Development and evaluation of selective, reversible LSD1 inhibitors derived from fragments

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Chemistry

General information

Flash chromatography was performed using pre-packed silica gel cartridges (KP-Sil SNAP, Biotage, Hengoed, UK) on a CombiFlash Rf200 automated purification system (Teledyne ISCO Inc., Lincoln, NE, USA). Thin layer chromatography (TLC) was conducted with 5 × 10 cm plates coated with Merck Type 60 F_{254} silica gel to a thickness of 0.25 mm. All of the reagents used in the current paper were obtained from commercial sources and used without further purification. Anhydrous solvents were obtained from the Sigma-Aldrich or Fisher and used as supplied. HPLC grade solvents were obtained from Fisher.

All of the compounds synthesized in the current paper were > 95% purity as determined by examination of both the LC-MS and ^{1}H NMR spectra unless otherwise indicated. Where Cl or Br were present, expected isotopic distribution patterns were observed.

NMR Spectroscopy

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a 300 MHz Bruker spectrometer (Bruker, Karlsruhe, Germany). Solutions were typically prepared in either deuterochloroform ($CDCl_3$) or deuterated dimethylsulfoxide (d^6-DMSO) with chemical shifts referenced to tetramethylsilane (TMS) or deuterated solvent as an internal standard. ^{1}H NMR data are reported indicating the chemical shift (δ), the integration (e.g., 1H), the multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; dd, doublet of doublets) and the coupling constant (J) in Hz (app implies apparent coupling on broadened signals). Deuterated solvents were obtained from the Sigma-Aldrich, Goss or Fluorochem.

Analytical LC-MS

LC-MS analyses were performed on a Waters Acquity UPLC system (Waters, Wythensure, UK) fitted with BEH C18 1.7 μM columns (2.1 × 50 mm) and a UV diode array detector (210–400 nm). Positive and negative mass ion detection was performed using a Waters SQD detector. Analyses were performed with either buffered acidic or basic solvents and the gradients as detailed below:

Low pH:

Solvent A – Water + 10mM ammonium formate + 0.1% formic acid.

Solvent B – Acetonitrile + 5% water + 0.1% formic acid.

High pH:

Solvent A – Water + 10mM ammonium hydrogen carbonate + 0.1% ammonia solution.

Solvent B – Acetonitrile + 0.1% ammonia solution.

Gradient:

Time	Flow rate (mL/min)	% Solvent A	% Solvent B
0	0.6	95	5
1.2	0.6	5	95
1.7	0.6	5	95
1.8	0.6	95	5

Preparative HPLC

Some compounds were purified by preparative HPLC on a Waters FractionLynx MS autopurification system, with a Waters XBridge 5 µm C18 column (100 × 19 mm, i.d.), running at a flow rate of 20 mL/min with UV diode array detection (210–400 nm) and mass-directed collection in the positive and negative ion modes.

Purifications were performed using buffered acidic or basic solvent systems as appropriate. Compound retention times on the system were routinely assessed using a 30-50 µL test injection and a standard gradient, then purified using an appropriately chosen focused gradient as detailed below, based upon observed retention time.

Low pH:

Solvent A – Water + 10mM ammonium formate + 0.1% formic acid.

Solvent B – Acetonitrile + 5% water +0.1% formic acid.

High pH:

Solvent A – Water + 10mM ammonium formate + 0.1% ammonia solution.

Solvent B – Acetonitrile + 5% water + 0.1% ammonia solution.

Standard Gradient:

Time	Flow rate (mL/min)	% Solvent A	% Solvent B
0	20	90	10
0.3	20	90	10
8.5	20	2	98
12	20	2	98
12.5	0	2	98

Chemical Synthesis

***trans*-*N*-[(2-Methoxy-3-pyridyl)methyl]-2-phenyl-cyclopropanamine (3)**[1]

2-Methoxynicotinaldehyd (1.27 mL, 10.73 mmol) was added to a magnetically stirred solution of *trans*-2-phenylcyclopropanamine (1.50 g, 11.26 mmol) in dichloromethane (20 mL) at 20°C, and the resulting mixture was agitated at ambient for 30 minutes (turns cloudy). Molecular sieves were then added (3Å) and mixture was agitated for 15 minutes. Sodium triacetoxyborohydride (3.18 g, 15.02 mmol) was then added in a single portion and the mixture was agitated for 16 hrs. The mixture was then filtered and the solvent removed *in vacuo* to give the crude product as a gum which was partitioned between saturated $NaHCO_3$ (20 mL) and dichloromethane (20 mL). The organic phase was collected, dried (Na_2SO) and distilled to dryness to give a crude residue, which was purified by automated column chromatography (50 g SNAP cartridge; 1:9 - EtOAc:hexane eluent) to afford the desired compound as a colorless oil (2.2 g, 8.45 mmol, 78.8%).

^{1}H NMR (DMSO): δ (ppm) 0.89 – 1.07 (m, 2H), 1.76 – 1.85 (m, 1H), 2.18 – 2.26 (m, 1H), 3.72 (s 2H), 3.81 (s, 3H), 6.91 – 6.96 (dd, *J* = 7.2, 5.0 Hz, 1H), 6.97 – 7.01 (m, 2H), 7.07 – 7.13 (m, 1H), 7.18 – 7.23 (m, 2H), 7.62 – 7.66 (m, 1H), 8.01 – 8.04 (dd, *J* = 5.0, 1.9 Hz, 1H).

General procedure for the synthesis of the aminothiazoles (12)

A mixture of α-bromo ketone (**8**) (1.0 mmol) and N-substituted thiourea (**9**) (0.61 mmol) in ethanol (2mL) was heated with agitation at 80°C for 60 mins. The mixture was then cooled to ambient temperature and the resulting precipitate collected by filtration. The filter cake was then washed with ethanol (2 × 10 mL) before being suspended in a bi-phasic mixture of DCM (20 mL) and saturated sodium bicarbonate (20 mL). The resulting mixture was agitated for 15 mins and the organic phase collected and distilled to dryness to give the desired material as a white solid.

_N_-(4-Chlorophenyl)-4-ethyl-thiazol-2-amine (10a)

A mixture of N-(4-chlorophenyl)thiourea (100 mg, 0.54 mmol) and 1-bromobutan-2-one (0.05 mL, 0.54 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (76%)

^{1}H NMR (DMSO): δ (ppm) 1.17 – 1.24 (t, *J* = 7.5 Hz, 3H), 2.53 – 2.63 (dq, *J* = 7.5, 0.9 Hz, 2H), 7.31 – 7.37 (m, 2H), 7.62 – 7.69 (m, 2H), 10.23 (s, 1H).

[2-(4-Chloroanilino)thiazol-4-yl]-phenyl-methanone (10c)

A mixture of 3-bromo-1-phenyl-propane-1,2-dione (100. mg, 0.44 mmol) and *N*-(4-chlorophenyl)thiourea (82.21 mg, 0.44 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (66%)

^{1}H NMR (DMSO): δ (ppm) 7.13 – 7.19 (m, 2H), 7.34 – 7.39 (m, 2H), 7.44 – 7.50 (m, 3H), 7.64 (s, 1H), 7.84 – 7.87 (m, 2H), 10.38 (s, 1H).

_N_-(4-Chlorophenyl)-4-(4-fluorophenyl)thiazol-2-amine (10d)

A mixture of *p*-Fluorophenacyl bromide (100. mg, 0.46 mmol) and *N*-(4-chlorophenyl)thiourea (86 mg, 0.46 mmol) in Ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (86%)

^{1}H NMR (DMSO): δ (ppm) 7.23 – 7.27 (m, 2H), 7.36 (s, 1H), 3.37 – 7.43 (m, 2H), 7.73 – 7.79 (m, 2H), 7.93 – 7.99 (m, 2H), 10.42 (s, 1H).

1-(2-Anilinothiazol-4-yl)ethanone (10e)

A mixture of 1-bromobutane-2,3-dione (100 mg, 0.61 mmol) and *N*-phenylthiourea (92.26 mg, 0.61 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (75%)

^{1}H NMR (DMSO): δ (ppm) 2.52 (s, 3H), 6.95 – 7.01 (t, m, 1H), 7.31 – 7.37 (m, 2H), 7.66 – 7.69 (m, 2H), 7.81 (s, 1H), 10.38 (s, 1H).

1-[2-(4-Chloroanilino)thiazol-4-yl]ethanone (6)

A mixture of 1-bromobutane-2,3-dione (100 mg, 0.61 mmol) and *N*-(4-chlorophenyl)thiourea (113.13 mg, 0.61 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (52%).

