Electronic Supplementary Material (ESI) for MedChemComm.

The design, synthesis and evaluation of low molecular weight acidic sulfonamides as URAT1 inhibitors for the treatment of gout.

Andy Pike,[a] R. Ian Storer,[b*] Robert M. Owen,[b] Emma Armstrong,[c] Caroline L. Benn,[c] Magda Bictash,[c] Kathy F. K. Cheung,[f] Kathryn Costelloe,[c] Emmanuel Dardennes,[f] Emma Impey,[c] Philip H. Milliken,[d] Elisabeth Mortimer,[d] Hannah J. Pearce[e]*

[a]Pharmacokinetics Dynamics and Metabolism, [b]Worldwide Medicinal Chemistry, [c]Discovery Biology, [d]Global Safety Group, [e]Pharmaceutical Sciences, Pfizer Ltd., The Portway, Granta Park, Cambridge, CB21 6GS. United UK; [f]Peakdale Molecular Ltd, Discovery Park House, Ramsgate Road, Sandwich, Kent, CT13 9ND, UK.

Contents

1. Abbreviations - The following abbreviations and definitions have been used:

br	broad
$CDCl_3$	Chloroform-d1
δ	Chemical shift
d	Doublet
DAD	Diode Array Detector
DCM	Dichloromethane
DMSO	Dimethylsulfoxide
ELSD	Evaporative Light Scattering Detector
eq	Equivalent
ESI	Electrospray ionisation (positive scan)
EtOAc	Ethyl acetate
h	Hour(s)
HPLC	High Performance Liquid chromatography
HRMS	High resolution mass spectrum
LRMS	Low resolution mass spectrum
M	Molarity
m	Multiplet
Me	Methyl
mg	Milligram
min	Minute(s)
MHz	Megahertz
mL	Millilitre
mmol	Millimole
m/z	Mass spectrum peak
N	Normal concentration
nm	Nuclear Magnetic Resonance
R_t	Retention time
s	Singlet
t	Triplet
UV	Ultraviolet

2. General Chemistry Experimental Prodecures[1]

^{1}H Nuclear magnetic resonance (NMR) spectra were in all cases consistent with the proposed structures. Characteristic chemical shifts (δ) are given in parts-per-million downfield from tetramethylsilane using conventional abbreviations for designation of major peaks: *e.g.* s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. The mass spectra (*m/z*) were recorded using either electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI). The following abbreviations have been used for common solvents: $CDCl_3$, deuterochloroform; d_6-DMSO, deuterodimethylsulphoxide; *d*4-methanol, deuteromethanol. All solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous magnesium sulfate or anhydrous sodium sulfate.

LCMS conditions:

System 1

A: 0.1% formic acid in water

B: 0.1% formic acid in acetonitrile

Column: C18 phase Phenomenex 20 x 4.0 mm with 3 micron particle size

Gradient: 98-2% or 98-10% A over 1.5 min, 0.3 min hold, 0.2 re-equilibration, 1.8 mL/min flow rate

UV: 210 nm – 450 nm DAD

Temperature: 75 °C

System 2

A: 0.1% formic acid in water

B: 0.1% formic acid in acetonitrile

Using either:

Column: Agilent Extend C18 phase 50 x 3 mm with 3 micron particle size

Gradient: 95-0% A over 3.5 min, 1 min hold, 0.4 min re-equilibration, 1.2 mL/min flow rate

Or

Column: C18 phase Waters Sunfire 50 x 4.6 mm with 5 micron particle size

Gradient: 95-5% A over 3 min, 1 min hold, 2 min re-equilibration, 1 mL/min flow rate
UV: 210 nm – 450 nm DAD
Temperature: 50 °C

Preparative HPLC:

Where singleton compounds are purified by preparative HPLC, there are two methods used, shown below:

Method 1 acidic conditions

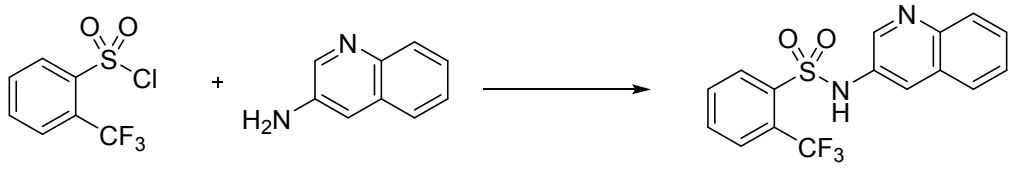
Column Gemini NX C18, 5 μm 21.2 x 100 mm
Temperature Ambient
Detection ELSD-MS
Mobile Phase A 0.1% formic acid in water
Mobile Phase B 0.1% formic acid in acetonitrile
Gradient initial 0% B, 1 min – 5% B; 7 min – 98% B; 9 min – 98% B; 9.1 min – 5% B; 10 min – 5% B
Flow rate 18 mL/min
Injection volume 1000 μL

Method 2 basic conditions

Column Gemini NX C18, 5 μm 21.2 x 100mm
Temperature Ambient
Detection ELSD-MS
Mobile Phase A 0.1% diethylamine in water
Mobile Phase B 0.1% diethylamine in acetonitrile
Gradient initial 0% B, 1 min – 5% B; 7 min – 98% B; 9 min – 98% B; 9.1 min – 5% B; 10 min – 5% B
Flow rate 18 mL/min
Injection volume 1000 μL

