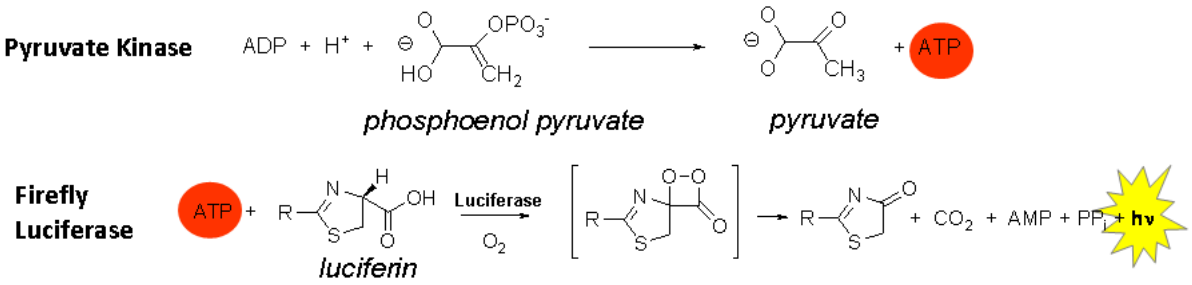


Supporting Figure 1. Pyruvate kinase-luciferase coupled assay

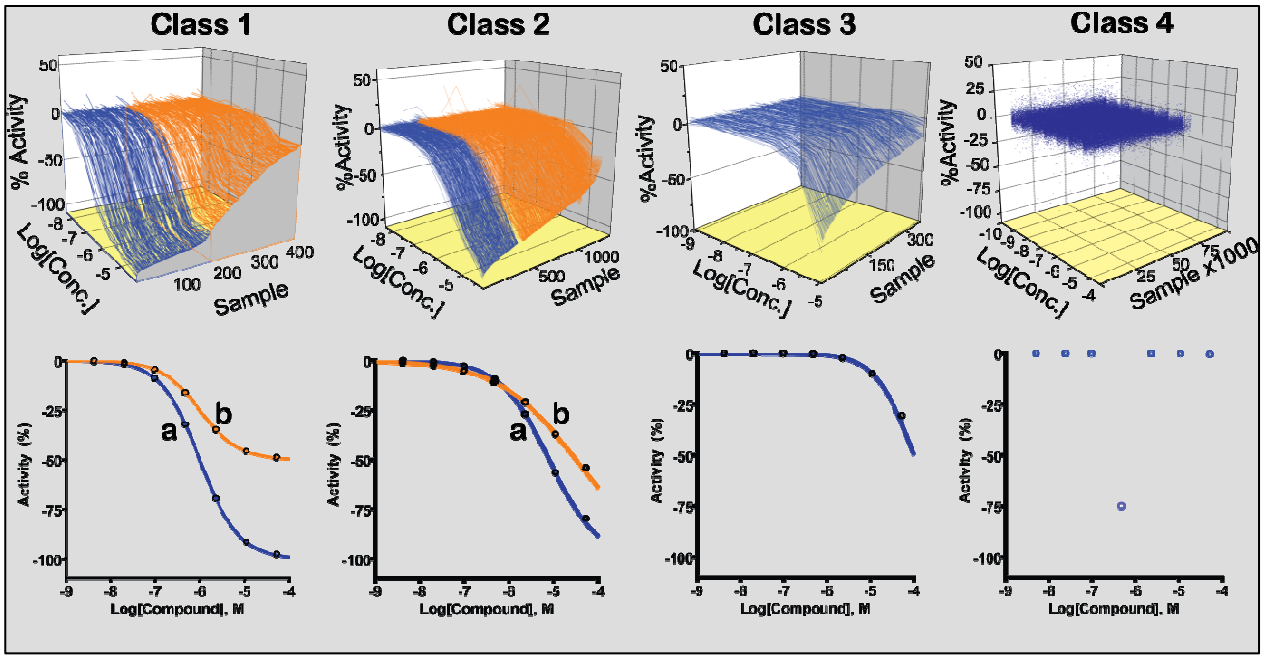
Supporting Table 1: Final 1536-well assay protocol

Step	Parameter	Value	Description
1	Reagent	3 µL	PEP/hPK-M2 buffer (0.5 mM and 0.1 nM final,
2	Controls	23 nl	Activator control (NCGC00031955)
3	Library compounds	23 nl	57 µM to 0.4 nM dilution series
4	Reagent	1 µL	ADP buffer (0.1 mM final)
5	Incubation time	1 hr	Room temperature
6	Reagent	2 µL	Kinase-Glo
7	Assay readout	luminescence	ViewLux

Step	Notes
1	Black walled clear bottom Greiner white solid plates; 4 tips dispense to all wells except column 3 of buffer 0.133 nM hPK-M2, 0.67 mM PEP, 50 mM Imidazole pH 7.2, 50 mM KCl, 7 mM MgCl2, 0.01% Tween, 0.05% BSA. Column 3, 1 tip of same buffer without hPK-M2.
2	Column 1, NCGC0031955 Activator titration 57 µM start, 16 points in duplicate 1:2 dilutions; Column 2 neutral, DMSO only; Column 3 no enzyme; Column 4, top 16 rows are NCGC0031955 at 57 µM, bottom 16 rows are DMSO only.
3	Pintool transfer (tip wash sequence; DMSO, iPA, MeOH, 3-s vacuum dry)
4	ADP in same 4 tips dispense to all wells of buffer containing 0.4 mM ADP (final assay 0.1 mM), 50 mM Imidazole pH 7.2, 50 mM KCl, 7 mM MgCl2, 0.01% Tween, 0.05% BSA.
5	Plates covered with stainless steel rubber gasket-lined lids containing pin holes for gas
6	Kinase-Glo detection. Luciferase-based detection of ATP product

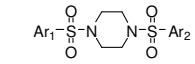
7 Perkin Elmer ViewLux, clear filter luminescent read.

*Identification of NCGC00030335 (**2**):* Following the qHTS the CRC data was subjected to a classification scheme to rank the quality of the CRCs as described by Inglese and co-workers (*Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11473-11478)(see scheme 1). Agents, including NCGC00030335 (**2**), were chosen for follow-up based upon their curve class ranking. Briefly, CRCs are placed into four classes. Class 1 contains complete CRCs showing both upper and lower asymptotes and r^2 values > 0.9. Class 2 contains incomplete CRCs lacking the lower asymptote and shows r^2 values greater than 0.9. Class 3 curves are of the lowest confidence because they are defined by a single concentration point where the minimal acceptable activity is set at 3 SD of the mean activity calculated from the lowest tested concentration. Finally, class 4 contains compounds that do not show any CRCs and are therefore classified as inactive.



Scheme 1: Example qHTS data and classification scheme for assignment of resulting curve-fit data into classes. **Top**, qHTS curve-fit data from AID 361 binned into curve classifications 1-4 based classification criteria. **Below**, Examples of curves fitting the following classification criteria: Class 1 curves display two asymptotes, an inflection point, and r2 ≥0.9; subclasses 1a (blue) vs. 1b (orange) are differentiated by full (>80%) vs. partial (≤ 80%) response. Class 2 curves display a single left-hand asymptote and inflection point; subclasses 2a (blue) and 2b (orange) are differentiated by a max response and r2, >80% and >0.9 or <80% and <0.9, respectively. Class 3 curves have a single left-hand asymptote, no inflection point, and a response >3SD the mean activity of the sample field. Class 4 defines those samples showing no activity across the concentration range.

Table 1. SAR of selected *N*,*N*'-diarylsulfonamides with selected aryl substitutions.



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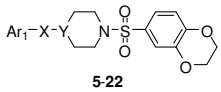
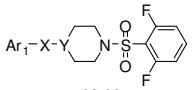


5-22

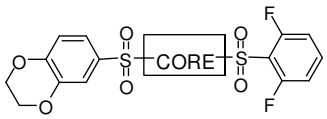


23-33

#	X	Y	Ar_1	Ar_2	*hPK, M2 AC_{50} (μM)*[a]	*hPK, M2 Max. Res.*[b]
2	NA	NA	6-(2,3-dihydrobenzo[*b*][1,4]dioxine)	4-methoxybenzene	0.111 ± 0.03	92.2 ± 12.0
3	NA	NA	6-(2,3-dihydrobenzo[*b*][1,4]dioxine)	6-(2,3-dihydrobenzo[*b*][1,4]dioxine)	0.270 ± 0.08	89.7 ± 2.2
4	NA	NA	4-methoxybenzene	4-methoxybenzene	0.171 ± 0.01	87.6 ± 16.2
5	SO_2	N	4-cyanobenzene	NA	0.029 ± 0.02	44.1 ± 6.1
6	SO_2	N	4-chlorobenzene	NA	0.154 ± 0.08	99.6 ± 2.5
7	SO_2	N	4-fluorobenzene	NA	0.094 ± 0.03	99.7 ± 4.2
8	SO_2	N	3-fluorobenzene	NA	0.316 ± 0	106.7 ± 8.8
9	SO_2	N	2-fluorobenzene	NA	0.089 ± 0.03	114.4 ± 4.0
10	SO_2	N	2,6-difluorobenzene	NA	0.065 ± 0.03	94.4 ± 2.8
11	SO_2	N	2,4,5-trifluorobenzene	NA	0.090 ± 0.01	104.9 ± 7.6
12	SO_2	N	2,6-difluoro-4-methoxybenzene	NA	0.028 ± 0.01	91.8 ± 9.6
13	SO_2	N	2,5-difluoro-4-propylbenzene	NA	0.757 ± 0.22	69.2 ± 10.4
14	SO_2	N	2,6-difluoro-3-phenol	NA	0.052 ± 0.01	95.3 ± 8.4
15	SO_2	N	2,4-difluorobenzene	NA	0.124 ± 0.03	112.9 ± 6.3
16	SO_2	N	phenyl	NA	0.202 ± 0.04	108.2 ± 4.3
17	SO_2	N	3-(trifluoromethyl)benzene	NA	0.209 ± 0.07	39.3 ± 6.2
18	SO_2	N	3-methoxybenzene	NA	0.113 ± 0.04	90.0 ± 4.5
19	SO_2	N	2-pyridine	NA	0.542 ± 0.04	103.1 ± 6.6
20	SO_2	N	2-pyridine 1-oxide	NA	> 10	81.5 ± 3.2
21	SO_2	CH	2,6-difluorobenzene	NA	0.254 ± 0.05	104.3 ± 5.1
22	CO	N	2,6-difluorobenzene	NA	inactive	NA
23	SO_2	N	4-methoxybenzene	NA	0.090 ± 0.02	102.0 ± 9.2
24	SO_2	N	2,6-difluorobenzene	NA	0.066 ± 0.01	74.3 ± 9.8
25	SO_2	N	7-(3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine)	NA	0.103 ± 0.03	100.4 ± 6.6
26	SO_2	N	5-(benzo[*d*][1,3]dioxole)	NA	0.191 ± 0.06	61.0 ± 1.2
27	SO_2	N	7-(4-methyl-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine)	NA	2.71 ± 0.18	93.5 ± 5.9
28	SO_2	N	2-naphthalene	NA	0.066 ± 0	138.0 ± 11.3
29	SO_2	N	6-(2,2-dimethylchroman)	NA	0.093 ± 0.01	119.3 ± 5.4
30	SO_2	N	5-(1-methyl-1*H*-indole)	NA	0.387 ± 0.07	91.1 ± 4.7
31	SO_2	N	6-(2-methylbenzo[*d*]thiazole)	NA	0.086 ± 0.01	103.6 ± 5.8
32	SO_2	CH	6-(2,3-dihydrobenzo[*b*][1,4]dioxine)	NA	0.863 ± 0.12	110.0 ± 5.4
33	CO	N	6-(2,3-dihydrobenzo[*b*][1,4]dioxine)	NA	inactive	NA