^{1}H NMR (DMSO): δ (ppm) 2.51 (s, 3H), 7.36 – 7.41 (t, m, 2H), 7.69 – 7.75 (m, 2H), 7.85 (s, 1H), 10.54 (s, 1H).

1-[2-(3-Chloroanilino)thiazol-4-yl]ethanone (10f)

A mixture of 1-bromobutane-2,3-dione (50 mg, 0.30 mmol) and 3-chlorophenylthiourea (56.56 mg, 0.30 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (69%).

^{1}H NMR (DMSO): δ (ppm) 2.51 (s, 3H), 7.01 – 7.04 (t, dd, *J* = 16.7, 8.1 Hz, 1H), 7.33 – 7.38 (m, 1H), 7.49 – 7.53 (m, 1H), 7.79 (s, 1H), 7.95 – 7.97 (t, *J* = 2.1 Hz, 1H), 10.63 (s, 1H).

1-[2-(2-Chloroanilino)thiazol-4-yl]ethanone (7)

A mixture of 2-chlorophenyl-thiourea (135. mg, 0.7200 mmol) and 1-bromobutane-2,3-dione (0.07 mL, 0.7200 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (70%).

^{1}H NMR ($CDCl_3$): δ (ppm) 2.64 (s, 3H), 7.01 – 7.07 (m, 1H), 7.32 – 7.37 (m, 1H), 7.42 – 7.45 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.58 (s, 1H), 8.16 – 8.19 (t, *J* = 8.3, 1.3 Hz, 1H).

1-[2-(2-Methylanilino)thiazol-4-yl]ethanone (10g)

A mixture of 1-bromobutane-2,3-dione (50 mg, 0.30 mmol) and 1-(2-methylphenyl)thiourea (50.37 mg, 0.30 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (72%).

^{1}H NMR (DMSO): δ (ppm) 2.23 (s, 3H), 2.43 (s, 3H), 7.02 – 7.08 (m, 1H), 7.19 – 7.25 (m, 2H), 7.75 (s, 1H), 7.87 – 7.89 (m, 1H), 9.55 (bs, 1H).

1-[2-(3,4-Dichloroanilino)thiazol-4-yl]ethanone (10h)

A mixture of 1-bromobutane-2,3-dione (50. mg, 0.30 mmol) and 3,4-dichlorophenylthiourea (67. mg, 0.30 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (65%).

^{1}H NMR (DMSO): δ (ppm) 2.53 (s, 3H), 7.50 – 7.60 (m, 2H), 7.91 (s, 1H), 8.17 – 8.18 (d, *J* = 2.4 Hz, 1H), 10.72 (bs, 1H).

1-[2-(3,5-Dichloroanilino)thiazol-4-yl]ethanone (10i)

A mixture of 1-bromobutane-2,3-dione (50. mg, 0.30 mmol) and 3,5-dichlorophenylthiourea (67. mg, 0.30 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (68%).

^{1}H NMR (DMSO): δ (ppm) 2.52 (s, 3H), 7.16 – 7.18 (dd, *J* = 1.85 Hz, 1H), 7.76 – 7.77 (d, *J* = 1.85 Hz, 1H), 7.93 (s, 1H), 10.79 (bs, 1H).

1-[2-(2,6-Dimethylanilino)thiazol-4-yl]ethanone (10j)

A mixture of 1-bromobutane-2,3-dione (50. mg, 0.3000 mmol) and 1-(2,6-Dimethylphenyl)thiourea (54.62 mg, 0.3000 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (68%).

^{1}H NMR (DMSO): δ (ppm) 2.21 (s, 6H), 2.42 (s, 3H), 7.17 (s, 3H), 7.62 (s, 1H), 9.52 (bs, 1H).

1-[2-(4-Bromoanilino)thiazol-4-yl]ethanone (10k)

A magentically stirred solution of 1-bromobutane-2,3-dione (100. mg, 0.61 mmol) and 1-(4-Bromophenyl)thiourea (140.08 mg, 0.61 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (73%).

^{1}H NMR (DMSO): δ (ppm) 2.53 (s, 3H), 7.49 – 7.54 (m, 2H), 7.65 – 7.70 (m, 2H), 7.87 (s, 1H), 10.38 (bs, 1H).

1-[2-(4-Hydroxyanilino)thiazol-4-yl]ethanone (10l)

A mixture of 1-bromobutane-2,3-dione (50. mg, 0.30 mmol) and 1-(4-hydroxyphenyl)thiourea (50.97 mg, 0.30 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (63%).

^{1}H NMR (DMSO): δ (ppm) 2.48 (s, 3H), 6.72 – 6.77 (m, 2H), 7.37 – 7.44 (m, 2H), 7.69 (s, 1H), 10.04 (bs, 1H).

1-[2-(3-Hydroxyanilino)thiazol-4-yl]ethanone (10m)

A mixture of 1-bromobutane-2,3-dione (100. mg, 0.61 mmol) and 1-(3-hydroxyphenyl)thiourea (101.96 mg, 0.61 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (77%).

^{1}H NMR (DMSO): δ (ppm) 2.52 (s, 3H), 6.37 – 6.41 (m, 1H), 7.00 – 7.13 (m, 2H), 7.18 – 7.20 (dd, *J* = 2.1 Hz, 1H), 7.78 (s, 1H), 10.27 (bs, 1H).

1-[2-(4-Methoxyanilino)thiazol-4-yl]ethanone (10n)

A mixture of 1-bromobutane-2,3-dione (100. mg, 0.61 mmol) and *N*-(4-methoxyphenyl)thiourea (110.46 mg, 0.61 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (71%).

^{1}H NMR (DMSO): δ (ppm) 2.50 (s, 3H), 3.73 (s, 3H), 6.91 – 6.96 (m, 2H), 7.52 – 7.61 (m, 2H), 7.73 (s, 1H), 10.17 (bs, 1H).

4-[(4-Acetylthiazol-2-yl)amino]benzonitrile (10o)

A mixture of 1-bromobutane-2,3-dione (100. mg, 0.61 mmol) and 1-(4-Cyanophenyl)thiourea (107.42 mg, 0.61 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (69%).

^{1}H NMR (DMSO): δ (ppm) 2.54 (s, 3H), 7.77 – 7.87 (m, 4H), 7.76 (s, 1H), 10.92 (bs, 1H).

1-[2-(4-Nitroanilino)thiazol-4-yl]ethanone (10p)

A mixture of 1-bromobutane-2,3-dione (100. mg, 0.6100 mmol) and 1-(4-nitrophenyl)thiourea (119.53 mg, 0.6100 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (72%).

^{1}H NMR (DMSO): δ (ppm) 2.57 (s, 3H), 7.87 – 7.90 (m, 2H), 8.03 (s, 1H), 8.23 – 8.29 (m, 2H), 11.17 (bs, 1H).

1-[2-(4-Pyridylamino)thiazol-4-yl]ethanone (10q)

A mixture of 1-bromobutane-2,3-dione (50. mg, 0.30 mmol) and *N*-(4-pyridyl)thiourea (46.42 mg, 0.30 mmol) in ethanol (2 mL) was subjected to the general procedure described above to give the desired compound as a white solid (65%).

^{1}H NMR (DMSO): δ (ppm) 2.55 (s, 3H), 7.63 – 7.66 (m, 2H), 7.95 (s, 1H), 8.38 (s, 1H).8.39 – 8.42 (m, 2H).

1-[2-(4-Chloroanilino)thiazol-4-yl]ethanol (10b)

Sodium borohydride (29.9 mg, 0.7900 mmol) was added in a portion-wise manner to a stirred solution of **6** (20. mg, 0.0800 mmol) in methanol (4 mL), and the resulting mixture was agitated at ambient temperature for 2. The reaction mixture was then distilled to dryness to give a residue, which was partitioned between saturated sodium bicarbonate (10 mL) and DCM (10 mL). The organic layer was collected and the aqueous washed with DCM (2 × 2 mL). The combined organic extracts were concentrated under reduced pressure to give the crude product as a residue, which was purified by column chromatography over silica gel using a mixture of methanol and DCM (1:9 – v/v) to give the desired compound as a white solid (10.1 mg, 51%).

^{1}H NMR ($CDCl_3$): δ (ppm) 3.52 – 3.53 (d, *J* = 2.79 Hz, 3H), 7.11 – 7.15 (d, *J* = 8.4 Hz, 2H), 7.28 (s, 1H), 7.63 – 7.64 (d, *J* = 1.83 Hz, 1H), 7.86 – 7.90 (dd, *J* = 8.4, 1.86 Hz, 2H).