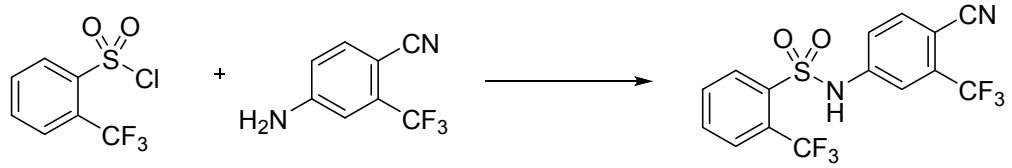
3. Chemistry experimental procedures and analytical data for additional intermediates and test compounds

N-(Quinolin-3-yl)-2-(trifluoromethyl)benzenesulfonamide (12)



A solution of quinolin-3-amine (36 mg, 0.25 mmol) in pyridine (0.5 mL) was cooled to 0 °C followed by addition of 2-(trifluoromethyl)benzene-1-sulfonyl chloride (**48**, 49 mg, 0.20 mmol). The reaction mixture was stirred at rt for 1 hour then concentrated in vacuo to give a thick gum which was purified by high throughput automated HPLC to give the title compound as a beige solid (26 mg, 37%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.19 (br s, 1H), 8.70 (d, *J* = 2.6 Hz, 1H), 8.19-8.16 (m, 1H), 8.03-7.81 (m, 6H), 7.65-7.61 (m, 1H), 7.55-7.51 (m, 1H); HPLC (system 1, 4.5 min, acidic) R_t 2.70 minutes, ELSD >95% purity; LRMS *m/z* 353.00 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{16}H_{12}F_3N_2O_2S$ 353.0566, found 353.0566.

N-(4-Cyano-3-(trifluoromethyl)phenyl)-2-(trifluoromethyl)benzenesulfonamide (13)



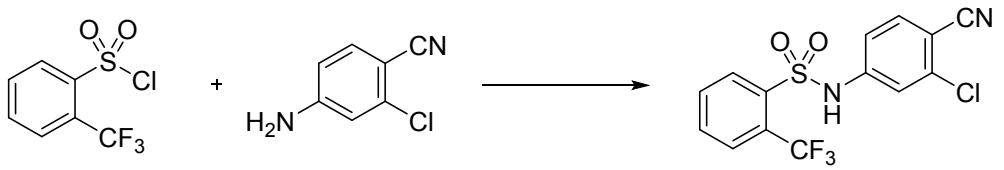
A solution of 4-amino-2-(trifluoromethyl)benzonitrile (380 mg, 2.04 mmol), 2-(trifluoromethyl)benzene-1-sulfonyl chloride (**48**, 500 mg, 2.04 mL) and DMAP (25 mg, 0.20 mmol) in pyridine (5 mL) was heated to 30 °C for 18 h. The reaction mixture was reduced in vacuo and the residue dissolved in DCM (50 mL). The organic phase was washed with 1M HCl (3x50 mL) and brine (50 mL), dried over $MgSO_4$ and solvent removed in vacuo. The crude material was purified by reverse phase chromatography (120 g C18 column, 0-50% MeCN in water for 20 minutes, isocratic at 50% for 15 minutes, 50-85% over 20 minutes) to yield the title compound as an off-white solid in 51% yield (410 mg). ^{1}H NMR (400 MHz, *d*4-methanol) δ 8.28 (dd, *J* = 6.0,

4.1 Hz, 1H), 7.97 (dd, *J* = 5.4, 3.4 Hz, 1H), 7.84-7.81 (m, 3H), 7.56 (d, *J* = 2.1 Hz, 1H), 7.48 (dd, *J* = 8.6, 2.0 Hz, 1H); ^{19}F NMR (376 MHz, *d*4-methanol) δ -59.11, -64.03: HPLC (system 2, 4.5 min, acidic) R_t 3.16 minutes, ELSD >95% purity; LRMS *m/z* 393.06 $[M-H]^-$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{15}H_9F_6N_2O_2S$ 395.0283, found 395.0281.

_N_-(3-Cyano-4-(trifluoromethyl)phenyl)-2-(trifluoromethyl)benzenesulfonamide (22)

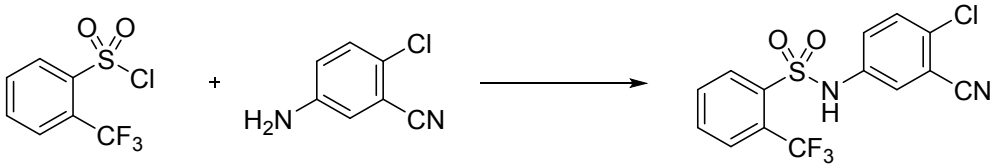
A solution of 5-amino-2-(trifluoromethyl)benzonitrile (380 mg, 2.04 mmol), 2-(trifluoromethyl)benzene-1-sulfonyl chloride (**48**, 500 mg, 2.04 mmol) and DMAP (25 mg, 0.204 mmol) in pyridine (5 mL) was stirred at rt for 3 days. The reaction was quenched by addition of aqueous 2M HCl (50 mL) and the product was extracted with EtOAc (50 mL). The organic layer was washed with aqueous 2M HCl (50 mL) and the solvent was removed in vacuo. Purification via reverse phase chromatography (10-60% MeCN in water (0.1% NH_4OH)), yielded the title compound as a white solid (318 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (dd, *J* = 6.9, 1.1 Hz, 1H), 7.91 (dd, *J* = 7.0, 1.4 Hz, 1H), 7.78-7.64 (m, 2H), 7.62 (d, *J* = 8.6 Hz, 1H), 7.56 (d, *J* = 1.8 Hz, 1H), 7.40 (dd, *J* = 8.5, 1.3 Hz, 1H), 7.19 (s, 1H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -62, -58: HPLC (system 1, 4.5 min, buffered) R_t 2.79 minutes, ELSD >95% purity; LRMS *m/z* 393.92, $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{15}H_9F_6N_2O_2S$ 395.0283, found 395.0280.