[a]AC50 values were determined utilizing the luminescent pyruvate kinase-luciferase coupled assay (ref. 22) and the data represents the results from three separate experiments. See the supporting information section for comparative values from the fluorescent pyruvate kinase-lactate dehydrogenase coupled secondary assay (ref. 27). Max Res. (Maximum Response) is % activity that represents the % activation at 57 μM of compound. See Methods for normalization.

Table 2. SAR of selected *N*,*N*'-diarylsulfonamides with divergent diamine moieties.

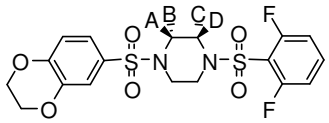


10, 34-47

#	CORE	*hPK, M2 AC_{50} (μM)*[a]	*hPK, M2 Max. Res.*[b]
10	piperazine	0.065 ± 0.03	94.4 ± 2.8
34	1,4-diazepane	0.866 ± 0.15	119.9 ± 7.3
35	*N*,*N*'-(ethane-1,2-diyl)	> 15	60.3 ± 20.6
36	*N*,*N*'-(propane-1,3-diyl)	3.85 ± 0.53	105.7 ± 5.1
37	*N*,*N*'-(butane-1,4-diyl)	7.97 ± 4.05	113.0 ± 14.6
38	*N*,*N*'-(pentane-1,5-diyl)	2.33 ± 0.16	113.9 ± 1.4
39	*N*,*N*'-(hexane-1,6-diyl)	4.83 ± 0.31	110.4 ± 3.0
40	*N*,*N*'-((trans)-cyclohexane-1,4-diyl)	2.11 ± 0.48	90.8 ± 12.4
41	*N*,*N*'-((cis)-cyclohexane-1,4-diyl)	> 35	60.7 ± 5.6
42		9.00 ± 4.5	99.6 ± 3.1
43		3.69 ± 1.26	100.9 ± 1.9
44		> 10	83.7 ± 24.2
45		> 15	82.4 ± 18
46		3.05 ± 0.2	108.3 ± 5.3
47		4.47 ± 0	93.3 ± 9

[a]AC50 values were determined utilizing the luminescent pyruvate kinase-luciferase coupled assay (ref. 22) and the data represents the results from three separate experiments. See the supporting information section for comparative values from the fluorescent pyruvate kinase-lactate dehydrogenase coupled secondary assay (ref. 27). Max Res. (Maximum Response) is % activity at 57 μM of compound. See Methods for normalization.

Table 3. SAR of selected *N*,*N*'-diarylsulfonamides with substitutions on the piperazine ring.



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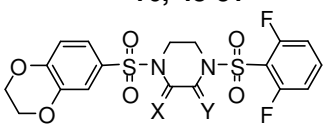
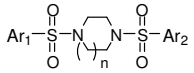


52, 53

#	A	B	C	D	X	Y	*hPK, M2 AC_{50} (μM)*[a]	*hPK, M2 Max. Res.*[b]
10	H	H	H	H	NA	NA	0.065 ± 0.03	94.4 ± 2.8
48	Me	H	H	H	NA	NA	4.34 ± 0.74	109.0 ± 9.4
49	H	Me	H	H	NA	NA	3.10 ± 1.17	98.8 ± 2.6
50	H	H	Me	H	NA	NA	9.18 ± 2.56	107.6 ± 9.9
51	H	H	H	Me	NA	NA	2.93 ± 0.2	107.9 ± 8.4
52	NA	NA	NA	NA	O	NA	0.114 ± 0.02	105.1 ± 9
53	NA	NA	NA	NA	NA	O	2.42 ± 0.16	96.9 ± 5.6

[a]AC50 values were determined utilizing the luminescent pyruvate kinase-luciferase coupled assay (ref. 22) and the data represents the results from three separate experiments. See the supporting information section for comparative values from the fluorescent pyruvate kinase-lactate dehydrogenase coupled secondary assay (ref. 27). Max Res. (Maximum Response) is % activity at 57 μM of compound. See Methods for normalization.

Table 4. SAR of selected *N*,*N*'-diarylsulfonamides including solubility assessment.

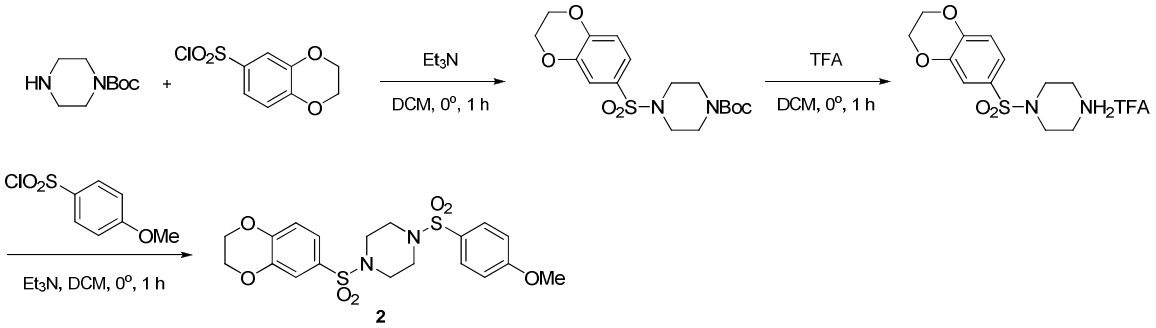


10, 34, 54-59

#	n	Ar_1	Ar_2	*hPK, M2 AC_{50} (μM)*[a]	*hPK, M2 Max. Res.*[b]	Solubility[c] *(μM)*	Solubility[c] *($\mu g/mL$)*
10	1	2,6-difluorobenzene	6-(2,3-dihydrobenzo[*b*][1,4]dioxine)	0.065 ± 0.03	94.4 ± 2.8	< 1.1	< 0.5
34	2	2,6-difluorobenzene	6-(2,3-dihydrobenzo[*b*][1,4]dioxine)	0.866 ± 0.15	119.9 ± 7.3	5.6	2.7
54	1	3-aniline	2,6-difluoro-4-methoxybenzene	0.026 ± 0.003	90.5 ± 2.1	< 0.7	< 0.4
55	1	3-aniline	6-(2,3-dihydrobenzo[*b*][1,4]dioxine)	0.043 ± 0.003	83.7 ± 2.4	7.3	4.1
56	1	3-aniline	2,6-difluorobenzene	0.099 ± 0.006	84.4 ± 3.9	5.7	3.0
57	2	3-aniline	2,6-difluoro-4-methoxybenzene	0.223 ± 0.014	92.5 ± 2.5	26.3	15.1
58	2	3-aniline	6-(2,3-dihydrobenzo[*b*][1,4]dioxine)	0.038 ± 0.007	81.7 ± 3.3	51.2	29.0

[a]AC50 values were determined utilizing the luminescent pyruvate kinase-luciferase coupled assay (ref. 22) and the data represents the results from three separate experiments. See the supporting information section for comparative values from the fluorescent pyruvate kinase-lactate dehydrogenase coupled secondary assay (ref. 27). [b] Max. Res. value represents the % activation at 57 μM of compound. [c] kinetic solubility analysis was performed by Analiza Inc. and are based upon quantitative nitrogen detection as described (www.analiza.com). The data represents results from three separate experiments with an average intraassay %CV of 4.5%. [b] LogD analysis was performed by Analiza Inc. and are based upon octanol/buffer partitioning and quantitative nitrogen detection of sample content as described (www.analiza.com). NT = not tested.

General Methods. All air or moisture sensitive reactions were performed under positive pressure of nitrogen with oven-dried glassware. Anhydrous solvents such as tetrahydrofuran (THF), toluene, dichloromethane, *N,N*-dimethylforamide (DMF), acetonitrile, methanol and triethylamine were obtained by purchasing from Sigma-Aldrich. Preparative purification was performed on a Waters semi-preparative HPLC. The column used was a Phenomenex Luna C18 (5 micron, 30 x 75 mm) at a flow rate of 45 mL/min. The mobile phase consisted of acetonitrile and water (each containing 0.1% trifluoroacetic acid). A gradient of 10% to 50% acetonitrile over 8 minutes was used during the purification. Fraction collection was triggered by UV detection (220 nM). Analytical analysis was performed on an Agilent LC/MS (Agilent Technologies, Santa Clara, CA). Method 1: A 7 minute gradient of 4% to 100% Acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with an 8 minute run time at a flow rate of 1 mL/min. A Phenomenex Luna C18 column (3 micron, 3 x 75 mm) was used at a temperature of 50°C. Method 2: A 3 minute gradient of 4% to 100% Acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with a 4.5 minute run time at a flow rate of 1 mL/min. A Phenomenex Gemini Phenyl column (3 micron, 3 x 100 mm) was used at a temperature of 50 °C. Purity determination was performed using an Agilent Diode Array Detector. Mass determination was performed using an Agilent 6130 mass spectrometer with electrospray ionization in the positive mode. ^{1}H NMR spectra were recorded on Varian 400 MHz spectrometers. Chemical Shifts are reported in ppm with tetramethylsilane (TMS) as internal standard (0 ppm) for CDCl3 solutions or undeuterated solvent (DMSO-h6 at 2.49 ppm) for DMSO-d6 solutions. All of the analogs for assay have purity greater than 95% based on both analytical methods. High resolution mass spectrometry was recorded on Agilent 6210 Time-of-Flight LC/MS system. Confirmation of molecular formula was accomplished using electrospray ionization in the positive mode with the Agilent Masshunter software (version B.02).