General procedure for the synthesis of the aminothiazole sulfonamides (14)

A mixture of 4-(4-chlorophenyl)thiazol-2-amine (1.0 mmol) and an aryl sulfonyl chloride (1.0 mmol) in pyridine (2 mL) was heated at 80°C for 8 hr. The mixture was then cooled and partitioned between DCM (10 mL) and saturated sodium bicarbonate (10 mL). The organic phase was collected and washed with water (10 mL) before being dried ($MgSO_4$) to give the crude product as a residue, which was purified by preparatory HPLC (low pH method) to give the desired product as a white solid.

***N*-[4-(4-Chlorophenyl)thiazol-2-yl]methanesulfonamide (14a)**

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100. mg, 0.47 mmol) and methane sulfonyl chloride (0.04 mL, 0.47 mmol) in pyridine was subjected to the procedure described above to give the desired product as a white solid (48%).

^{1}H NMR (DMSO): δ (ppm) 3.33 (s, 3H), 7.30 (bs, 1H), 7.51 – 7.56 (m, 2H), 7.76 – 7.80 (m, 2H), 12.96 (bs, 1H).

***N*-[4-(4-Chlorophenyl)thiazol-2-yl]-1,1,1-trifluoro-methanesulfonamide (14b)**

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100. mg, 0.47 mmol) and triflic anhydride (0.18 mL, 1.04 mmol) in pyridine was subjected to the procedure described above to give the desired product as a white solid (24%).

^{1}H NMR (DMSO): δ (ppm) 7.51 (s, 1H), 7.52 – 7.56 (m, 2H), 7.79 – 7.84 (m, 2H).

***N*-[4-(4-Chlorophenyl)thiazol-2-yl]benzenesulfonamide (11f)**

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100. mg, 0.47 mmol) and benzenesulphonyl chrloride (0.06 mL, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (48%).

^{1}H NMR (DMSO): δ (ppm) 7.29 (s, 1H), 7.49 – 7.62 (m, 5H), 7.71 – 7.76 (m, 2H), 7.82 – 7.87 (m, 2H), 13.27 (bs, 1H).

***N*-[4-(4-Chlorophenyl)thiazol-2-yl]-1-phenyl-methanesulfonamide (14e)**

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100 mg, 0.47 mmol) (100. mg, 0.4700 mmol) and phenylmethanesulfonyl chloride (90.49 mg, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (46%).

^{1}H NMR (DMSO): δ (ppm) 4.37 (bs, 2H), 7.20 (bs, 1H), 7.28 – 7.29 (m, 5H), 7.51 – 7.55 (m, 2H), 7.75 – 7.78 (m, 2H), 13.06 (bs, 1H).

_N_-[4-(4-Chlorophenyl)thiazol-2-yl]-2-methyl-benzenesulfonamide (14f)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100 mg, 0.47 mmol) and *ortho*-toluenesulfonyl chloride (0.07 mL, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (46%).

^{1}H NMR (DMSO): δ (ppm) 2.61 (s, 3H), 7.24 (s, 1H), 7.34 – 7.39 (m, 2H), 7.46 – 7.54 (m, 3H), 7.72 – 7.76 (m, 2H), 7.92 – 7.95 (m, 2H), 13.20 (bs, 1H).

_N_-[4-(4-Chlorophenyl)thiazol-2-yl]-3-methyl-benzenesulfonamide (14g)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100 mg, 0.47 mmol) and *meta*-toluenesulfonyl chloride (0.07 mL, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (46%).

^{1}H NMR (DMSO): δ (ppm) 2.38 (s, 3H), 7.28 (s, 1H), 7.41 – 7.46 (m, 2H), 7.61 – 7.67 (m, 2H), 7.70 – 7.76 (m, 2H), 13.23 (bs, 1H).

_N_-[4-(4-Chlorophenyl)thiazol-2-yl]-4-methyl-benzenesulfonamide (14h)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100 mg, 0.47 mmol) and *para*-toluenesulfonyl chloride (0.07 mL, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (46%).

^{1}H NMR (DMSO): δ (ppm) 2.36 (s, 3H), 7.28 (bs, 1H), 7.34 – 7.37 (m, 2H), 7.49 – 7.56 (m, 2H), 7.71 – 7.75 (m, 4H), 13.21 (bs, 1H).

_N_-[4-(4-Chlorophenyl)thiazol-2-yl]-3-methoxy-benzenesulfonamide (14i)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100 mg, 0.47 mmol) and 3-methoxybenzenesulfonyl chloride (0.07 mL, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (44%).

^{1}H NMR (DMSO): δ (ppm) 3.81 (s, 3H), 7.16 – 7.21 (m, 1H), 7.29 (s, 1H), 7.31 – 7.33 (m, 1H), 7.40 – 7.54 (m, 4H), 7.71 – 7.76 (m, 2H), 13.29 (bs, 1H).

_N_-[4-(4-Chlorophenyl)thiazol-2-yl]-4-methoxy-benzenesulfonamide (14j)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100 mg, 0.47 mmol) and 4-methoxybenzenesulfonyl chloride (0.07 mL, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (46%).

^{1}H NMR (DMSO): δ (ppm) 3.81 (s, 3H), 7.05 – 7.09 (m, 2H), 7.28 (s, 1H), 7.49 – 7.53 (m, 2H), 7.72 – 7.85 (m, 4H), 13.16 (bs, 1H).

_N_-[4-(4-Chlorophenyl)thiazol-2-yl]-3-phenyl-benzenesulfonamide (14k)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (50 mg, 0.24 mmol) and biphenyl-3-sulfonyl chloride (59.98 mg, 0.24 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (47%).

^{1}H NMR (DMSO): δ (ppm) 7.28 (s, 1H), 7.41 – 7.54 (m, 5H), 7.64 – 7.75 (m, 5H), 7.83 – 7.93 (m, 2H), 8.06 (m, 1H), 13.30 (bs, 1H).

_N_-[4-(4-Chlorophenyl)thiazol-2-yl]-4-phenyl-benzenesulfonamide (14l)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (50 mg, 0.24 mmol) and biphenyl-4-sulfonyl chloride (59.98 mg, 0.24 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (46%).

^{1}H NMR (DMSO): δ (ppm) 7.31 (s, 1H), 7.40 – 7.53 (m, 5H), 7.70 – 7.77 (s, 4H), 7.83 – 7.94 (m, 4H), 13.31 (bs, 1H).

2-Chloro-_N_-[4-(4-chlorophenyl)thiazol-2-yl]benzenesulfonamide (14m)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (60 mg, 0.28 mmol) and 2-Chlorobenzenesulfonyl chloride (0.06 mL, 0.43 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (48%).

^{1}H NMR (DMSO): δ (ppm) 7.31 (s, 1H), 7.40 – 7.53 (m, 5H), 7.70 – 7.77 (s, 4H), 7.83 – 7.94 (m, 4H), 13.31 (bs, 1H).

3-Chloro-*N*-[4-(4-chlorophenyl)thiazol-2-yl]benzenesulfonamide (14n)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (60 mg, 0.28 mmol) and 3-chlorobenzenesulfonyl chloride (0.06 mL, 0.43 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (46%).

^{1}H NMR (DMSO): δ (ppm) 7.32 (s, 1H), 7.49 – 7.55 (m, 2H), 7.57 – 7.64 (s, 1H), 7.67 – 7.77 (m, 3H), 7.78 – 7.83 (m, 2H), 13.41 (bs, 1H).

4-Chloro-*N*-[4-(4-chlorophenyl)thiazol-2-yl]benzenesulfonamide (14o)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100. mg, 0.47 mmol) and 4-chlorobenzenesulfonyl chloride (100.19 mg, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (40%).

^{1}H NMR (DMSO): δ (ppm) 7.31 (s, 1H), 7.49 – 7.54 (m, 2H), 7.61 – 7.66 (s, 2H), 7.71 – 7.77 (m, 2H), 7.82 – 7.87 (m, 2H), 13.37 (bs, 1H).

***N*-[4-(4-Chlorophenyl)thiazol-2-yl]-2-(trifluoromethyl)benzenesulfonamide (14p)**

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100. mg, 0.47 mmol) and 2-(trifluoromethyl)benzenesulfonyl chloride (0.07 mL, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (42%).

^{1}H NMR (DMSO): δ (ppm) 7.30 (s, 1H), 7.50 – 7.55 (m, 2H), 7.73 – 7.76 (m, 2H), 7.80 – 7.89 (m, 2H), 7.92 – 7.98 (m, 1H), 8.22 – 8.27 (m, 1H), 13.41 (bs, 1H).

***N*-[4-(4-Chlorophenyl)thiazol-2-yl]-3-(trifluoromethyl)benzenesulfonamide (14q)**

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100 mg, 0.47 mmol) and 3-(trifluoromethyl)benzenesulfonyl chloride (0.07 mL, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (42%).