_N_-(3-Chloro-4-cyanophenyl)-2-(trifluoromethyl)benzenesulfonamide (23)



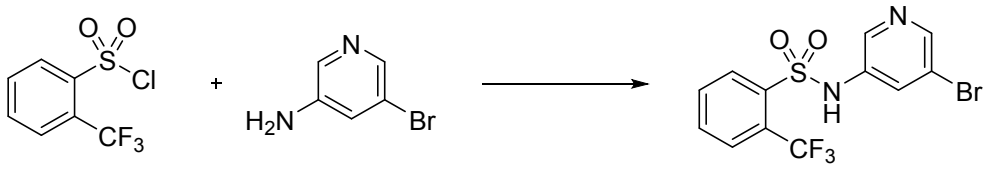
A solution of 4-amino-2-chlorobenzonitrile (100 mg, 0.65 mmol) and 2-(trifluoromethyl)benzene-1-sulfonyl chloride (**48**, 176 mg, 0.72 mmol) in DCM (5 mL) was treated with pyridine (0.11 mL, 1.31 mmol) and stirred at rt for 16 h. The reaction mixture was washed with 1M HCl (2 x 5 mL), saturated aqueous $NaHCO_3$ (2 x 10 mL) and brine (2 x 10 mL), then dried ($MgSO_4$), filtered and concentrated. The resulting solid was purified by high throughput automated chromatography to afford the title compound as a white solid (18.7 mg, 8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23-8.20 (m, 1H), 7.93-7.90 (m, 1H), 7.78-7.71 (m, 2H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 2.4 Hz, 1H), 7.08 (dd, *J* = 8.4, 2.4 Hz, 1H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.99; HPLC (system 2, 4.5 min, acidic) R_t 3.03 minutes, ELSD >95% purity; LRMS *m/z* 359.06, 361.02 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{14}H_8ClF_3N_2O_2S$ 361.0020, found 361.0015.

_N_-(4-Chloro-3-cyanophenyl)-2-(trifluoromethyl)benzenesulfonamide (24)



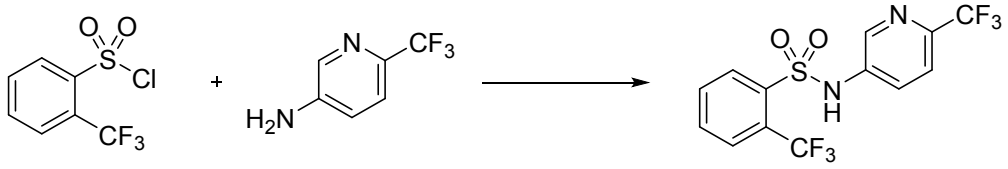
A solution of 5-amino-2-chlorobenzonitrile (312 mg, 2.04 mmol), 2-(trifluoromethyl)benzene-1-sulfonyl chloride (**48**, 500 mg, 2.04 mmol) and DMAP (25 mg, 0.204 mmol) in pyridine (7.5 mL) was heated to 30 °C for 18 h. The reaction mixture was reduced in vacuo and the residue dissolved in DCM (50 mL). The organic phase was washed with 1M HCl (3 x 50 mL) and brine (50 mL), then dried ($MgSO_4$), filtered and solvent removed in vacuo. The crude material was purified by reverse phase chromatography (120 g C18 column, 0-50% MeCN in water for 20 minutes, isocratic at 50% for 15 minutes, 50-85% over 20 minutes) to yield the title compound as an off-white solid in (420 mg, 57%). ^{1}H NMR (400 MHz, *d*4-methanol) δ 8.18-8.16 (m, 1H), 7.97-7.95 (m, 1H), 7.80-7.77 (m, 2H), 7.50-7.47 (m, 2H), 7.39 (dd, *J* = 8.8 Hz, 1H); ^{19}F NMR (376 MHz, *d*4-methanol) δ -59.06; HPLC (system 2, 4.5 min, acidic) R_t 3.09 minutes, ELSD >95% purity; LRMS *m/z* 359.06, 361.02 $[M+H]^+$; HRMS (ESI) *m/z*: $[MNa]^+$ Calcd for $C_{14}H_8ClF_3N_2NaO_2S$ 382.9839, found 382.9858.

_N_-(5-Bromopyridin-3-yl)-2-(trifluoromethyl)benzenesulfonamide (45)



A solution of 2-(trifluoromethyl)benzene-1-sulfonyl chloride (1 g, 4.09 mmol, 1) and 5-bromopyridin-3-amine (**48**, 707 mg, 4.09 mmol) in MeCN (15 mL) was treated with pyridine (660 µL, 8.18 mmol). The solution was stirred at rt for 24 h. Water (25 mL) was added and the mixture was extracted with EtOAc (2 x 40 mL). The combined organics were washed with saturated aqueous NH_4Cl (20 mL) and brine (20 mL), dried (Na_2SO_4), filtered and concentrated. The crude residue was triturated with DCM, filtered and washed with DCM to give the title compound as a white solid (335 mg, 21%). ^{1}H NMR (400 MHz, *d*6-DMSO) δ 11.24 (br s, 1H), 8.38 (d, *J* = 2.0 Hz, 1H), 8.29 (d, *J* = 2.2 Hz, 1H), 8.15-8.12 (m, 1H), 8.03-8.00 (m, 1H), 7.92-7.84 (m, 2H), 7.65 (t, *J* = 2.2 Hz, 1H); ^{19}F NMR (376 MHz, *d*6-DMSO) δ -56.28: HPLC (system 1, 4.5 min, acidic) R_t 3.10 minutes, ELSD >95% purity; LRMS *m/z* 378.94, 380.97 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{12}H_9BrF_3N_2O_2S$ 380.9515, found 380.9517.