Most bis-sulfonamides were synthesized by a three-step, two-pot procedure (Method A and Method B) exemplified by the synthesis of **2**, shown below:

Method A:

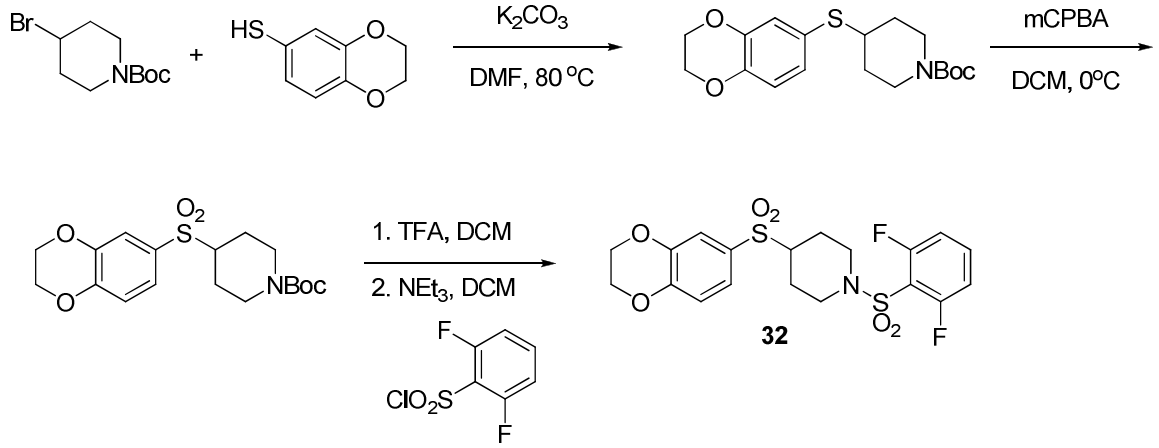
1-Boc-piperazine (250 mg, 1.34 mmol, 1 equiv.) was dissolved in dichloromethane (2.5 mL) and cooled in an ice bath under nitrogen atmosphere. Triethylamine (375 µl, 2.68 mmol, 2.0 equiv.) was added followed by portionwise addition of 2,3-

dihydrobenzo[b][1,4]dioxine-6-sulfonyl chloride (346 mg, 1.48 mmol, 1.1 equiv.). The reaction was stirred in the ice bath for one hour, then quenched with saturated aqueous ammonium chloride (~3 mL). The organic layer was washed twice with saturated ammonium chloride, once with brine, dried over sodium sulfate and concentrated *in vacuo* and then purified on silica gel chromatography using a 95/5 – 5/95, hexane/EtOAc (v/v) gradient to give 1-boc-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazine as a white powder (516 mg, 89% yield).

Method B:

1-Boc-4-(2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonyl)piperazine (400 mg, 1.04 mmol) was dissolved in dichloromethane (1 mL) and cooled in an ice bath. Trifluoroacetic acid (1 mL) was then added and the solution was stirred in the ice bath. The reaction was monitored by TLC and showed completion after one hour. The solution was removed from the ice bath and the solvents removed on *in vacuo* to yield the TFA salt of 1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazine, which was carried onto the next step without purification. The oily residue was dissolved in dichloromethane (2 mL) and cooled in an ice bath. Triethylamine (580 µl, 4.16 mmol, 4 equiv.) was added followed by portionwise addition of 4-methoxybenzene-1-sulfonyl chloride (236 mg, 1.14 mmol, 1.1 equiv.). The progress was monitored by TLC and showed completion after 1 hour. The reaction was quenched with saturated aqueous ammonium chloride (~3 mL). The organic layer was washed twice with saturated ammonium chloride, once with brine, dried over sodium sulfate and concentrated *in vacuo* and then dissolved in DMSO and purified by reverse phase HPLC.

Synthesis of sulfones 21 and 32:



Exemplified by **32**

4-bromo-1-boc piperidine (500 mg, 1.89 mmol, 1 equiv.) and 2,3-dihydrobenzo[b][1,4]dioxine-6-thiol (318 mg, 1.89 mmol, 1 equiv.) were dissolved in DMF (4 mL). Potassium carbonate (392 mg, 2.84 mmol, 1.5 equiv.) was then added and the solution was stirred at 80 °C for 5 hours. The reaction was cooled to room temperature, diluted with ethyl acetate (~10 mL) and water (~10 mL). The organic layer

was washed with saturated aqueous sodium bicarbonate, brine, dried over sodium sulfate and the solvents removed. The crude sulfide was dissolved in dichloromethane (6 mL) and cooled to 0 °C. Solid *m*-CPBA (720 mg, 4.16 mmol, 2.2 equiv. based on initial thiol) was then added and the suspension stirred at 0 °C for 2 hours. The suspension was then filtered and the filtrate was washed with 10% aqueous sodium thiosulfate, aqueous sodium bicarbonate, brine and dried over sodium sulfate. The solvent was removed and the residue was purified by silica gel chromatography using a 95/5 – 5/95, hexane/EtOAc (v/v) gradient to give the desired sulfone. Method B (see above) was then used to cleave the boc-group and introduce the sulfonamide moiety (by using 2,6-difluorobenzenesulfonyl chloride) to give product **32** which was dissolved in DMSO and purfied by reverse phase HPLC.

Synthesis of amides 22 and 33:

These amides were synthesized with the same procedure used for bis-sulfonamides (Method A, then Method B) using with benzoyl chlorides in place of a sulfonyl chloride in Method B.

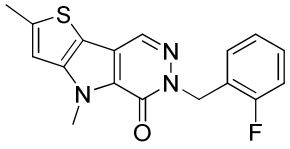
Synthesis of oxo-piperazine derivatives 52 and 53

Exemplified by **52.**

Method A was used to introduce the 2,6-difluorosulfonyl group.

4-(2,6-difluorophenylsulfonyl)piperazin-2-one (500 mg, 1.81 mmol, 1 equiv.) was dissolved in THF (5 mL) and cooled to -78 ^{o}C. LHMDS (1.85 mL of 1.0 M THF solution, 1.9 mmol, 1.05 equiv.) was then added dropwise and the solution stirred at -78 ^{o}C for 1 h. A solution of 2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonyl chloride (510 mg, 2.17 mmol, 1.2 equiv.) in THF (2 mL) was then added drop-wise to the cold solution. The reaction was stirred at -78 ^{o}C for 15 minutes then allowed to warm to room temperature and stirred an additional 1 h. The reaction was carefully quenched with saturated aqueous ammonium chloride (~5 mL), and diluted with ethyl acetate (~15 mL). The organic layer was washed twice with saturated aqueous ammonium chloride, once with brine, dried over sodium sulfate and concentrated. The residue was dissolved in DMSO and purified by reverse phase HPLC.

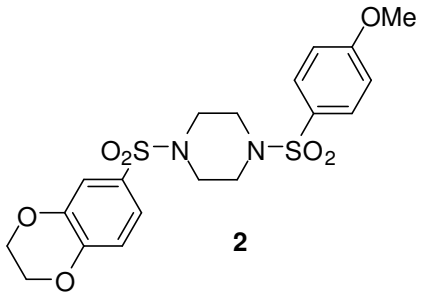
Compound Data:



1

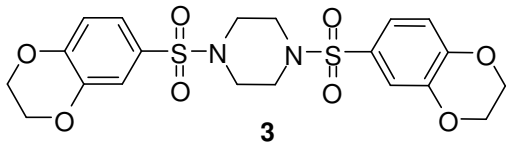
2,4-Methyl-6-[(2-fluorophenyl)methyl]-4H-thieno[3,2-b]pyrrole[3,2-d]pyridazinone (1). For the synthesis of **1** see supporting information in the article directly following this manuscript. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.26-7.19 (m, 2H), 7.09-7.02 (m, 2H), 6.92 (q, 1 H, *J*= 1.2 Hz), 5.53 (s, 2H), 4.27 (s, 3H), 2.64 (d, 3H, *J*= 1.2 Hz);

LC/MS: Method 1, retention time: 6.313 min; Method 2, retention time: 3.992 min; HRMS: *m/z* ($M+H^+$) = 328.0925 (Calculated for $C_{17}H_{15}FN_3OS$ = 328.0920).



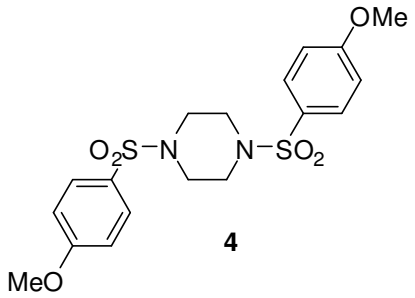
2

1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-4-(4-methoxyphenylsulfonyl)piperazine (**2**). ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 7.67-7.57 (m, 2H), 7.19-7.08 (m, 4H), 7.08-7.01 (m, 1H), 4.45-4.23 (m, 4H), 3.86 (s, 3H), 2.94 (m, 8H). LC/MS: Method 1, retention time: 5.744 min; Method 2, retention time: 3.889 min. HRMS: *m/z* (M+) = 454.0872 (Calculated for $C_{19}H_{22}N_2O_7S_2$ = 454.0868).



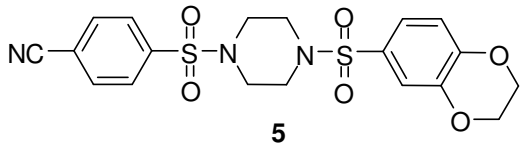
3

1,4-bis(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazine (3). ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 7.70-7.61 (m, 2H), 7.61-7.52 (m, 1H), 7.22-7.12 (m, 2H), 7.12-7.05 (m, 1H), 4.35 (m, 8H), 3.44-3.36 (m, 4H), 3.00 - 2.88 (m, 4H). LC/MS: Method 1, retention time: 6.114 min; Method 2, retention time: 3.961 min. HRMS: *m/z* (M+) = 482.0816 (Calculated for $C_{20}H_{22}N_2O_8S_2$ 482.0818).