^{1}H NMR (DMSO): δ (ppm) 7.33 (s, 1H), 7.50 – 7.54 (d, 2H), 7.71 – 7.77 (d, 2H), 7.81 – 7.86 (dd, 1H), 8.0 – 8.07 (m, 2H), 8.14 – 8.17 (m, 1H), 13.44 (bs, 1H).

***N*-[4-(4-Chlorophenyl)thiazol-2-yl]-4-(trifluoromethyl)benzenesulfonamide (14r)**

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100. mg, 0.47 mmol) and 4-(trifluoromethyl)benzenesulfonyl chloride (0.07 mL, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (42%).

^{1}H NMR (DMSO): δ (ppm) 7.33 (s, 1H), 7.50 – 7.55 (m, 2H), 7.72 – 7.76 (s, 2H), 7.93 – 7.96 (m, 2H), 8.04 – 8.07 (m, 2H), 13.47 (bs, 1H).

***N*-[4-(4-Chlorophenyl)thiazol-2-yl]-2-nitro-benzenesulfonamide (14s)**

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (60 mg, 0.28 mmol) and 2-nitrobenzolsulfonylchlorid (94.67 mg, 0.4300 mmol) in pyridine (2 mL) was subjected to the procedure described above to give the desired product as a tan solid (32%).

^{1}H NMR (DMSO): δ (ppm) 7.36 (s, 1H), 7.52 – 7.57 (m, 2H), 7.74 – 7.85 (m, 4H), 7.89 – 7.93 (m, 1H), 8.07 – 8.11 (m, 1H), 13.58 (bs, 1H).

***N*-[4-(4-Chlorophenyl)thiazol-2-yl]-3-nitro-benzenesulfonamide (14t)**

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (60 mg, 0.28 mmol) and 3-nitrobenzolsulfonylchlorid (94.67 mg, 0.4300 mmol) in pyridine (2 mL) was subjected to the procedure described above to give the desired product as a tan solid (43%).

^{1}H NMR (DMSO): δ (ppm) 7.34 (s, 1H), 7.50 – 7.54 (m, 2H), 7.72 – 7.76 (m, 2H), 7.85 – 7.90 (dd, *J* =8.0 Hz, 1H), 8.24 – 8.28 (m, 1H), 8.43 – 8.48 (m, 1H), 8.50 – 8.54 (dd, *J* = 1.9 Hz, 1H), 13.51 (bs, 1H).

***N*-[4-(4-Chlorophenyl)thiazol-2-yl]-4-nitro-benzenesulfonamide (14u)**

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (60 mg, 0.28 mmol) and 4-nitrobenzolsulfonylchlorid (94.67 mg, 0.4300 mmol) in pyridine (2 mL) was subjected to the procedure described above to give the desired product as a tan solid (47%).

^{1}H NMR (DMSO): δ (ppm) 7.34 (s, 1H), 7.50 – 7.55 (m, 2H), 7.72 – 7.82 (m, 2H), 8.06 – 8.11 (m, 2H), 8.36 – 8.40 (m, 2H), 13.56 (bs, 1H).

_N_-[4-(4-Chlorophenyl)thiazol-2-yl]thiophene-2-sulfonamide (14v)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100 mg, 0.47 mmol) and 2-thienylsulfonyl chloride (86.7 mg, 0.47 mmol) in pyridine (2 mL) was subjected to the procedure described above to give the desired product as a white solid (40%).

^{1}H NMR (DMSO): δ (ppm) 6.93 – 6.96 (dd, *J* = 4.9, 3.6 Hz, 1H), 7.04 (s, 1H), 7.34 – 7.40 (m, 3H), 7.51 – 7.55 (dd, *J* = 4.9, 1.3 Hz, 1H), 7.78 –7.82 (m, 2H), 8.22 (s, 1H).

5-Bromo-*N*-[4-(4-chlorophenyl)thiazol-2-yl]pyridine-3-sulfonamide (14w)

A mixture of 4-(4-chlorophenyl)-2-thiazolamine (100 mg, 0.47 mmol) and 5-bromopyridine-3-sulfonyl chloride (121.75 mg, 0.47 mmol) in pyridine (2 mL) was subjected to the procedure described above to give the desired product as a white solid (40%).

^{1}H NMR (DMSO): δ (ppm) 7.35 (s, 1H), 7.50 – 7.54 (m, 2H), 7.73 – 7.77 (m, 2H), 8.36 – 8.38 (dd, *J* = 2.1 Hz, 1H), 8.87 – 8.97 (dd, *J* = 6.2, 2.2 Hz, 1H), 13.59 (bs, 1H)

_N_-[4-(4-Chlorophenyl)thiazol-2-yl]-3-(2-methylpyrazol-3-yl)benzenesulfonamide (14x)

To a mixture of 4-(4-chlorophenyl)-2-thiazolamine (40 mg, 0.19 mmol) and 3-(1-methyl-1H-pyrazol-5-yl)benzenesulfonyl chloride (48.74 mg, 0.19 mmol) in pyridine (2 mL) was subjected to the procedure described above to give the desired product as a white solid (66%).

^{1}H NMR (DMSO): δ (ppm) 3.99 (s, 3H), 6.43 – 6.45 (d, *J* = 2.0 Hz, 1H), 7.41 – 7.49 (m, 4H), 7.53 – 7.55 (d, *J* = 2.0 Hz, 1H), 7.60 – 7.67 (m, 3H), 8.00 – 8.06 (m, 2H)

_N_-[4-(4-Chlorophenyl)thiazol-2-yl]-3-(2-methylpyrimidin-4-yl)benzenesulfonamide (14y)

A mixture of 4-(4-Chlorophenyl)-2-thiazolamine (50. mg, 0.24 mmol) and 3-(2-methyl-4-pyrimidinyl)benzenesulfonyl chloride (63.77 mg, 0.24 mmol) in pyridine (2 mL) was subjected to the procedure described above to give the desired product as a white solid (38%).

^{1}H NMR (DMSO): δ (ppm) 2.71 (s, 3H), 7.29 (s, 1H), 7.48 – 7.52 (m, 2H), 7.71 – 7.77 (m, 3H), 7.94 – 7.96 (d, *J* = 5.3 Hz, 1H), 7.99 – 8.03 (m, 1H), 8.36 – 8.41 (m, 1H), 8.67 – 8.69 (dd, *J* = 1.6 Hz, 1H), 8.80 – 8.82 (d, *J* = 5.3 Hz, 1H), 13.35 (bs, 1H).

3-Chloro-*N*-(4-phenylthiazol-2-yl)benzenesulfonamide (17a)

A mixture of 4-phenylthiazol-2-amine hydrobromide (164. mg, 0.64 mmol) and 3-chlorobenzenesulfonyl chloride (0.09 mL, 0.64 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (25%).

^{1}H NMR (DMSO): δ (ppm) 7.36 (s, 1H), 7.48 – 7.58 (m, 3H), 7.68 – 7.74 (dd, *J* = 8.1 Hz, 1H), 7.78 – 7.85 (m, 3H), 7.89 – 7.94 (m, 2H), 13.48 (bs, 1H)

3-Chloro-*N*-[4-(2-chlorophenyl)thiazol-2-yl]benzenesulfonamide (17b)

A mixture of 4-(2-chlorophenyl)thiazol-2-amine hydrobromide (149.26 mg, 0.51 mmol) and 3-chlorobenzenesulfonyl chloride (0.07 mL, 0.51 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (41%).

^{1}H NMR (DMSO): δ (ppm) 7.06 (s, 1H), 7.41 – 7.52 (m, 2H), 7.53 – 7.66 (m, 3H), 7.68 – 7.74 (m, 1H), 7.79 – 7.84 (m, 2H), 13.26 (bs, 1H)

3-Chloro-*N*-[4-(3-chlorophenyl)thiazol-2-yl]benzenesulfonamide (17c)

A mixture of 4-(3-chlorophenyl)thiazol-2-amine hydrobromide (149.26 mg, 0.51 mmol) and 3-chlorobenzenesulfonyl chloride (0.07 mL, 0.51 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (43%).

^{1}H NMR (DMSO): δ (ppm) 7.41 (s, 1H), 7.44 – 7.49 (m, 2H), 7.57 – 7.64 (dd, *J* = 8.1 Hz, 1H), 7.67 – 7.74 (m, 2H), 7.78 – 7.83 (m, 2H), 7.84 – 7.87 (m, 1H), 13.41 (bs, 1H)

3-Chloro-*N*-[4-(2-nitrophenyl)thiazol-2-yl]benzenesulfonamide (17e)

A mixture of 4-(2-nitrophenyl)thiazol-2-amine hydrobromide (170 mg, 0.56 mmol) and 3-chlorobenzenesulfonyl chloride (0.08 mL, 0.56 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (36%).