2-(Trifluoromethyl)-N-(5-(trifluoromethyl)pyridin-2-yl)benzenesulfonamide (46)



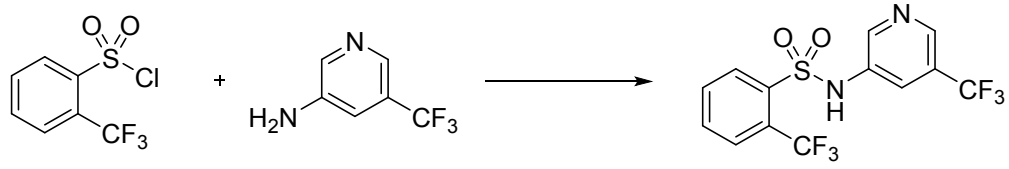
Small scale synthesis batch:

A solution of 6-(trifluoromethyl)pyridin-3-amine (300 mg, 1.85 mmol), pyridine (0.45 mL, 5.55 mmol). and 2-(trifluoromethyl)benzene-1-sulfonyl chloride (**48**, 498 mg, 2.04 mmol) in MeCN (8 mL) was stirred at rt for 16 h. Solvent was removed in vacuo to leave a residue which was treated with 2N HCl(aq) (10 mL) and extracted with DCM (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude material was purified by silica chromatography (10% to 30% ethyl acetate/heptanes) to give the title compound (598 mg, 87%) as a white solid.

Scale up synthesis batch:

A solution of 6-(trifluoromethyl)pyridin-3-amine (50 g, 0.31 mol), pyridine (74.5 mL, 0.92 mol) and 2-(trifluoromethyl)benzene-1-sulfonyl chloride (**48**, 52.4 mL, 0.34 mol) in MeCN (500 mL) was stirred at rt for 16 h. Solvent was removed in vacuo and the residue was treated with 20% aq. citric acid (500 mL) and extracted into EtOAc (2 x 500 mL). The organic phase was washed with saturated sodium bicarbonate solution (500 mL) and brine (500 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated to give 140 g of crude mixture. The crude material was purified by filtering through a silica plug washing with DCM then concentrating in vacuo. The residue (100 g) was dissolved in TBME (150 mL) at 70 °C. Heptane (150 mL) was then added and the material was allowed to crystallise overnight. The crystals were isolated by filtration to afford the title compound as an orange solid (80 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, J = 2.5 Hz, 1H), 8.15 (d, J = 7.7 Hz, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.76-7.72 (m, 2H), 7.68 (dt, J = 7.8, 1.2 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1 H), 7.10 (bs, 1H); ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.58 (s, 1H), 8.49 (d, J = 2.5 Hz, 1H), 8.20 (dd, J = 7.1, 2.0 Hz, 1H), 8.03 (dd, J = 6.9, 2.3 Hz, 1H), 7.95–7.83 (m, 2H), 7.81 (d, J = 8.6 Hz, 1H), 7.69 (dd, J = 8.6, 2.5 Hz, 1H); ^{13}C NMR (101 MHz, d_6-DMSO) δ 141.11 (q, J = 34.5 Hz), 140.31, 137.52 (q, J = 1.3 Hz), 137.29 (q, J = 1.0 Hz), 134.09 , 133.79 , 130.68 , 128.96 (q, J = 6.2 Hz), 126.34 , 126.31 (q, J = 32.9 Hz), 122.64 (q, J = 274.1 Hz), 121.62 (q, J = 2.7 Hz), 121.51 (q, J = 273.6 Hz); ^{19}F NMR (376 MHz, $CDCl_3$): -67, -58; HPLC (system 1, 4.5 min, acidic) R_t 3.12 minutes, ELSD >95% purity; LRMS *m/z* 371.04 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{13}H_9F_6N_2O_2S$ 371.0283, found 371.0279.

2-(Trifluoromethyl)-*N*-(5-(trifluoromethyl)pyridin-3-yl)benzenesulfonamide (47)



Small scale synthesis batch:

A solution of 5-(trifluoromethyl)pyridin-3-amine (196 mg, 1.21 mmol) and 2-(trifluoromethyl)benzene-1-sulfonyl chloride (**48**, 295 mg, 1.21 mmol) in pyridine (3 mL) was stirred at rt for 18 h. Solvent was removed under reduced pressure the residue purified by column

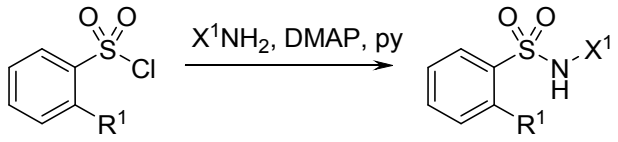
chromatography(heptane initially then 10-40% EtOAc-heptane) to afford the title compound as a colourless solid, 414 mg. A minor impurity was removed by dissolving the solid in DCM (20 mL) and washing with water (20 mL), 0.5 M aqueous citric acid (20 mL), aqueous NH_4Cl (20 mL) and water (20 mL), followed by drying over $MgSO_4$ and removal of solvent in vacuo to afford the title compound as a white solid (354 mg, 79%).