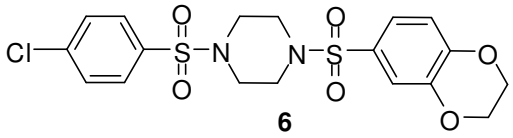
4

1,4-bis(4-methoxyphenylsulfonyl)piperazine (4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.58 (d, 4H, *J* = 6.9 Hz), 7.08 (d, 4H, *J* = 8.4 Hz), 3.82 (s, 6H), 2.91 (s, 8H). LC/MS: Method 1, retention time: 5.828 min; Method 2, retention time: 3.895 min.



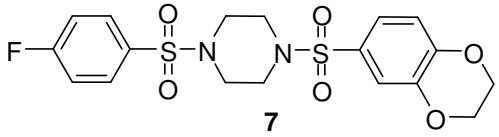
5

4-(4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazin-1-ylsulfonyl)benzonitrile (5). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.07 (d, 2H, *J* = 8.4 Hz), 7.84 (d, 2H, *J* = 8.4 Hz), 7.10 (m, 2H), 7.00 (m, 1H), 4.31 (m, 4H), 3.03 - 2.91 (m, 8H). LC/MS: Method 1, retention time: 5.671 min; Method 2, retention time: 3.879 min. HRMS: *m/z* (M+) = 449.0716 (Calculated for $C_{19}H_{19}N_3O_6S_2$ = 449.0715).



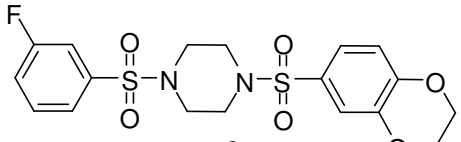
6

1-(4-chlorophenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazine (6). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 7.67 (b, 4H), 7.12 (m, 2H), 7.03 (m, 1H), 4.32 (m, 4H), 2.95 (m, 8H). LC/MS: Method 1, retention time: 6.114 min; Method 2, retention time: 3.959 min. HRMS: *m/z* (M+) = 458.0380 (Calculated for $C_{18}H_{19}ClN_2O_6S_2$ = 458.0373).



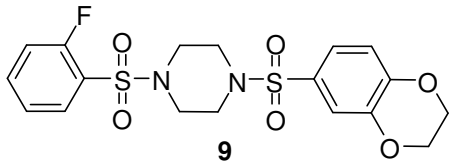
7

1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-4-(4-fluorophenylsulfonyl)piperazine (7). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 7.72 (m, 2H), 7.43 (m, 2H), 7.18-7.09 (m, 2H), 7.09-7.01 (m, 1H), 4.32 (m, 4H), 2.93 (m, 8H). LC/MS: Method 1, retention time: 5.813 min; Method 2, retention time: 3.893 min. HRMS: *m/z* (M+) = 442.0677 (Calculated for $C_{18}H_{19}FN_2O_6S_2$ = 442.0669).



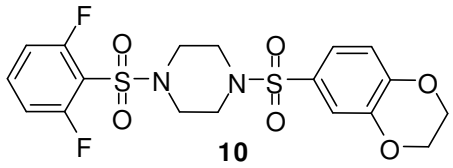
8

1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-4-(3-fluorophenylsulfonyl)piperazine (8). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 7.75-7.64 (m, 1H), 7.64-7.49 (m, 3H), 7.18-7.09 (m, 2H), 7.09-7.01 (m, 1H), 4.43-4.25 (m, 4 H), 3.12 - 3.00 (m, 4H), 2.99-2.82 (m, 4H). LC/MS: Method 1, retention time: 5.853 min; Method 2, retention time: 3.911 min. HRMS; *m/z* (M+) = 442.0662 (Calculated for $C_{18}H_{19}FN_2O_6S_2$ = 442.0669).



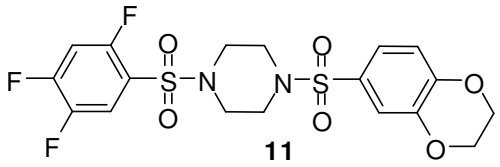
9

1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-4-(2-fluorophenylsulfonyl)piperazine (9). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 7.84 - 7.68 (m, 2H), 7.52 - 7.36 (m, 2H), 7.21 - 7.11 (m, 2H), 7.10 - 7.02 (m, 1H), 4.44 - 4.25 (m, 4H), 3.23 - 3.07 (m, 4H), 3.04 - 2.87 (m, 4H), LC/MS: Method 1, retention time: 5.775 min; Method 2, retention time: 3.891 min. HRMS; *m/z* (M+) = 442.0664 (Calculated for $C_{18}H_{19}FN_2O_6S_2$ = 442.0669).



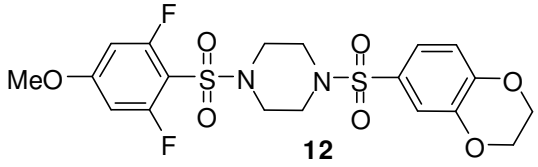
10

1-(2,6-difluorophenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazine (10). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.55 (m, 1H), 7.24 (m, 2H), 7.00 (m, 3H), 4.33 (m, 4H), 3.38 (m, 4H), 3.13 (m, 4H). LC/MS: Method 1, retention time: 5.781 min; Method 2, retention time: 3.889 min. HRMS: *m/z* (M+) = 460.0570 (Calculated for $C_{18}H_{18}F_2N_2O_6S_2$ = 460.0574).



11

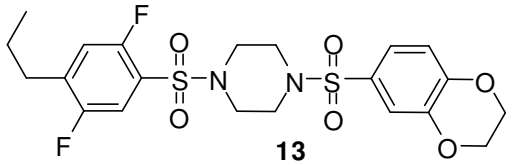
1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-4-(2,4,5-trifluorophenylsulfonyl)piperazine (11). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.00 - 7.76 (m, 2H), 7.22 - 7.12 (m, 2H), 7.11 - 7.04 (m, 1H), 4.34 (dd, 4H, *J*= 12.13, 5.09 Hz), 3.24 - 3.14 (m, 4H), 3.04 – 2.87 (m, 4H). LC/MS: Method 1, retention time: 6.076 min; Method 2, retention time: 3.936 min. HRMS; *m/z* (M+) = 478.0495 (Calculated for $C_{18}H_{17}F_3N_2O_6S_2$ = 478.0480).



12

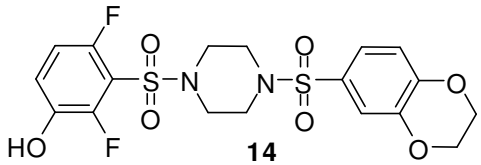
1-(2,6-difluoro-4-methoxyphenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazine (12). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.22 (m, 2H), 6.97 (m, 1H), 6.53 (d, 2H, *J* = 10.56 Hz), 4.26 (m, 4H), 3.87 (s, 3H), 3.31 (m, 4H), 3.11 (m, 4H).

LC/MS: Method 1, retention time: 5.922 min; Method 2, retention time: 3.911 min. HRMS; *m/z* (M+) = 490.0698 (Calculated for $C_{19}H_{20}F_2N_2O_7S_2$ = 490.0680).



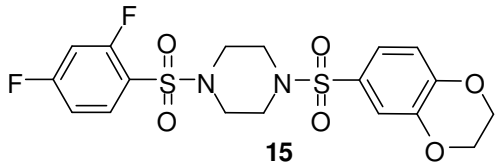
13

1-(2,5-difluoro-4-propylphenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazine (13). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44 (dd, 1H, *J* = 8.41, 5.67 Hz), 7.23 (m, 2H), 7.06 (dd, 1H, *J* = 10.17, 5.48 Hz), 6.98 (d, 1H, *J* = 8.22 Hz), 4.32 (m, 4H), 3.30 (m, 4H), 3.11 (m, 4H), 2.66 (t, 2H, *J* = 7.43 Hz), 1.66 (m, 2H), 0.98 (t, 3H, *J* = 7.43 Hz). LC/MS: Method 1, retention time: 6.737 min; Method 2, retention time: 4.055 min. HRMS: *m/z* (M+) = 502.1057 (Calculated for $C_{21}H_{24}F_2N_2O_6S_2$ = 502.1044).



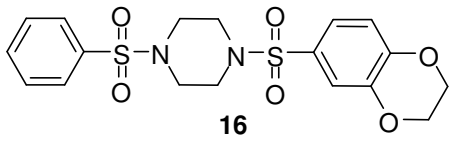
14

3-(4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazin-1-ylsulfonyl)-2,4-difluorophenol (14). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.25 (m, 3H), 7.05 (m, 2H), 4.33 (m, 4H), 3.37 (m, 4H), 3.13 (m, 4H), 1.84 (b, 1H). LC/MS: Method 1, retention time: 5.783 min; Method 2, retention time: 3.888 min. HRMS: *m/z* (M+) = 476.0542 (Calculated for $C_{18}H_{18}F_2N_2O_7S_2$ = 476.0523).



15

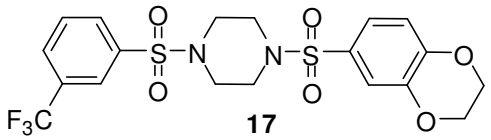
1-(2,4-difluorophenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazine (15). ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 7.81 (m, 1H), 7.57 (ddd, 1H, *J* = 10.96, 9.00, 2.35 Hz) 7.32 (td, 1H, *J* = 8.51, 2.15 Hz), 7.23-7.11 (m, 2H), 7.11-7.01 (m, 1H), 4.40 - 4.27 (m, 4H), 3.22 - 3.08 (m, 4H), 3.03 - 2.80 (m, 4H), LC/MS: Method 1, retention time: 5.910 min; Method 2, retention time: 3.910 min. HRMS: *m/z* (M+) = 460.0585 (Calculated for $C_{18}H_{18}F_2N_2O_6S_2$ = 460.0574).