^{1}H NMR (DMSO): δ (ppm) 7.04 (s, 1H), 7.58 – 7.65 (dd, *J* = 8.0 Hz, 1H), 7.67 – 7.88 (m, 6H), 8.16 – 8.22 (m, 1H), 13.30 (bs, 1H)

3-Chloro-*N*-[4-(3-nitrophenyl)thiazol-2-yl]benzenesulfonamide (17f)

A mixture of 4-(3-nitrophenyl)thiazol-2-amine hydrobromide (170 mg, 0.56 mmol) and 3-chlorobenzenesulfonyl chloride (0.08 mL, 0.56 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (36%).

^{1}H NMR (DMSO): δ (ppm) 7.60 (s, 1H), 7.60 – 7.65 (dd, *J* = 8.1 Hz, 1H), 7.69 – 7.78 (m, 2H), 7.79 – 7.85 (m, 2H), 8.16 – 8.25 (m, 1H), 8.16 – 8.63 (dd, *J* = 1.9 Hz, 1H), 13.61 (bs, 1H)

3-Chloro-*N*-[4-(4-nitrophenyl)thiazol-2-yl]benzenesulfonamide (17g)

A mixture of 4-(4-nitrophenyl)thiazol-2-amine hydrobromide (170 mg, 0.56 mmol) and 3-chlorobenzenesulfonyl chloride (0.08 mL, 0.56 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (36%).

^{1}H NMR (DMSO): δ (ppm) 7.57 – 7.63 (m, 2H), 7.68 – 7.72 (m, 1H), 7.80 – 7.83 (m, 2H), 7.98 – 8.03 (m, 2H), 8.25 – 8.31 (m, 1H), 13.59 (bs, 1H)

Ethyl 3-[2-(m-tolylsulfonylamino)thiazol-4-yl]benzoate (17h)

A mixture of ethyl 3-(2-aminothiazol-4-yl)benzoate (1.0 g, 4.03 mmol) and m-toluenesulfonyl chloride (584.34 μL, 4.03 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (80%).

^{1}H NMR (DMSO): δ (ppm) 2.38 (s, 3H), 7.38 (s, 1H), 7.41 – 7.49 (m, 2H), 7.56 – 7.70 (m, 3H), 7.92 – 8.01 (m, 2H), 7.98 – 8.03 (m, 2H), 8.27 – 8.31 (dd, *J* = 1.5 Hz, 1H), 13.34 (bs, 1H)

***N*-[4-(3-Bromophenyl)thiazol-2-yl]-3-chloro-benzenesulfonamide (17i)**

A mixture of 4-(3-bromophenyl)thiazol-2-amine hydrobromide (212. mg, 0.63 mmol) and 3-chlorobenzenesulfonyl chloride (0.09 mL, 0.63 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (42%).

^{1}H NMR (DMSO): δ (ppm) 7.37 – 7.44 (m, 2H), 7.57 – 7.64 (m, 2H), 7.68 – 7.76 (m, 2H), 7.78 – 7.83 (m, 2H), 7.96 – 8.00 (dd, *J* = 1.7 Hz, 1H), 13.40 (bs, 1H)

_N_-[4-(4-Bromophenyl)thiazol-2-yl]-3-chloro-benzenesulfonamide (17j)

A mixture of 4-(4-bromophenyl)thiazol-2-amine hydrobromide (212. mg, 0.63 mmol) and 3-chlorobenzenesulfonyl chloride (0.09 mL, 0.63 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (38%).

^{1}H NMR (DMSO): δ (ppm) 7.33 (s, 1H), 7.57 – 7.74 (m, 6H), 7.78 – 7.83 (m, 2H), 13.42 (bs, 1H)

3-Chloro-_N_-[4-(2-methoxyphenyl)thiazol-2-yl]benzenesulfonamide (17k)

A mixture of 4-(2-methoxyphenyl)thiazol-2-amine hydrobromide (168. mg, 0.58 mmol) and 3-chlorobenzenesulfonyl chloride (0.08 mL, 0.58 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (43%).

^{1}H NMR (DMSO): δ (ppm) 3.31 (s, 3H), 6.97 – 7.04 (m, 1H), 7.09 (s, 1H), 7.10 – 7.15 (m, 1H), 7.35 – 7.42 (m, 1H), 7.54 – 7.62 (m, 2H), 7.65 – 7.71 (m, 1H), 7.77 – 7.82 (m, 2H), 13.30 (bs, 1H)

3-Chloro-_N_-[4-(3-methoxyphenyl)thiazol-2-yl]benzenesulfonamide (17l)

A mixture of 4-(3-methoxyphenyl)thiazol-2-amine hydrobromide (168. mg, 0.58 mmol) and 3-chlorobenzenesulfonyl chloride (0.08 mL, 0.58 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (43%).

^{1}H NMR (DMSO): δ (ppm) 3.33 (s, 3H), 6.93 – 6.98 (m, 1H), 7.27 – 7.39 (m, 4H), 7.56 – 7.64 (dd, *J* = 8.1 Hz, 1H), 7.67 – 7.73 (m, 1H), 7.79 – 7.84 (m, 2H), 13.37 (bs, 1H)

3-Chloro-_N_-[4-(4-methoxyphenyl)thiazol-2-yl]benzenesulfonamide (17m)

A mixture of 4-(4-methoxyphenyl)thiazol-2-amine hydrobromide (168. mg, 0.58 mmol) and 3-chlorobenzenesulfonyl chloride (0.08 mL, 0.58 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (43%).

^{1}H NMR (DMSO): δ (ppm) 3.21 (s, 3H), 6.85 – 6.90 (m, 2H), 6.96 (s, 1H), 7.46 – 7.60 (m, 4H), 7.66 – 7.71 (m, 2H) 13.17 (bs, 1H)

3-Chloro-*N*-[4-(2,3-dihydro-1,4-benzodioxin-6-yl)thiazol-2-yl]benzenesulfonamide (17n)

A mixture of 4-(2,3-dihydro-1,4-benzodioxin-6-yl)thiazol-2-amine hydrobromide (157. mg, 0.50 mmol) and 3-chlorobenzenesulfonyl chloride (0.07 mL, 0.50 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (44%).

^{1}H NMR (DMSO): δ (ppm) 4.26 (s, 4H), 6.89 – 6.92 (d, *J* = 8.4 Hz, 1H), 7.11 (s, 1H), 7.17 – 7.22 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.26 – 7.27 (d, *J* = 2.2 Hz, 1H), 7.57 – 7.63 (dd, *J* = 8.1, 1H), 7.68 – 7.72 (m, 1H), 7.78 – 7.82 (m, 2H), 13.25 (bs, 1H)

3-Chloro-*N*-(5-methyl-4-phenyl-thiazol-2-yl)benzenesulfonamide (17o)

A mixture of 5-methyl-4-phenyl-thiazol-2-amine hydrobromide (147. mg, 0.54 mmol) and 3-chlorobenzenesulfonyl chloride (0.08 mL, 0.54 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (42%).

^{1}H NMR (DMSO): δ (ppm) 3.33 (s, 3H), 7.40 – 7.49 (m, 5H), 7.57 – 7.63 (m, 1H), 7.67 – 7.72 (m, 1H), 7.77 – 7.82 (m, 2H), 12.99 (bs, 1H).

3-Chloro-*N*-(4,5-diphenylthiazol-2-yl)benzenesulfonamide (17p)

A mixture of 4,5-diphenylthiazol-2-amine hydrobromide (168 mg, 0.50 mmol) and 3-chlorobenzenesulfonyl chloride (0.07 mL, 0.50 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (34%).

^{1}H NMR (DMSO): δ (ppm) 7.21 – 7.26 (m, 2H), 7.30 – 7.42 (m, 9H), 7.59 – 7.65 (m, 1H), 7.69 – 7.75 (m, 1H), 7.82 – 7.87 (m, 2H), 13.31 (bs, 1H)

3-Chloro-*N*-[5-(p-tolyl)-1,3,4-thiadiazol-2-yl]benzenesulfonamide (18)

A mixture of 3-chlorobenzenesulfonyl chloride (0.08 mL, 0.58 mmol) and 5-(p-tolyl)-1,3,4-thiadiazol-2-amine (100. mg, 0.52 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (42%).