Scale up synthesis batch:

Pyridine (28.4 mL, 352.5 mmol) was added to a cooled solution (0 °C) of 5-(trifluoromethyl)pyridin-3-amine hydrochloride (14.0 g, 70.5 mmol) in MeCN (140 mL). A solution of 2-(trifluoromethyl)benzene-1-sulfonyl chloride (**48**, 18.11 g, 74.0 mmol) in MeCN (30 mL) was added dropwise maintaining the internal temperature below 5 °C. The reaction mixture was then stirred at rt for 72 h. The solvent was removed in vacuo and the resulting residue was dissolved in EtOAc (250 mL) and washed with 1M citric acid (2 x 250 mL). The aqueous layers were extracted with EtOAc (250 mL). The combined organic layers were washed with sat. $NaHCO_3$ (250 mL) and brine (250 mL), dried ($MgSO_4$), filtered and concentrated to give the crude. The crude was purified by recrystallisation from TBME (290 mL) and heptane (60 mL). The crystals were collected by filtration and washed with 1:1 TBME/heptane (150 mL) and dried under reduced pressure to give the title compound as a pale brown solid (16.16 g, 46%) which required further purification. This material was combined with another batch of the same compound to total 21.5 g and was dissolved in EtOAc (600 mL) was washed with water (3 x 600 mL), dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in methanol (420 mL) and charcoal (10.5 g) was added. The resulting mixture was heated at 50 °C for 1 h. After this time, the mixture was cooled to rt, filtered through arbocel and washed with methanol (600 mL). The filtrate was concentrated under reduced pressure to give the title compound (20.31 g, 94% recovery) as an off-white solid. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.46 (br.s, 1H), 8.67 (d, J = 0.9 Hz, 1H), 8.59 (d, J = 2.4 Hz, 1H), 8.17 (dd, J = 1.6, 7.3 Hz, 1H), 8.01 (dd, J = 1.7, 7.2 Hz, 1H), 7.88 (overlapped m, 2H), 7.74 (t, J = 1.9 Hz, 1H); ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.44 (s, 1H), 8.66–8.63 (m, 1H), 8.58 (d, J = 2.3 Hz, 1H), 8.18 (dd, J = 7.7, 1.6 Hz, 1H), 8.01 (dd, J = 7.4, 1.8 Hz, 1H), 7.93–7.83 (m, 2H), 7.73 (t, J = 2.3 Hz, 1H); ^{13}C NMR (101 MHz, d_6-DMSO) δ 144.56 (q, J = 1.6 Hz), 141.02 (q, J = 4.1 Hz), 137.71 (q, J = 1.6 Hz), 134.74, 133.99, 133.73 (q, J = 1.0 Hz), 130.71, 128.81 (q, J = 6.3 Hz), 126.34 (q, J = 32.9 Hz), 125.31 (q, J = 32.4 Hz), 123.08 (q, J = 274.2 Hz), 122.61 (q,

J = 273.9 Hz), 122.59 (q, *J* = 3.8 Hz); ^{19}F NMR (376 MHz, d_6-DMSO) δ -61.37, -56.34; HPLC (system 1, 4.5 min, acidic) R_t 3.17 minutes, ELSD >95% purity; LRMS *m/z* 371.00 $[M+H]^+$; HRMS (ESI) *m/z*: [M+H]+ Calcd for $C_{13}H_9F_6N_2O_2S$ 371.0283, found 371.0277.

Library Protocol 1



To a solution of the appropriate amine (X^1NH_2, 100 μmol) in anhydrous pyridine (300 μL) was added a 0.33M solution of the appropriate sulfonyl chloride in anhydrous pyridine (300 μL, 100 μmol) followed by DMAP (10 μmol). The reaction mixture was shaken at 30 °C for 16 hours before concentrating *in vacuo* and purifying by one of the three preparative HPLC methods described below. The organic gradient used for each compound is described in the following table.

Preparative HPLC Method A: Phenomenex Gemini C18 250 x 21.2 mm, 8 μm; mobile phase A: MeCN, mobile phase B: 0.225% formic acid in water. Gradient time 10 min; flow rate 30 mL/min.

Preparative HPLC Method B: YMC-Actus Triart C18 150 x 30 mm, 5 μm; mobile phase A: MeCN, mobile phase B: 0.225% formic acid in water. Gradient time 10 min; flow rate 30 mL/min.

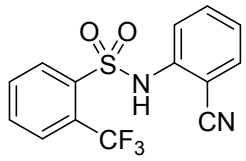
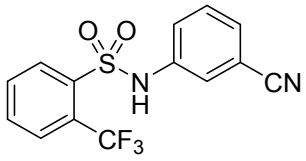
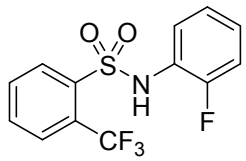
Preparative HPLC Method C: DIKMA Diamonsil (2) C18; mobile phase A: MeCN, mobile phase B: 0.225% formic acid in water. Gradient time 8 min; flow rate 35 mL/min.