16

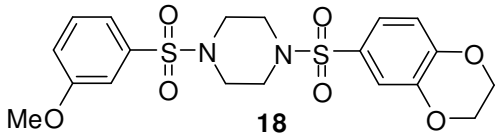
1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-4-(phenylsulfonyl)piperazine (16). ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 7.73 – 7.64 (m, 3H), 7.59 (m, 2H), 7.13 - 7.07 (m,

2H), 7.01 (m, 1H) 4.30 (m, 4H), 2.98 – 2.88 (m, 8H). LC/MS: Method 1, retention time: 5.706 min; Method 2, retention time: 3.883 min. HRMS: *m/z* (M+) = 424.0769 (Calculated for $C_{18}H_{20}N_2O_6S_2$ = 424.0763).



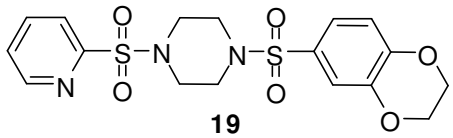
17

1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-4-(3-(trifluoromethyl)phenylsulfonyl)piperazine (17). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.89 (m, 2H), 7.70 (m, 1H), 7.20 (m, 2H), 6.96 (d, 1H, *J* = 8.61 Hz), 4.31 (m, 4H), 3.11 (m, 8H). LC/MS: Method 1, retention time: 6.249 min; Method 2, retention time: 3.920 min. HRMS: *m/z* (M+) = 492.0654 (Calculated for $C_{19}H_{19}F_3N_2O_6S_2$ = 492.0637).



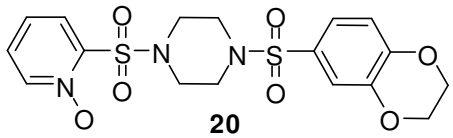
18

1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-4-(3-methoxyphenylsulfonyl)piperazine (18). ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 7.50 (m, 1H), 7.24 (m, 2H), 7.09 (m, 3H), 7.01 (m, 1H) 4.31 (m, 4H), 3.80 (s, 3H), 2.99 (m, 4H), 2.89 (m, 4H). LC/MS: Method 1, retention time: 5.819 min; Method 2, retention time: 3.902 min. HRMS: *m/z* (M+) = 454.0878 (Calculated for $C_{19}H_{22}N_2O_7S_2$ = 454.0868).



19

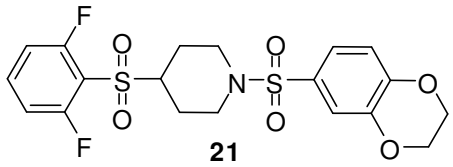
1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-4-(pyridin-2-ylsulfonyl)piperazine (19). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.68 (d, 1H, *J* = 4.7 Hz), 7.92 (m, 2H), 7.51 (m, 1H), 7.24 (m, 2H), 6.99 (d, 1H, *J* = 8.61 Hz), 4.33 (m, 4H), 3.44 (m, 4H), 3.09 (m, 4H). LC/MS: Method 1, retention time: 5.205 min; Method 2, retention time: 3.772 min. HRMS: *m/z* (M+) = 425.0720 (Calculated for $C_{17}H_{19}N_3O_6S_2$ = 425.0715).



20

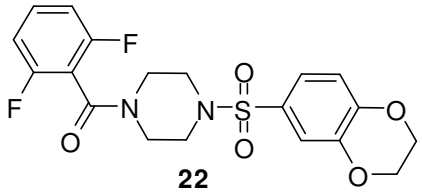
2-(4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazin-1-ylsulfonyl)pyridine 1-oxide (20). ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 8.27 (m, 1H), 7.90 (m, 1H), 7.57 (m, 1H), 7.41 (m, 1H), 7.06 (m, 3H), 4.30 (m, 4H), 3.40 (m, 4H), 2.87 (m, 4H). LC/MS: Method 1,

retention time: 4.618 min; Method 2, retention time: 3.630 min. HRMS: *m/z* (M+) = 441.0669 (Calculated for $C_{17}H_{19}N_3O_7S_2$ = 441.0664).



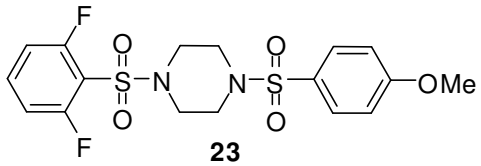
21

4-(2,6-difluorophenylsulfonyl)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperidine (21). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.63 (m, 1H), 7.24 (m, 2H), 7.06 (t, 2H, *J* = 8.61 Hz), 6.96 (d, 1H, *J* = 8.61 Hz), 4.31 (m, 4H), 3.88 (d, 2H, *J* = 12.1 Hz), 3.08 (m, 1H), 2.40 (td, 2H, *J* = 11.93, 2.35 Hz), 2.14 (m, 2H).1.96 (m, 2H). LC/MS: Method 1, retention time: 5.561 min; Method 2, retention time: 3.847 min. HRMS: *m/z* (M+) = 459.0634 (Calculated for $C_{19}H_{19}F_2NO_6S_2$ = 459.0622).



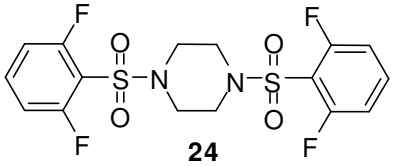
22

1-(2,6-difluorophenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazin-2-one (22). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 (m, 1H), 7.23 (m, 2H), 6.95 (m, 3H), 4.34 (m, 4H), 3.92 (m, 2H), 3.20 (m, 2H), 3.12 (m, 2H), 3.02 (m, 2H). LC/MS: Method 1, retention time: 5.387 min; Method 2, retention time: 3.815 min. HRMS: *m/z* (M+) = 424.0901 (Calculated for $C_{19}H_{18}F_2N_2O_5S$ = 424.0919).



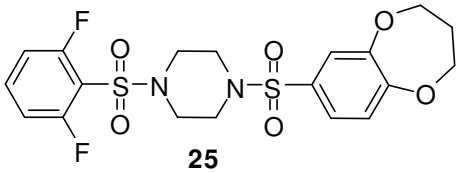
23

1-(2,6-difluorophenylsulfonyl)-4-(4-methoxyphenylsulfonyl)piperazine (23). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (m, 2H), 7.54 (m, 2H), 7.02 (m, 3H), 3.88 (s, 3H), 3.35 (m, 4H), 3.09 (m, 4H). LC/MS: Method 1, retention time: 5.829 min; Method 2, retention time: 3.904 min. HRMS: *m/z* (M+) = 432.0633 (Calculated for $C_{17}H_{18}F_2N_2O_5S_2$ = 432.0625).



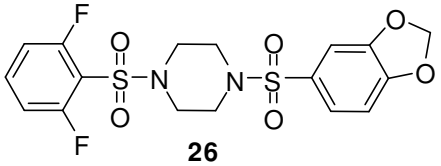
24

1,4-bis(2,6-difluorophenylsulfonyl)piperazine (24). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54 (m, 2H), 7.04 (t, 4H, *J* = 12 Hz), 3.40 (s, 8H). LC/MS: Method 1, retention time: 5.851 min; Method 2, retention time: 3.911 min. HRMS: *m/z* (M+) = 438.0331 (Calculated for $C_{16}H_{14}F_4N_2O_4S_2$ = 438.034).



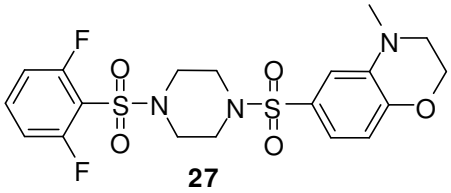
25

1-(2,6-difluorophenylsulfonyl)-4-(3,4-dihydro-2H-benzo[b][1,4]dioxepin-7-ylsulfonyl)piperazine (25). ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 7.72 (m, 1H), 7.31 – 7.18 (m, 4H), 7.11 (d, 1H, *J* = 8.4 Hz), 4.22 (dt, *J* = 17.8 Hz, 4 Hz), 3.18 (m, 4H), 2.98 (m, 4H), 2.14 (m, 2H). LC/MS: Method 1, retention time: 5.973 min; Method 2, retention time: 3.925 min. HRMS: *m/z* (M+) = 474.0747 (Calculated for $C_{19}H_{20}F_2N_2O_6S_2$ = 474.0731).



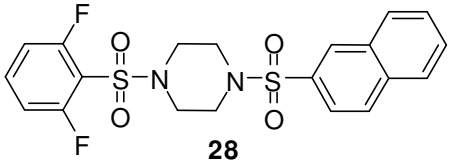
26

1-(benzo[d][1,3]dioxol-5-ylsulfonyl)-4-(2,6-difluorophenylsulfonyl)piperazine (26). ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 7.75 (m, 1H), 7.29 (m, 2H), 7.22 (m, 1H), 7.16 (m, 1H), 7.07 (d, 1H, *J* = 8.2 Hz), 6.17 (s, 2H), 3.17 (m, 4H), 2.99 (m, 4H). LC/MS: Method 1, retention time: 5.741 min; Method 2, retention time: 3.879 min. HRMS: *m/z* (M+) = 446.0427 (Calculated for $C_{17}H_{16}F_2N_2O_6S_2$ = 446.0418).



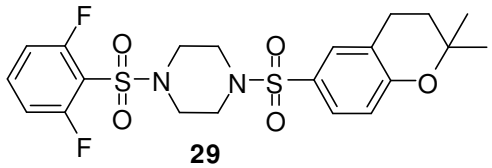
27

6-(4-(2,6-difluorophenylsulfonyl)piperazin-1-ylsulfonyl)-4-methyl-3,4-dihydro-2H-benzo[b][1,4]oxazine (27). ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 7.74 (m, 1H), 7.29 (m, 2H), 6.89 - 6.76 (m, 3H), 4.27 (m, 2H), 3.28 (m, 2H), 3.17 (m, 4H), 2.96 (m, 4H), 2.84 (s, 3H). LC/MS: Method 1, retention time: 5.514 min; Method 2, retention time: 3.813 min. HRMS: *m/z* (M+) = 473.0897 (Calculated for $C_{19}H_{21}F_2N_3O_5S_2$ = 473.0891).