^{1}H NMR (DMSO): δ (ppm) 2.38 (s, 3H), 7.34 – 7.37 (d, *J* = 8.0 Hz, 2H), 7.58 – 7.64 (dd, *J* = 8.0 Hz, 1H), 7.70 – 7.75 (m, 3H), 7.80 – 7.84 (m, 2H), 14.49 (bs, 1H)

_N_-[5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl]-3-methyl-benzenesulfonamide (19)

A mixture of m-toluenesulfonyl chloride (0.07 mL, 0.47 mmol) and 5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (100 mg, 0.47 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (49%).

^{1}H NMR (DMSO): δ (ppm) 2.20 (s, 3H), 7.25 – 7.30 (m, 2H), 7.40 – 7.47 (m, 4H), 7.65 – 7.71 (m, 2H), 14.29 (bs, 1H)

3,5-Dichloro-_N_-(1-phenyl-1H-pyrazol-3-yl)benzenesulfonamide (20)

This material was purchased from Enamine (Kiev, Ukraine) and used without further purification.

^{1}H NMR (DMSO): δ (ppm) 6.30 – 6.32 (d, J = 2.6 Hz, 1H), 7.25 – 7.30 (m, 1H), 7.43 – 7.51 (m, 2H), 7.64 – 7.69 (m, 2H), 7.85 – 7.87 (d, J = 1.9 Hz, 2H), 7.98 – 7.99 (dd, J = 1.9 Hz, 1H), 8.39 – 8.40 (d, J = 2.6 Hz, 1H), 11.17 (bs, 1H)

_N_-[1-(4-Chlorophenyl)pyrazol-3-yl]-3-(trifluoromethyl)benzenesulfonamide (21)

A mixture of 1-(4-chlorphenyl)-1H-pyrazol-3-amin (100 mg, 0.52 mmol) and 3-(trifluoromethyl)benzenesulfonyl chloride (0.08 mL, 0.52 mmol) in pyridine (1 mL) was subjected to the procedure described above to give the desired product as a white solid (48%).

^{1}H NMR (DMSO): δ (ppm) 6.29 – 6.30 (d, J = 2.6 Hz, 1H), 7.47 – 7.53 (m, 2H), 7.64 – 7.69 (m, 2H), 7.82 – 7.87 (m, 1H), 8.03 – 8.06 (m, 1H), 8.13 – 8.17 (m, 2H), 8.39 – 8.40 (d, J = 2.6 Hz, 1H), 11.21 (bs, 1H)

_N_-[4-(4-Chlorophenyl)thiazol-2-yl]-N,3-dimethyl-benzenesulfonamide (14c)

To an agitated solution of potassium carbonate (121.21 mg, 0.88 mmol) and **14g** (160. mg, 0.4400 mmol) in DMF (2 mL) was added iodomethane (0.03 mL, 0.48 mmol), and the resulting mixture was stirred for 1 hr at ambient temperature. The reaction mixture was then poured into water (10 mL) and the resulting mixture was extracted with DCM (2 × 20 mL). The combined organics were then distilled to dryness to give the crude product, which was purified by preparatory HPLC (high pH) to give the desired product as a tan powder (20%).

^{1}H NMR (DMSO): δ (ppm) 3.47 (s, 3H), 7.44 – 7.49 (m, 2H), 7.51 – 7.57 (m, 2H), 7.64 – 7.72 (m, 3H).

_N_-[(E)-1-(5-Chloro-2-hydroxy-phenyl)ethylideneamino]-3-morpholinosulfonyl-benzamide (22)[2]

This compound was synthesized according to the route described in the literature to give title compound as an off-white solid.[2]

^{1}H NMR (DMSO): δ (ppm) 2.88 – 2.90 (m, 4H), 3.57 (s, 3H), 3.63 – 3.67 (m, 4H), 6.95 – 6.98 (d, *J* = 8.8 Hz, 1H), 7.34 – 7.38 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.67 – 7.69 (d, *J* = 2.5 Hz, 1H), 7.83 – 7.88 (dd, *J* = 7.8 Hz, 1H), 7.95 – 8.00 (m, 1H), 8.20 (bs, 1H), 8.26 – 8.32 (m, 1H), 11.71 (s, 1H), 13.33 (bs, 1H).

4-[2-(p-Tolyl)-5-(pyrrolidin-3-ylmethoxy)-3-pyridyl]benzonitrile hydrochloride (23)[3]

This material was synthesized according to standard procedures to give the title compounds as an off-white solid. The chirality in the compound was introduced form the chiral starting material (R)-1-Boc-3-hydroxymethylpyrrolidine, which was purchased from Fluorochem (Hadfield, UK).

^{1}H NMR (DMSO): δ (ppm) 1.69 – 1,84 (m, 1H), 2.07 – 2.18 (m, 1H), 2.27 (s, 3H), 2.73 – 2.83 (m, 1H), 2.99 – 3.09 (m, 1H), 3.18 – 3.44 (m, 3H), 4.13 – 4.26 (m , 2H), 7.04 – 7.12 (m, 4H), 7.38 – 7.42 (d, *J* = 8.3 Hz, 1H), 7.45 – 7.47 (d, *J* = 2.8 Hz, 1H), 7.80 – 7.83 (d, *J* = 8.3 Hz, 1H), 8.44 – 8.46 (d, *J* = 2.8 Hz, 1H), 9.04 (bs, 1H).

LCMS data

Table S1 – LCMS data for compounds **3**, **6**, **7** and **10a-q**

Compound	LC-MS Data								NMR Purity
	pH 4				*pH 10*				
	RT	Observed MW	Adduct	Purity	RT	Observed MW	Adduct	Purity	
3	0.75	255.5	[M+H]+	>95	1.17	255.5	[M+H]+	>95	>95
6	1.11	253.4	[M+H]+	>95	1.10	255.5	[M+H]+	>95	>95
7	1.14	253.4	[M+H]+	>95	1.06	253.4	[M+H]+	>95	>95
10a	1.17	247.5	[M+H]+	>95	1.17	247.5	[M+H]+	>95	>95
10b	1.08	255.4	[M+H]+	>95	1.05	255.4	[M+H]+	>95	>95
10c	1.36	315.5	[M+H]+	>95	1.34	315.4	[M+H]+	>95	>95
10d	1.49	305.5	[M+H]+	>95	1.46	305.5	[M+H]+	>95	>95
10e	1.00	219.4	[M+H]+	>95	1.01	219.4	[M+H]+	>95	>95
10f	1.18	253.4	[M+H]+	>95	1.10	253.4	[M+H]+	>95	>95
10g	1.18	253.4	[M+H]+	>95	1.09	253.4	[M+H]+	>95	>95
10h	1.14	253.4	[M+H]+	>95	1.06	253.4	[M+H]+	>95	
10i	1.07	233.4	[M+H]+	>95	0.99	233.4	[M+H]+	>95	>95
10j	1.30	287.4	[M]+	>95	1.20	287.4	[M]+	>95	>95
10k	1.35	287.4	[M]+	>95	1.23	287.4	[M]+	>95	>95
10l	1.09	247.4	[M+H]+	>95	1.01	247.4	[M+H]+	>95	>95
10m	1.17	297.4	[M]+	>95	1.16	297.4	[M]+	>95	>95
10n	0.77	235.4	[M+H]+	>95	0.73	235.4	[M+H]+	>95	>95
10o	0.81	235.4	[M+H]+	>95	0.81	235.4	[M+H]+	>95	>95
10p	0.96	249.4	[M+H]+	>95	0.98	249.4	[M+H]+	>95	>95
10q	0.96	244.4	[M+H]+	>95	0.96	244.4	[M+H]+	>95	>95