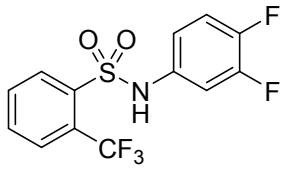
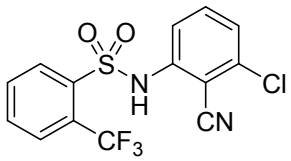
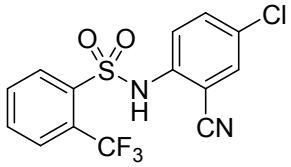
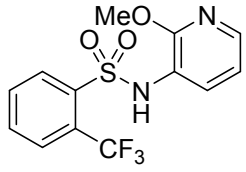
The retention times quoted in the table below were obtained using one of the following three LCMS methods:

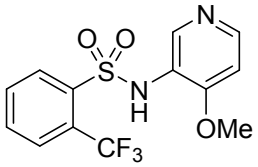
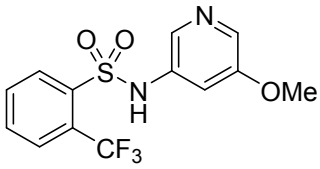
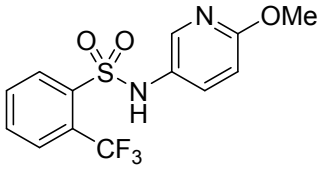
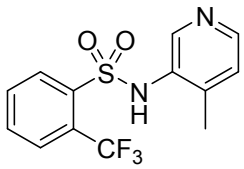
LCMS Method A: XBRIDGE 50 x 2.1 mm, 5 μm; mobile phase A: 0.0375% TFA in water; mobile phase B: 0.01875% TFA in MeCN; gradient from 1% B to 5% B at 0.60 min, further to 100% B at 4.00 min and finally returning to 1% B at 4.30-4.70 min; flow rate 0.8 mL/min.

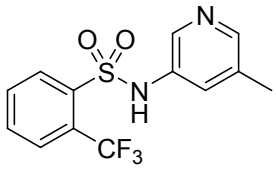
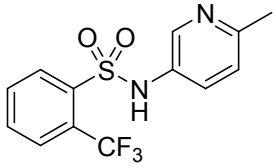
LCMS Method B: XBRIDGE 50 x 2.1 mm, 5 µm; mobile phase A: 0.0375% TFA in water; mobile phase B: 0.01875% TFA in MeCN; gradient from 10% B to 100% B at 4.00 min and finally returning to 1% B at 4.30-4.70 min; flow rate 0.8 mL/min.

The following compounds were prepared according to the method described for **Library Protocol 1** using 2-(trifluoromethyl)benzenesulfonyl chloride (**48**) and the appropriate amine. Where stated the title compounds were isolated as formate salts.

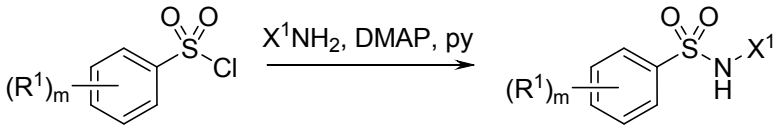
No.	Name	Data/SM
14	*N*-(2-Cyanophenyl)-2-(trifluoromethyl)benzenesulfonamide 	MS *m/z* 327 $[M+H]^+$ Rt = 3.045 minutes. LCMS Method A, Prep HPLC Method B with 42-72% organic gradient. 2-cyanoaniline.
15	*N*-(3-Cyanophenyl)-2-(trifluoromethyl)benzenesulfonamide 	MS *m/z* 327 $[M+H]^+$ Rt = 3.113 minutes. LCMS Method A, Prep HPLC Method B with 45-75% organic gradient. 3-cyanoaniline.
17	*N*-(2-Fluorophenyl)-2-(trifluoromethyl)benzenesulfonamide 	MS *m/z* 320 $[M+H]^+$ Rt = 3.205 minutes. LCMS Method A, Prep HPLC Method C with 38-68% organic gradient. 2-fluoroaniline.

No.	Name	Data/SM
18	*N*-(3,4-Difluorophenyl)-2-(trifluoromethyl)benzenesulfonamide 	MS *m/z* 338 $[M+H]^+$ Rt = 3.148 minutes. LCMS Method B, Prep HPLC Method B with 51-81% organic gradient. 3,4-difluoroaniline.
25	*N*-(3-Chloro-2-cyanophenyl)-2-(trifluoromethyl)benzene sulfonamide 	MS *m/z* 361 $[M+H]^+$ Rt = 3.041 minutes. LCMS Method B, Prep HPLC Method B with 48-78% organic gradient. 3-chloro-2-cyanoaniline.
26	*N*-(4-Chloro-2-cyanophenyl)-2-(trifluoromethyl)benzene sulfonamide 	MS *m/z* 361 $[M+H]^+$ Rt = 3.103 minutes. LCMS Method A, Prep HPLC Method C with 50-80% organic gradient. 4-chloro-2-cyanoaniline.
38	*N*-(2-Methoxypyridin-3-yl)-2-(trifluoromethyl) benzenesulfonamide formate salt 	MS *m/z* 333 $[M+H]^+$ Rt = 3.098 minutes. LCMS Method A, Prep HPLC Method A with 40-70% organic gradient. 2-methoxypyridin-3-amine.