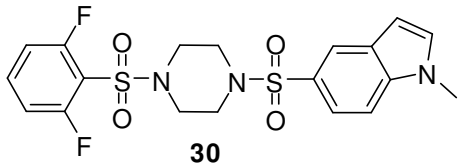
28

1-(2,6-difluorophenylsulfonyl)-4-(naphthalen-2-ylsulfonyl)piperazine (28). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.39 (s, 1H), 8.18 – 8.03 (m, 3H), 7.76 – 7.56 (m, 4H), 7.14 (m, 2H), 3.20 – 3.17 (m, 8H). LC/MS: Method 1, retention time: 5.532 min; Method 2, retention time: 3.814 min. HRMS: *m/z* (M+) = 452.0673 (Calculated for $C_{20}H_{18}F_2N_2O_4S_2$ = 452.0676).



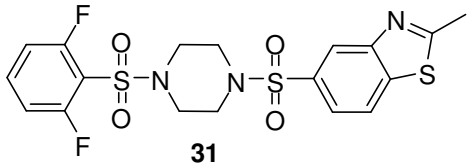
29

1-(2,6-difluorophenylsulfonyl)-4-(2,2-dimethylchroman-6-ylsulfonyl)piperazine (29). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54 (m, 1H), 7.43 (m, 2H), 7.03 (m, 2H), 6.5 (d, 1H, *J* = 8.4 Hz), 3.36 (m, 4H), 3.11 (m, 4H), 2.81 (m, 2H), 1.84 (m, 2H), 1.36 (s, 6H). LC/MS: Method 1, retention time: 5.514 min; Method 2, retention time: 3.811 min. HRMS: *m/z* (M+) = 486.1100 (Calculated for $C_{21}H_{24}F_2N_2O_5S_2$ = 486.1095).



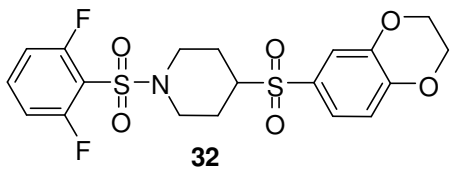
30

5-(4-(2,6-difluorophenylsulfonyl)piperazin-1-ylsulfonyl)-1-methyl-1H-indole (30). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 7.94 (s, 1H), 7.62 (m, 2H), 7.54 (d, 1H, *J* = 3.1 Hz), 7.42 (m, 1H), 7.19 (t, 2H, *J* = 9.0 Hz), 6.63 (d, 1H, *J* = 2.9 Hz), 3.85 (s, 3H), 3.15 (m, 4H), 2.95 (m, 4H). LC/MS: Method 1, retention time: 5.893 min; Method 2, retention time: 3.914 min. HRMS: *m/z* (M+) = 455.0793 (Calculated for $C_{19}H_{19}F_2N_3O_4S_2$ = 455.0785).



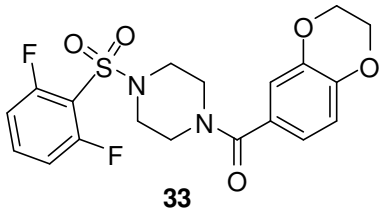
31

5-(4-(2,6-difluorophenylsulfonyl)piperazin-1-ylsulfonyl)-2-methylbenzo[d]thiazole (31). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 8.51 (s, 1H), 8.07 (m, 1H), 7.73 (m, 1H), 7.64 (m, 1H), 7.20 (m, 2H), 3.09 (m, 8H), 2.86 (s, 3H). LC/MS: Method 1, retention time: 5.729 min; Method 2, retention time: 3.882 min; HRMS: *m/z* (M+) = 473.0353 (Calculated for $C_{18}H_{17}F_2N_3O_4S_3$ = 473.0349).



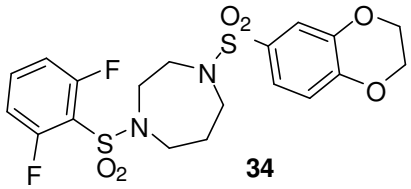
32

1-(2,6-difluorophenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperidine (32). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (m, 1H), 7.35 – 7.26 (m, 2H), 7.07 – 6.97 (m, 3H), 4.40 – 4.28 (m, 4H), 4.08 - 4.00 (m, 2H), 2.92 (m, 1H), 2.66 (t, 2H, *J* = 11.93 Hz), 2.17 – 2.08 (m, 2H), 1.80 – 1.67 (m, 2H). LC/MS: Method 1, retention time: 5.584 min; Method 2, retention time: 3.853 min; HRMS: *m/z* (M+) = 459.0631 (Calculated for $C_{19}H_{19}F_2NO_6S_2$ = 459.0622).



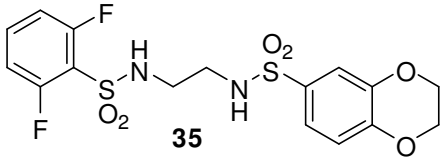
33

(4-(2,6-difluorophenylsulfonyl)piperazin-1-yl)(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)methanone (33). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.56 (m, 1H), 7.06 (m, 2H), 6.91 (s, 1H), 6.87 (m, 2h), 4.27 (m, 4H), 3.74 (m, 4H), 3.26 (m, 4H), 3.12 (m, 2H), 3.02 (m, 2H). LC/MS: Method 1, retention time: 5.279 min; Method 2, retention time: 3.792 min. HRMS: *m/z* (M+) = 424.0897 (Calculated for $C_{19}H_{18}F_2N_2O_5$ = 424.0904).



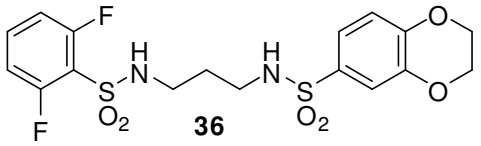
34

1-(2,6-difluorophenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-1,4-diazepane (34). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.50 (m, 1H), 7.28 (m, 2H), 7.02 (m, 2H), 6.96 (d, 1H, *J* = 8.6 Hz), 4.32 (m, 4H), 3.56 (m, 4H), 3.41 (m, 4H), 2.05 (m, 2H). LC/MS: Method 1, retention time: 5.812 min; Method 2, retention time: 3.891 min. HRMS: *m/z* (M+) = 474.0731 (Calculated for $C_{19}H_{20}F_2N_2O_6S_2$ = 474.0731).



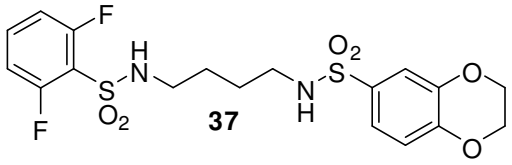
35

N-(2-(2,6-difluorophenylsulfonamido)ethyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (35). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54 (m, 1H), 7.35 (m, 2H), 7.06 (m, 2H), 6.96 (d, 1H, *J* = 8.2 Hz), 5.37 (b, 1H), 4.73 (b, 1H), 4.31 (m, 4H), 3.25 (m, 2H), 3.14 (m, 2H). LC/MS: Method 1, retention time: 4.986 min; Method 2, retention time: 3.711 min. HRMS: *m/z* (M+) = 434.0434 (Calculated for $C_{16}H_{16}F_2N_2O_6S_2$ = 434.0418).



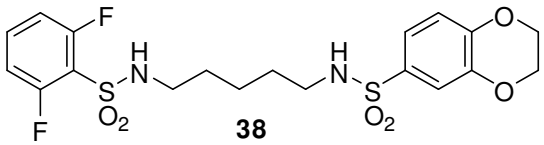
36

N-(3-(2,6-difluorophenylsulfonamido)propyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (36). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (m, 1H), 7.34 (m, 2H), 7.04 (m, 2H), 6.96 (d, 1H, *J* = 8.22 Hz), 5.43 (t, 1H, *J* = 6.46 Hz), 4.85 (b, 1H), 4.31 (m, 4H), 3.21 (q, 2H, *J* = 6.26 Hz), 3.05 (t, 2H, *J* = 6.06 Hz), 1.74 (m, 2H). LC/MS: Method 1, retention time: 5.115 min; Method 2, retention time: 3.730 min. HRMS: *m/z* (M+) = 448.0571 (Calculated for $C_{17}H_{18}F_2N_2O_6S_2$ = 448.0574).



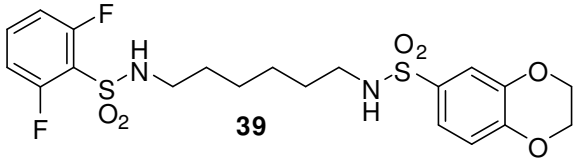
37

N-(4-(2,6-difluorophenylsulfonamido)butyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (37). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49 (m, 1H), 7.31 (m, 2H), 7.02 (m, 2H), 6.91 (d, 1H, *J* = 8.22 Hz), 5.03 (m, 1H), 4.47 (m, 1H), 4.28 (m, 4H), 3.06 (m, 2H), 2.89 (m, 2H), 1.54 (m, 4H). LC/MS: Method 1, retention time: 5.238 min; Method 2, retention time: 3.757 min. HRMS: *m/z* (M+) = 462.0739 (Calculated for $C_{18}H_{20}F_2N_2O_6S_2$ = 462.0731).



38

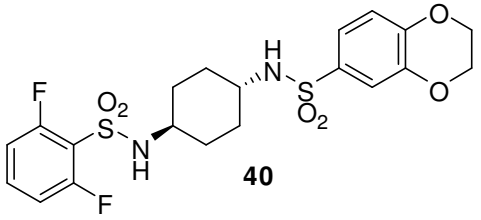
N-(5-(2,6-difluorophenylsulfonamido)pentyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (38). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (m, 1H), 7.35 (m, 2H), 7.04 (m, 2H), 6.96 (d, 1H, *J* = 8.61 Hz), 5.00 (b, 1H), 4.32 (m, 4H), 3.07 (q, 1H, *J* = 6.65 Hz), 2.91 (t, 1H, *J* = 6.85 Hz), 2.70 (b, 1H), 1.50 (m, 4H), 1.32 (m, 2H). LC/MS: Method 1, retention time: 5.450 min; Method 2, retention time: 3.798 min. HRMS: *m/z* (M+) = 476.0899 (Calculated for $C_{19}H_{22}F_2N_2O_6S_2$ = 476.0877).