Table S2 – LCMS data for compounds **14a-y**

Compound	LC-MS Data								NMR Purity
	pH 4				*pH 10*				
	RT	Observed MW	Adduct	Purity	RT	Observed MW	Adduct	Purity	
14a	0.94	289.3	[M+H]+	>95	0.80	286.4	[M+H]+	>95	>95
14b	1.20	341.5	[M-H]-	87	0.99	341.5	[M-H]-	>95	>95
14c	1.51	379.5	[M+H]+	>95	1.53	379.5	[M+H]+	>95	>95
11f	1.14	351.4	[M+H]+	>95	0.92	351.4	[M+H]+	>95	>95
14e	1.15	365.5	[M+H]+	>95	0.94	365.5	[M+H]+	>95	>95
14f	1.21	365.4	[M+H]+	>95	0.95	365.4	[M+H]+	>95	>95
14g	1.20	365.4	[M+H]+	>95	0.94	365.4	[M+H]+	90-95	>95
14h	1.20	365.4	[M+H]+	>95	0.94	365.4	[M+H]+	>95	>95
14i	1.17	381.4	[M+H]+	>95	0.92	381.4	[M+H]+	>95	>95
14j	1.16	381.4	[M+H]+	95	0.93	381.4	[M+H]+	>95	>95
14k	1.05	427.4	[M+H]+	>95	1.05	427.4	[M+H]+	>95	>95
14l	1.06	427.4	[M+H]+	>95	1.06	427.5	[M+H]+	>95	>95
14m	1.24	385.3	[M]+	>95	0.98	385.3	[M]+	>95	>95
14n	1.24	385.3	[M]+	>95	0.98	385.3	[M]+	>95	>95
14o	1.24	385.3	[M]+	>95	0.99	385.4	[M]+	>95	>95
14p	1.24	419.4	[M+H]+	>95	0.98	419.4	[M+H]+	>95	>95
14q	1.27	419.4	[M+H]+	>95	1.01	419.4	[M+H]+	>95	>95
14r	1.28	419.4	[M+H]+	>95	1.03	419.4	[M+H]+	>95	>95
14s	1.11	396.4	[M+H]+	>95	0.90	396.4	[M+H]+	>95	>95
14t	1.18	396.4	[M+H]+	>95	0.96	396.4	[M+H]+	>95	>95
14u	1.18	396.4	[M+H]+	>95	0.97	396.4	[M+H]+	>95	>95
14v	1.10	357.4	[M+H]+	>95	0.91	357.4	[M+H]+	>95	>95
14w	1.16	430.3	[M]+	>95	0.94	430.3	[M]+	>95	>95
14x	1.09	429.5	[M-H]-	100	0.90	429.5	[M-H]-	100	>95
14y	1.13	443.4	[M+H]+	>95	0.92	443.4	[M+H]+	>95	>95

Table S3 – LC-MS data for compounds **17a-p** and **18-23**

Compound	LC-MS Data								NMR Purity
	pH 4				*pH 10*				
	RT	Observed MW	Adduct	Purity	RT	Observed MW	Adduct	Purity	
17a	1.16	351.4	[M+H]+	>95	0.92	351.4	[M+H]+	>95	>95
17b	1.20	385.4	[M+H]+	>95	0.96	385.4	[M+H]+	>95	>95
17c	1.25	385.4	[M+H]+	>95	0.98	385.4	[M+H]+	>95	>95
17d	1.24	385.3	[M]+	>95	0.98	385.3	[M]+	>95	>95
17e	1.11	396.4	[M+H]+	>95	0.90	396.4	[M+H]+	>95	>95
17f	1.17	396.4	[M+H]+	>95	0.93	396.4	[M+H]+	>95	>95
17g	1.18	396.4	[M+H]+	>95	0.94	396.4	[M+H]+	>95	>95
17h	1.21	403.5	[M+H]+	90-95	1.15	403.5	[M+H]+	>95	90-95
17i	1.27	429.3	[M]+	>95	1.01	429.3	[M]+	>95	>95
17j	1.27	429.3	[M]+	>95	1.01	429.3	[M]+	>95	>95
17k	1.20	381.5	[M+H]+	>95	0.93	381.5	[M+H]+	>95	>95
17l	1.19	381.4	[M+H]+	90-95	0.93	381.5	[M+H]+	>95	90-95
17m	1.18	381.5	[M+H]+	90-95	0.91	381.5	[M+H]+	>95	90-95
17n	1.17	409.4	[M+H]+	>95	0.91	409.4	[M+H]+	>95	>95
17o	1.23	365.5	[M-H]-	>95	0.95	365.5	[M+H]+	90-95	>95
17p	1.37	427.4	[M+H]+	>95	1.06	427.4	[M+H]+	>95	>95
18	1.27	366.4	[M+H]+	>95	0.92	366.5	[M+H]+	>95	>95
19	1.26	366.4	[M+H]+	90-95	0.96	366.5	[M+H]+	>95	>95
20	0.79	368.4	[M]+	90-95	0.79	368.4	[M]+	>95	>95
21	0.85	401.5	[M+H]+	>95	0.86	401.5	[M+H]+	>95	>95
22	1.02	438.5	[M+H]+	>95	1.01	438.5	[M+H]+	>95	>95
23	0.94	370.6	[M+H]+	>95	1.40	370.6	[M+H]+	>95	>95

Biology

LSD1 screening Methods

Protein Production

The coding sequence for residues M13 to M852 of LSD1, cloned into pET30a to utilise the N-terminal His tag, was kindly provided by the laboratory of Ernest Laue. LSD1 was expressed in Rosetta™ 2 cells grown in auto induction medium overnight at 25°C. Cell pellets were resuspended in 3 volumes of IMAC Buffer A (40 mM Tris, 300 mM NaCl, 1 mM MgCl2, 5% Glycerol, pH 8.0) containing 1 × Complete EDTA-free protease inhibitors, 1 mg/mL lysozyme and 25 U/mL Benzonase®. Lysate was prepared by sonication and clarification by centrifugation @ 45,000 xg, 45 min then filtration of the supernatant through a 1.2 μM syringe filter. Clarified lysate was purified over Talon® resin (5 mL/L of culture) using a batch/gravity protocol. Following a 25 column (CV) wash with IMAC, protein was eluted with 5 CV of IMAC A containing 250 imidazole and subsequently supplemented with 1 mM EDTA & DTT. The eluted protein was concentrated and applied to a Superdex 200 HR 26/60 column equilibrated in 20 mM Tris, 150 mM NaCl, 0.5 mM EDTA, 1 mM DTT, 5% glycerol @ pH8.0. Peak fractions were pooled and diluted with IEX Buffer A (20 mM Tris, pH 8.0) to reduced [NaCl] to 30 mM then loaded onto a HR5/5 Mono-Q column. Following a 25 CV wash with IEX Buffer A, protein was eluted by developing a NaCl gradient from 50 mM to 300 mM over 45 CV. Peak fractions were pooled & frozen on dry ice prior to storage at 80°C.

Compound Handling

All compounds for the fragment screening and hit validation were dispensed from source plates, containing compounds at 20 or 100 mM in 100% (v/v) DMSO or 100% DMSO, directly into assay plates using an ECHO 550 Acoustic dispenser (Labcyte Inc™, Sunnyvale, CA, USA) to obtain the desired final assay concentrations of compound and DMSO.

Primary fragment screen

LSD1 protein (in house) was screened against the ICR library of 2446 fragments using a biochemical assay based on AlphaScreen technology. Compounds were screened at a final concentration of 300 μM and 1.5% (v/v) DMSO in white 384-well Optiplate (#6007290; Perkin Elmer Life Sciences). The enzyme reaction (total volume 20 μL) was carried out with 100 nM monomethylated peptide (ARTK(Me)QTARKSTGGKAPRKQLA-Biotin), 12.5 nM LSD1 in 40 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT 0.02% (v/v) Tween 20, 10 % (v/v) glycerol. Each 384 well plate contained 32 control wells for total enzyme activity, plus 32 wells of no enzyme blanks. The reaction was incubated for 90 min then stopped by the addition of 5 μL bead mix containing mouse IgG detection

kit beads (#6760606; Perkin Elmer Life Sciences) and anti-histone H3(unmodified Lys4) cloneCMA301 (#05-1341; Millipore) in 40 mM HEPES pH 7.5, 40 mM NaCl, 5% (v/v) glycerol and 0.125% BSA to give final well concentrations of 20 μg/mL beads and 1nM antibody. The plate were sealed and incubated overnight at room temperature in the dark before being read on an Envision plate reader (PerkinElmer Life Sciences). Primary screening data was analysed in ActivityBase (IDBS, Guildford, UK). The percentage inhibition was calculated relative to total wells (containing enzyme plus DMSO) and blank wells (containing no enzyme plus DMSO). Compounds with a percentage inhibition of 50% or better were classed as initial hits.

Confirmation and interference screens

The 104 initial hits were cherry picked directly from the 20 mM source plates and dispensed into assay plates in duplicate using the ECHO 550 acoustic dispenser and assayed as for the primary screen. Additionally an interference screen was run by testing the fragments at 300 μM in the assay in duplicate in the absence of the LSD1 protein. Interference was determined by calculating the % decrease from the DMSO only control well.

IC_{50} follow up

IC_{50} determinations for the 7 fragments with confirmed activity and showing <50% assay interference were carried out as for the primary assay. Duplicate 10-point dilution curves were dispensed directly into white 384-well Optiplate plates to give a final assay concentrations in the range 0.05 - 1000 μM in 1.0% (v/v) DMSO. Tranylcypromine was included as a control compound. Additionally, the fragments were further tested for assay interference by performing IC_{50} determinations under the same assay conditions minus LSD1 and substituting the 100 nM mono-methylated peptide (with 100 nM unmethylated peptide (ARTKQTARKSTGGKAPRKQLA-Biotin). IC_{50} values were calculated from a four-parameter logistics fit of percentage inhibition versus Log concentration using GraphPad Prism 5 (GraphPad Software, Inc., CA, USA).