No.	Name	Data/SM
39	*N*-(4-Methoxypyridin-3-yl)-2-(trifluoromethyl) benzenesulfonamide formate salt 	MS *m/z* 333 $[M+H]^+$ Rt = 2.266 minutes. LCMS Method A, Prep HPLC Method A with 17-47% organic gradient. 4-methoxypyridin-3-amine.
40	*N*-(5-Methoxypyridin-3-yl)-2-(trifluoromethyl) benzenesulfonamide formate salt 	MS *m/z* 333 $[M+H]^+$ Rt = 2.645 minutes. LCMS Method A, Prep HPLC Method C with 25-55% organic gradient. 5-methoxypyridin-3-amine.
41	*N*-(6-Methoxypyridin-3-yl)-2-(trifluoromethyl) benzenesulfonamide formate salt 	MS *m/z* 333 $[M+H]^+$ Rt = 3.013 minutes. LCMS Method A, Prep HPLC Method A with 37-67% organic gradient. 6-methoxypyridin-3-amine.
42	*N*-(4-Methylpyridin-3-yl)-2-(trifluoromethyl)benzene sulfonamide formate salt 	MS *m/z* 317 $[M+H]^+$ Rt = 2.334 minutes. LCMS Method A, Prep HPLC Method A with 19-49% organic gradient. 4-methylpyridin-3-amine.

No.	Name	Data/SM
43	*N*-(5-Methylpyridin-3-yl)-2-(trifluoromethyl)benzenesulfonamide formate salt 	MS *m/z* 317 $[M+H]^+$ Rt = 2.444 minutes. LCMS Method A, Prep HPLC Method A with 11-51% organic gradient. 5-methyl-3-aminopyridine.
44	*N*-(6-Methylpyridin-3-yl)-2-(trifluoromethyl)benzene sulfonamide formate salt 	MS *m/z* 317 $[M+H]^+$ Rt = 2.351 minutes. LCMS Method A, Prep HPLC Method C with 18-48% organic gradient. 6-methylpyridin-3-amine

Library Protocol 2



To a 0.25M solution of the appropriate amine (X^1NH_2) in anhydrous pyridine (300 µL, 75 µmol) was added a 0.275M solution of the appropriate sulfonyl chloride in anhydrous pyridine (300 µL, 82.5 µmol) followed by DMAP (1.8 mg, 15 µmol). The reaction mixture was shaken at 60 °C for 16 hours before concentrating *in vacuo* and purifying by one of the five preparative HPLC methods described below. The organic gradient used for each compound is described in the following table.

Preparative HPLC Method A: Phenomenex Gemini C18 250 x 21.2 mm, 8 µm; mobile phase A: MeCN, mobile phase B: 0.225% formic acid in water. Gradient time 8 min; flow rate 30 mL/min.

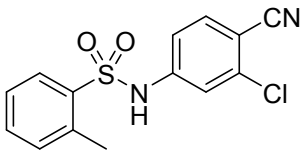
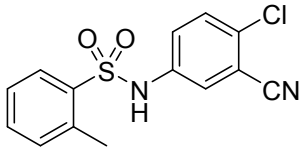
Preparative HPLC Method B: Waters Sunfire C8 150 x 30 mm, 5 μm; mobile phase A: MeCN, mobile phase B: 0.225% formic acid in water. Gradient time 8 min; flow rate 40 mL/min.

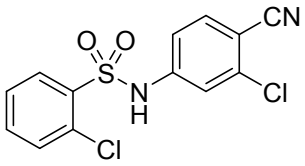
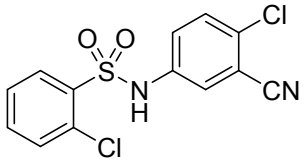
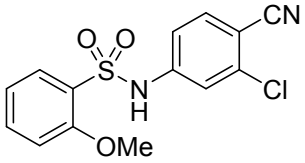
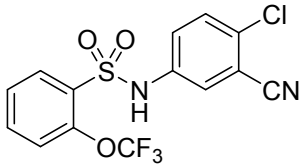
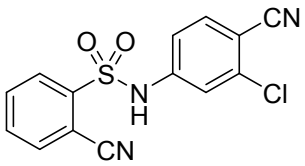
The retention times quoted in the table below were obtained using the following LCMS method:

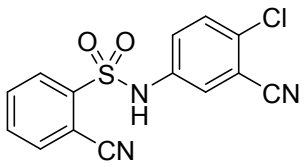
LCMS Method A: XBRIDGE 50 x 2.1 mm, 5 μm; mobile phase A: 0.05% NH_4OH in water; mobile phase B: 100% MeCN; gradient from 5% B to 100% B at 3.40 min, hold at 100% B to 4.20 min and finally returning to 5% B at 4.21-4.70 min; flow rate 0.8 mL/min.

The following compounds were prepared according to the method described for **Library Protocol 2** using one of the following two amines and the appropriate sulfonyl chloride as described in the table below.

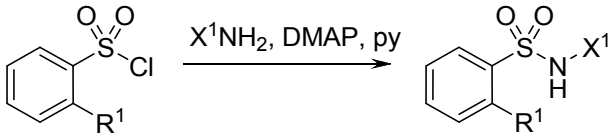
Amines: 5-amino-2-chlorobenzonitrile or 4-amino-2-chlorobenzonitrile.

No.	Name	Data/SM
30	*N*-(3-Chloro-4-cyanophenyl)-2-methylbenzenesulfonamide 	MS *m/z* 305 $[M-H]^-$; Rt = 2.052 minutes. LCMS Method A, Prep HPLC Method A with 40-70% organic gradient. 2-methylbenzenesulfonyl chloride.
31	*N*-(4-Chloro-3-cyanophenyl)-2-methylbenzenesulfonamide 	MS *m/z* 305 $[M-H]^-$; Rt = 2.102 minutes. LCMS Method A, Prep HPLC Method A with 41-71% organic gradient. 2-methylbenzenesulfonyl chloride.