39

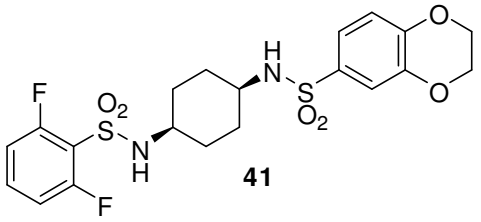
N-(6-(2,6-difluorophenylsulfonamido)hexyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (39). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (m, 1H), 7.36 (m, 2H), 7.04 (m, 2H), 6.96 (d, 1H, *J* = 8.61 Hz), 4.99 (b, 1H), 4.32 (m, 4H), 3.08 (m, 2H), 2.91 (m, 2H), 1.72 (b, 1H), 1.47 (m, 4H), 1.27 (m, 4H). LC/MS: Method 1, retention time: 5.629 min;

Method 2, retention time: 3.836 min. HRMS: *m/z* (M+) = 490.1056 (Calculated for $C_{20}H_{24}F_2N_2O_6S_2$ = 490.1044).



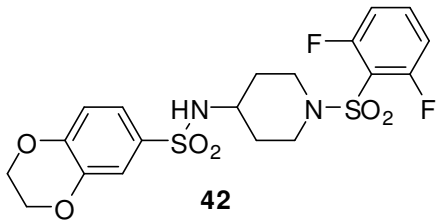
40

N-((*trans*)-4-(2,6-difluorophenylsulfonamido)cyclohexyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (40). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49 (m, 1H), 7.30 (m, 2H), 7.00 (m, 2H), 6.91 (d, 1H, *J* = 8.61 Hz), 4.95 (m, 1H), 4.47 (m, 1H), 4.28 (m, 4H), 3.25 (b, 1H), 3.00 (b, 1H), 1.84 (m, 4H), 1.24 (m, 4H). LC/MS: Method 1, retention time: 5.290 min; Method 2, retention time: 3.760 min. HRMS: *m/z* (M+) = 488.0895 (Calculated for $C_{20}H_{22}F_2N_2O_6S_2$ = 488.0887).



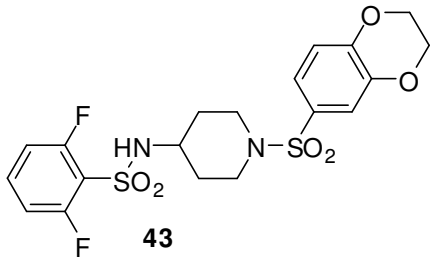
41

N-((*cis*)-4-(2,6-difluorophenylsulfonamido)cyclohexyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (41). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49 (m, 1H), 7.35 (m, 2H), 7.00 (m,2H), 6.90 (d, 1H, *J* = 8.61 Hz), 5.21 (m, 1H), 4.85 (m, 1H), 4.29 (m, 4H), 3.42 (b, 1H), 3.20 (b, 1H), 1.45 – 1.65 (m, 8H). LC/MS: Method 1, retention time: 5.507 min; Method 2, retention time: 3.803 min. HRMS: *m/z* (M+) = 488.0885 (Calculated for $C_{20}H_{22}F_2N_2O_6S_2$ = 488.0887).



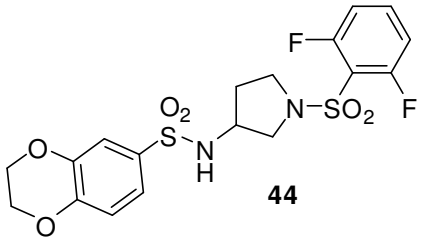
42

N-(1-(2,6-difluorophenylsulfonyl)piperidin-4-yl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (42). ^{1}H NMR ($CDCl_3$) δ: 7.50 (m, 1H), 7.33 (m, 2H), 7.00 (m, 2H), 6.93 (d, 1H, *J* = 8.61 Hz), 4.86 (d, 1H, *J* = 6.65 Hz), 4.30 (m, 4H), 3.67 (m, 2H), 3.22 (m, 1H), 2.83 (t, 2H, *J* = 10.37 Hz), 1.86 (m, 2H), 1.56 (m, 2H). LC/MS: Method 1, retention time: 5.514 min; Method 2, retention time: 3.825 min. HRMS: *m/z* (M+) = 474.0744 (Calculated for $C_{19}H_{20}F_2N_2O_6S_2$ = 474.0731).



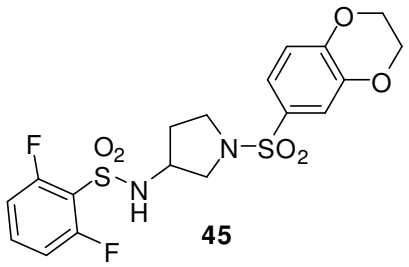
43

N-(1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperidin-4-yl)-2,6-difluorobenzenesulfonamide (43). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.50 (m, 1H), 7.31 (m, 2H), 7.01 (m, 2H), 6.96 (d, 1H, *J* = 8.6 Hz), 4.96 (d, 1H, *J* = 6.65 Hz), 4.37 (m, 4H), 3.64 (m, 2H), 3.20 (m, 1H), 2.80 (t, 2H, *J* = 10.4 Hz), 1.89 (m, 2H), 1.55 (m, 2H). LC/MS: Method 1, retention time: 5.511 min; Method 2, retention time: 3.825 min. HRMS: *m/z* (M+) = 474.0733 (Calculated for $C_{19}H_{20}F_2N_2O_6S_2$ = 474.0731).



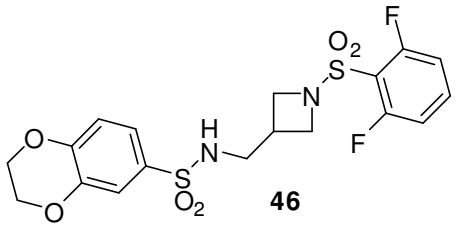
44

N-(1-(2,6-difluorophenylsulfonyl)pyrrolidin-3-yl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (44). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (m, 1H), 7.33 (m, 2H), 7.03 (m, 2H), 6.96 (d, 1H, *J* = 8.6 Hz), 4.85 (b, 1H), 4.32 (m, 4H), 3.84 (m, 1H), 3.53 (m, 2H), 3.42 (m, 1H), 3.19 (q, 1H, *J* = 4.7 Hz), 2.11 (m, 1H), 1.87 (m, 1H). LC/MS: Method 1, retention time: 5.339 min; Method 2, retention time: 3.789 min. HRMS: *m/z* (M+) = 460.0578 (Calculated for $C_{18}H_{18}F_2N_2O_6S_2$ = 460.0574).



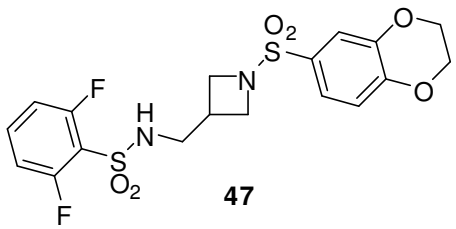
45

N-(1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)pyrrolidin-3-yl)-2,6-difluorobenzenesulfonamide (45). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51 (m, 1H), 7.33 (m, 2H), 7.03 (m, 2H), 6.95 (d, 1H, *J* = 8.6 Hz), 5.02 (b, 1H), 4.31 (m, 4H), 3.88 (m, 1H), 3.59 (m, 2H), 3.44 (m, 1H), 3.16 (q, 1H, *J* = 4.7 Hz), 2.08 (m, 1H), 1.88 (m, 1H). LC/MS: Method 1, retention time: 5.339 min; Method 2, retention time: 3.792 min. HRMS: *m/z* (M+) = 460.0587 (Calculated for $C_{18}H_{18}F_2N_2O_6S_2$ = 460.0574).



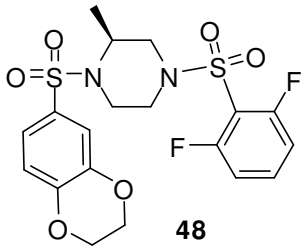
46

N-((1-(2,6-difluorophenylsulfonyl)azetidin-3-yl)methyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (46). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.53 (m, 1H), 7.30 (m, 2H), 7.05 (m, 2H), 6.94 (d, 1H, *J* = 8.6 Hz), 4.40 (m, 1H) 4.30 (m, 4H), 4.04 (t, 2H, *J* = 8.2 Hz), 3.66 (dd, 2H, *J* = 8.4, 5.65 Hz), 3.08 (t, 2H, *J* = 6.7 Hz), 2.69 (m, 1H). LC/MS: Method 1, retention time: 5.295 min; Method 2, retention time: 3.780 min. HRMS: *m/z* (M+) = 460.0582 (Calculated for $C_{18}H_{18}F_2N_2O_6S_2$ = 460.0574).



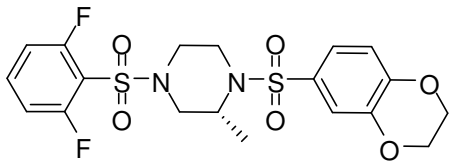
47

N-((1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)azetidin-3-yl)methyl)-2,6-difluorobenzenesulfonamide (47). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51 (m, 1H), 7.27 (m, 2H), 7.00 (m, 3H), 5.23 (t, 1H, *J* = 6.06 Hz), 4.31 (m, 4H), 3.78 (t, 2H, *J* = 8.22 Hz), 3.47 (dd, 2H, *J* = 8.41, 5.67 Hz), 3.10 (t, 2H, *J* = 6.7 Hz), 2.62 (m, 1H). LC/MS: Method 1, retention time: 5.234 min; Method 2, retention time: 3.767 min. HRMS: *m/z* (M+) = 460.0583 (Calculated for $C_{18}H_{18}F_2N_2O_6S_2$ = 460.0574).



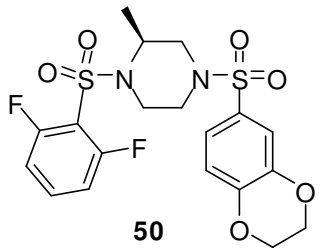
48

(S)-4-(2,6-difluorophenylsulfonyl)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-2-methylpiperazine (48). ^{1}H NMR (400 Hz, $CDCl_3$) δ: 7.55 (m, 1H), 7.26 (m, 2H), 7.04 (m, 2H), 6.92 (m, 1H), 4.30 (m, 4H), 4.21 (m, 1H), 3.84 (d, 1H, *J* = 12.1 Hz), 3.72 (d, 1H, *J* = 12.9 Hz), 3.61 (d, 1H, *J* = 12.1 Hz), 3.24 (td, *J* = 12.5, 3.13 Hz), 2.86 (dd, 1H, *J* = 12.1, 2.74 Hz), 2.72 (td, 1H, *J* = 11.9, 3.1 Hz), 1.13 (d, 3H, *J* = 6.7 Hz). LC/MS: Method 1, retention time: 5.873 min; Method 2, retention time: 3.905 min. HRMS: *m/z* (M+) = 474.0736 (Calculated for $C_{19}H_{20}F_2N_2O_6S_2$ = 474.0731).