Reversibility studies (pre-incubation)

For looking at the reversibility of binding of the compounds IC_{50} determinations were carried out as above, but with different preincubation times (0, 30 and 60 min) of the compound with enzyme before the addition of substrate to start the reaction. Tranylcypromine was used as an irreversible inhibitor control in these experiments

Orthogonal assays

Caliper assay

LSD1 activity was measured in a microfluidic assay which monitors the separation of a unmethylated product from its methylated substrate by employing a methylation-sensitive endoproteinase strategy (Wigle et al. 2010). The assay was performed on an EZ Reader II (PerkinElmer Life Sciences,

Waltham, MA, USA). For IC_{50} determinations of the fragments and tranylcypromine, 100 nL samples were dispensed directly into 384 well Griener polypropylene plate from 10 or 100 mM stock to generate duplicate 10-point dilution curves to give final assay concentrations in the range 0.05 - 1000 μM in 1.0% (v/v) DMSO. The enzyme reaction (total volume 10 μL) was carried out with 1 μM monomethylated peptide (ARTK(me)QTARK[FAM]STGGKAPRKQLAK), 50 nM LSD1 in 20 mM Tris HCl (pH 8), 25 mM NaCl, 1 mM DTT 0.02% (v/v) Tween 20. The control wells contained 1.0% (v/v) DMSO plus and minus LSD1. The reaction was incubated at room temperature for 70 min then stopped by the applying a heat kill step of 5 min incubation at 80°C. The plate was left to cool to room temperature for 15 min. The 10 μL of stopped reaction was transferred to a black 384 well shallow well microtitre plate containing 15 μL Endoproteinase-LysC (1 ng/ml in 25 mM Tris HCl pH 8). After 2 h incubation the plate was read on the EZ Reader II with instrument settings of -1.7 psi and 1500 ΔV. The percentage conversion of product from substrate was generated automatically and the percentage inhibition was calculated relative to blank wells (containing no enzyme and 1% (v/v) DMSO) and total wells (containing all reagents and 1% (v/v) DMSO). IC_{50} values were calculated in GraphPad Prism5 using a nonlinear regression fit of percentage inhibition versus Log concentration.

LCMS assay

For the LCMS assay the enzyme reaction (total volume 50 μL in a 96 well round bottomed polypropylene plate) was carried out with 10 μM monomethylated peptide (ARTK(Me)QTARKSTGGKAPRKQLA-Biotin) and 100 nM LSD1 in 5 mM potassium phosphate buffer (pH 7.5). The compounds were tested in duplicate at a range of final assay concentrations from 1.5-200 μM in 1% (v/v) DMSO alongside controls wells of buffer only, buffer plus substrate, and buffer plus substrate and enzyme The reaction was incubated for 90 minutes then stopped by the applying a heat kill step of 5 min incubation at 85°C. The samples were processed by LCMS

Ten microliter injections (with needle wash) of each well, were made onto a Merck Chromolith SpeedRod RP-18e column (50 × 4.6 mm, i.d., 151450, Darmstadt, Germany). Samples were refrigerated at 4°C in a G1367B auto-sampler with G1330B thermostat module prior to injection. Chromatographic separation at 30C was carried out using a 1200 Series HPLC (Agilent, Santa Clara, USA) over a 7 min gradient elution. Sample was loaded onto the column cartridge using a G1312A binary pump dispensing a gradient from 90:10 to 10:90 water and methanol (both modified with 0.1% formic acid) at a flow rate of 0.5 mL/min.

The post column eluent flow from the diode array detector was infused into a 6520 Series qToF mass spectrometer (G6520A) fitted with an ESI/APCI multimode ionisation source (Agilent, Santa Clara,

USA). LC eluent and nebulising gas was introduced into the grounded nebuliser with spray direction orthogonal to the capillary axis. 2 kV was applied to the charging electrode to generate a charged aerosol. The aerosol was dried by infrared emitters (200°C) and heated drying gas (5 L/min of nitrogen at 300°C, 15 psi), producing ions by ESI. Aerosol and ions were transferred by nebulising gas to the APCI zone where infrared emitters vaporized solvent and analyte. A corona discharge was produced between the corona needle and APCI counter electrode by applying a current of 2 µA, ionizing the solvent to transfer charge to analyte molecules, producing ions by APCI. ESI and APCI ions simultaneously entered the transfer capillary along which a potential difference of 2.5 kV was applied. The fragmentor voltage was set at 120 V and skimmer at 65 V. Signal was optimised by AutoTune.m. Profile mass spectrometry data was acquired in positive ionisation mode over a scan range of *m/z* 300-650 (scan rate 1.0) with reference mass correction at *m/z* 322.048121 (hexamethoxyphosphazene), and 622.02896 (hexakis(2,2-difluoroethoxy)phosphazene).

Raw data was processed using Agilent MassHunter Qualitative Analysis B.04.00. Peaks for the extracted ion chromatograms of the $[M+5H]^{5}+$ charged species of non-methylated and mono-methylated H3K4 were integrated between 2.8 and 3.6 minutes. Background was subtracted using corresponding average areas of blanks in wells either side of sample. Peak areas were normalised against the relative % ionisation of the $[M+5H]^{5}+$ charged species of non-methylated and mono-methylated H3K4. % inhibition of the demethylation was plotted from relative % of non-methylated and mono-methylated H3K4 at inhibitor concentrations. IC_{50} values were calculated in GraphPad Prism5 using a nonlinear regression fit of percentage inhibition versus Log concentration.

LSD1 HTRF assay

Assays were performed in 384-well plates in a 10 µL reaction volume consisting of 50 mM TrisHCl, 50 mM NaCl, 1 mM DTT, 0.01% Tween-20, 1% DMSO with or without an inhibitor at varying concentrations, 0.2 µM Histone H3 (1 – 21) K4(Me1) biotin peptide substrate (AnaSpec Inc., Freemont, CA) and 0.125 nM LSD1 (BPS Bioscience, San Diego, CA). The assay was allowed to proceed for 30 min at 25°C before stopping the reaction by the addition of 0.3 mM tranylcypromine and quantifying the level of demethylated peptide by the addition of 1 nM Europium-α-unmodified H3K4 antibody and 25 nM ULight Streptavidin (both from Perkin Elmer, Wltham, MA). Following a further 60 min incubation period the TR-FRET signal was read with excitation at 340 nm and emission at 665 nm.

MAO-A biochemical assay

Assays were performed in black 384-well plates in a 20 µL reaction volume consisting of 100 mM potassium phosphate buffer (pH 7.4), 1% DMSO, 40 µM Kynuramine dihydrobromide (Sigma-Aldrich) and 0.001U/mL MAO-A (Promega). The assay was allowed to proceed for 30 min at 25°C with gentle agitation, before stopping the reaction with 555 mM NaOH and quantifying the level of oxidative deamination of Kynuramine to 4-hydroxyquinoline (4-HQ), which is fluorescent at excitation 340 nm and emission at 440 nM.

Reversibility assay (dilution)

A number of compounds, representative of the different series, were chosen based on their potency and ligand efficiency. Reversibility studies were performed by pre-incubating the compound at 10× IC_{50} value with LSD1 at 100 nM (100× the standard assay concentration) for 20 min. The reaction was then diluted 100-fold in enzyme buffer (50 mM TrisHCl, 50 mM NaCl, 1 mM DTT, 0.01% Tween-20) supplemented with 0.2 µM Histone H3 (1 – 21) K4(Me1) biotin peptide substrate to initiate the reaction. The reaction was stopped at various time points by the addition of 0.3 mM tranylcypromine and the level of demethylated peptide was quantified by the addition of 1 nM europium-α-unmodified H3K4 antibody and 25 nM ULight streptavidin (both from Perkin Elmer, Waltham, MA, USA). Following a further 60 min incubation period the TR-FRET signal was read with excitation at 340 nm and emission at 665 nm on a PheraStar FS microplate reader. The % recovery in enzymatic activity following the 100-fold dilution of enzyme-inhibitor complex was monitored for 60 minutes post reaction initiation.

LSD1 Cellular assay

Cells were lysed in lysis buffer (150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1% Triton-X-100, 50 mM NaF, 20 mM Tris-HCl (pH 7.6), 1 × Phosphatase Inhibitor cocktail 2 and 3 (Sigma), 1 × Protease Inhibitor cocktail (Roche, Burgess Hill, UK)) and applied to a CD86 ELISA (ab45920, Abcam, Cambridge, UK). The assay was used in accordance with the manufacturer's instructions.