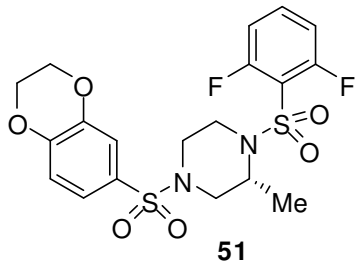
49

(R)-4-(2,6-difluorophenylsulfonyl)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-2-methylpiperazine (49). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.55 (m, 1H), 7.26 (m, 2H), 7.04 (m, 2H), 6.92 (m, 1H), 4.30 (m, 4H), 4.21 (m, 1H), 3.84 (d, 1H, *J* = 12.1 Hz), 3.72 (d, 1H, *J* = 12.9 Hz), 3.61 (d, 1H, *J* = 12.1 Hz), 3.24 (td, 1H, *J* = 12.5, 3.1 Hz), 2.86 (dd, 1H, J_1 = 12.1, 2.7 Hz), 2.72 (td, 1H, *J* = 11.9, 13.0 Hz), 1.13 (d, 3H, *J* = 6.7 Hz). LC/MS: Method 1, retention time: 5.872 min; Method 2, retention time: 3.905 min. HRMS: *m/z* (M+) = 474.0736 (Calculated for $C_{19}H_{20}F_2N_2O_6S_2$ = 474.0731).



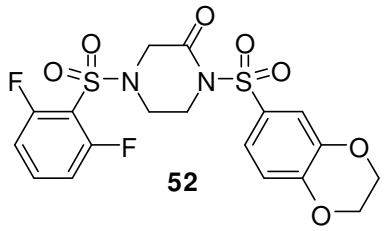
50

(S)-1-(2,6-difluorophenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-2-methylpiperazine (50). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.50 (m, 1H), 7.22 (m, 2H), 7.00 (m, 3H), 4.33 (m, 5H), 3.92 (d, 1H, *J* = 13.7 Hz), 3.70 (d, 1H, *J* = 11.4 Hz), 3.50 (d, 1H, *J* = 11.4 Hz), 3.38 (m, 1H), 2.53 (dd, 1H, *J* = 11.4, 3.5 Hz), 2.39 (td, *J* = 11.8, 3.3 Hz),1.22 (d, 3H, *J* = 7.0 Hz). LC/MS: Method 1, retention time: 5.912 min; Method 2, retention time: 3.910 min. HRMS: *m/z* (M+) = 474.0726 (Calculated for $C_{19}H_{20}F_2N_2O_6S_2$ = 474.0731).



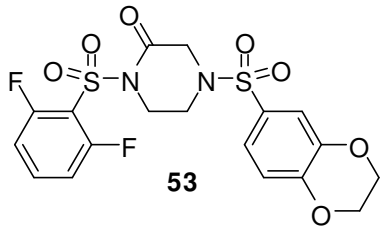
51

(R)-1-(2,6-difluorophenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-2-methylpiperazine (51). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.50 (m, 1H), 7.22 (m, 2H), 7.00 (m, 3H), 4.33 (m, 5H), 3.92 (d, 1H, *J* = 13.7 Hz), 3.70 (d, 1H, *J* = 11.4 Hz), 3.50 (d, 1H, *J* = 11.4 Hz), 3.38 (m, 1H), 2.53 (dd, 1H, *J* = 11.4, 3.5 Hz), 2.39 (td, 1H, *J* = 11.8, 3.3 Hz), 1.22 (d, 3H, *J* = 7.0 Hz). LC/MS: Method 1, retention time: 5.910 min; Method 2, retention time: 3.912 min. HRMS: *m/z* (M+) = 474.0727 (Calculated for $C_{19}H_{20}F_2N_2O_6S_2$ = 474.0731).



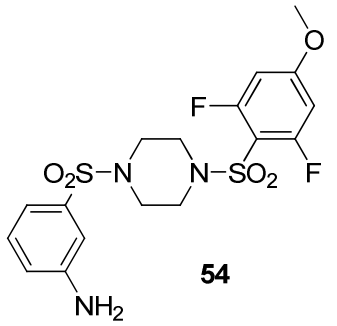
52

4-(2,6-difluorophenylsulfonyl)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazin-2-one (52). ^{1}H NMR (400 Hz, $CDCl_3$) δ: 7.58 (m, 1H), 7.29 (m, 3H), 7.03 (m, 2H), 4.31 (m, 4H), 4.07 (m, 2H), 3.81 (s, 2H), 3.47 (m, 2H). LC/MS: Method 1, retention time: 5.631 min; Method 2, retention time: 3.858 min. HRMS: *m/z* (M+) = 474.0372 (Calculated for $C_{18}H_{16}F_2N_2O_7S_2$ = 474.0367).



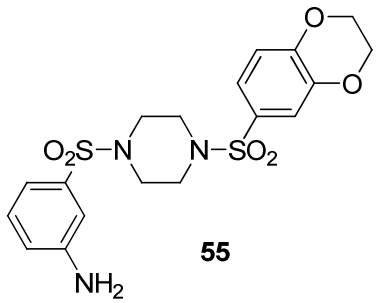
53

1-(2,6-difluorophenylsulfonyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazin-2-one (53). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58 (m, 1H), 7.29 (m, 3H), 7.03 (m, 2H), 4.33 (m, 4H), 4.07 (m, 2H), 3.78 (s, 2H), 3.44 (m, 2H). LC/MS: Method 1, retention time: 5.612 min; Method 2, retention time: 3.849 min. HRMS: *m/z* (M+) = 474.0366 (Calculated for $C_{18}H_{16}F_2N_2O_7S_2$ = 474.0367).



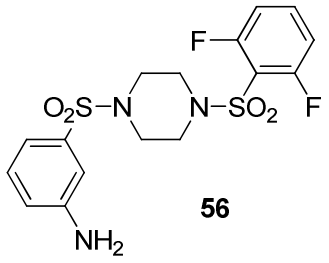
54

3-(4-(2,6-difluoro-4-methoxyphenylsulfonyl)piperazin-1-ylsulfonyl)aniline (54). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.18 (t, *J*=7.8 Hz, 1 H), 6.90 (d, *J*=11.5 Hz, 2 H), 6.83 (t, *J*=1.9 Hz, 1 H), 6.63 - 6.81 (m, 2 H), 5.62 (s, 2 H), 3.84 (s, 3 H), 3.12 (m, 4 H), 2.96 (m, 4 H). LC/MS: Method 1, retention time: 5.293 min; Method 2, retention time: 3.771 min. HRMS: *m/z* (M+) = 447.0743 (Calculated for $C_{17}H_{19}F_2N_3O_5S_2$ = 447.0734).



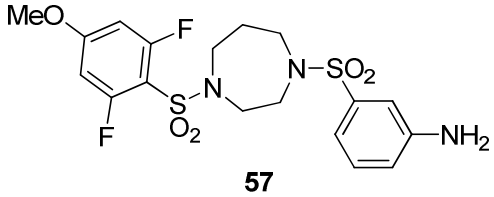
55

3-(4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazin-1-ylsulfonyl)aniline (55). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.18 (t, *J*=7.8 Hz, 1 H), 7.07 - 7.15 (m, 2 H), 7.00 - 7.07 (m, 1 H), 6.74 - 6.85 (m, 2 H), 6.70 (d, *J*=7.6 Hz, 1 H), 5.61 (s, 2 H), 4.30 (m, 4 H), 2.92 (br., 8 H). LC/MS: Method 1, retention time: 5.051 min; Method 2, retention time: 3.721 min. HRMS: *m/z* (M+) = 439.0878 (Calculated for $C_{18}H_{21}N_3O_6S_2$ = 439.0872).



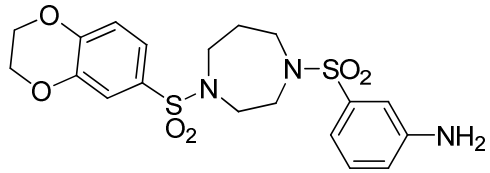
56

3-(4-(2,6-difluorophenylsulfonyl)piperazin-1-ylsulfonyl)aniline (56).
^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.66 - 7.83 (m, 1 H), 7.14 - 7.36 (m, 3 H), 6.68 - 6.96 (m, 3 H), 3.75 (br. s., 2 H), 3.10 - 3.26 (m, 4 H), 2.84 - 3.02 (m, 4 H). LC/MS: Method 1, retention time: 5.115 min; Method 2, retention time: 3.726 min. HRMS: *m/z* (M+) = 417.0638 (Calculated for $C_{16}H_{17}N_3O_4F_2S_2$ = 417.0629).



57

3-(4-(2,6-difluoro-4-methoxyphenylsulfonyl)-1,4-diazepan-1-ylsulfonyl)aniline (57).
^{1}H NMR (400 MHz, DMSO-d_6) δ: 1H NMR (400 MHz, DMSO-d_6) δ ppm: 7.17 (t, *J*=7.9 Hz, 1 H), 6.86 - 7.03 (m, 3 H), 6.65 - 6.86 (m, 2 H), 5.57 (br. s., 2 H), 3.82 (m, 4 H), 3.38 (m, 4 H), 1.68 - 1.87 (m, 2 H). LC/MS: Method 1, retention time: 5.257 min; Method 2, retention time: 3.753 min. HRMS: *m/z* (M+) = 461.0898 (Calculated for $C_{18}H_{21}N_3O_5F_2S_2$ = 461.0891).



58

3-(4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)-1,4-diazepan-1-ylsulfonyl)aniline (58). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.18 (m, 3 H), 6.98 - 7.09 (m, 2 H), 6.81 - 6.97 (m, 2 H), 4.18 - 4.36 (m, 4 H), 3.08 - 3.21 (m, 8 H), 1.66 - 1.83 (m, 2 H); LC/MS: Method 1, retention time, 5.017 min; Method 2, retention time 3.704 min; HRMS: *m/z* ($M+H^+$) = 453.1035 (Calculated for $C_{19}H_{23}N_3O_6S_2$ = 453.1028).