No.	Name	Data/SM
32	2-Chloro-*N*-(3-chloro-4-cyanophenyl)benzenesulfonamide 	MS *m/z* 325 $[M-H]^-$; Rt = 2.015 minutes. LCMS Method A, Prep HPLC Method A with 39-69% organic gradient. 2-chlorobenzenesulfonyl chloride.
33	2-Chloro-*N*-(4-chloro-3-cyanophenyl)benzenesulfonamide 	MS *m/z* 325 $[M-H]^-$; Rt = 2.055 minutes. LCMS Method A, Prep HPLC Method A with 40-70% organic gradient. 2-chlorobenzenesulfonyl chloride.
34	*N*-(3-Chloro-4-cyanophenyl)-2-methoxybenzenesulfonamide 	MS *m/z* 321 $[M-H]^-$; Rt = 1.949 minutes. LCMS Method A, Prep HPLC Method B with 37-67% organic gradient. 2-methoxybenzenesulfonyl chloride.
35	*N*-(4-Chloro-3-cyanophenyl)-2-(trifluoromethoxy)benzene sulfonamide 	MS *m/z* 375 $[M-H]^-$; Rt = 2.191 minutes. LCMS Method A, Prep HPLC Method A with 45-75% organic gradient. 2-trifluoromethoxybenzenesulfonyl chloride.
36	*N*-(3-Chloro-4-cyanophenyl)-2-cyanobenzenesulfonamide 	MS *m/z* 316 $[M-H]^-$; Rt = 1.960 minutes. LCMS Method A, Prep HPLC Method A with 34-64% organic gradient. 2-cyanobenzenesulfonyl chloride.

No.	Name	Data/SM
37	*N*-(4-Chloro-3-cyanophenyl)-2-cyanobenzenesulfonamide 	MS *m/z* 316 $[M-H]^-$; Rt = 2.000 minutes. LCMS Method A, Prep HPLC Method A with 35-65% organic gradient. 2-cyanobenzenesulfonyl chloride.

Library Protocol 3



To a solution of the appropriate amine (X^1NH_2, 100 μmol) in anhydrous pyridine (600 μL) was added a 1.2M solution of the appropriate sulfonyl chloride in anhydrous pyridine (100 μL, 120 μmol) followed by DMAP (2 mg, 20 μmol). The reaction mixture was shaken at 30 °C for 2 hours followed by 60 °C for 16 hours before concentrating *in vacuo* and purifying by one of the three preparative HPLC methods described below. The organic gradient used for each compound is described in the following table.

Preparative HPLC Method A: Waters Sunfire C8 150 x 30 mm, 5 μm; mobile phase A: MeCN, mobile phase B: 0.225% formic acid in water. Gradient time 8 min; flow rate 40 mL/min.

Preparative HPLC Method B: YMC-Actus Triart C18 150 x 30 mm, 5 μm; mobile phase A: MeCN, mobile phase B: 0.225% formic acid in water. Gradient time 9 min; flow rate 30 mL/min.

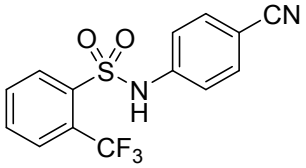
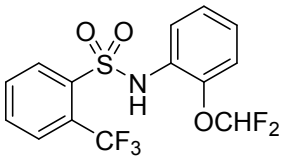
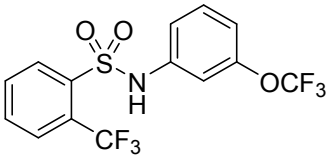
Preparative HPLC Method C: Agela DuraShell C18 150 x 21.2 mm, 5 μm; mobile phase A: MeCN, mobile phase B: 0.225% formic acid in water. Gradient time 10 min; flow rate 30 mL/min.

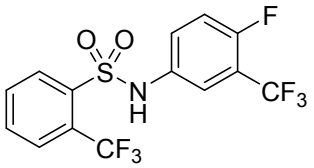
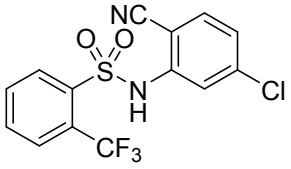
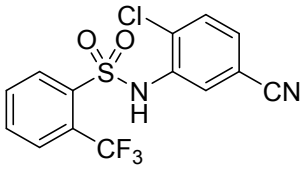
The retention times quoted in the table below were obtained using one of the following two LCMS methods:

LCMS Method A: XBRIDGE 50 x 2.1 mm, 5 µm; mobile phase A: 0.0375% TFA in water; mobile phase B: 0.01875% TFA in MeCN; gradient from 10% B to 100% B at 4.00 min and finally returning to 1% B at 4.30-4.70 min; flow rate 0.8 mL/min.

LCMS Method B: XBRIDGE 50 x 2.1 mm, 5 µm; mobile phase A: 0.05%NH_4OH in water; mobile phase B: 100% MeCN; gradient from 5% B to 100% B at 3.40 min, hold at 100% B to 4.20 min and finally returning to 5% B at 4.21-4.70 min; flow rate 0.8 mL/min.

The following compounds were prepared according to the method described for **Library Protocol 3** using 2-(trifluoromethyl)benzenesulfonyl chloride and the appropriate amine as described below. Where stated the title compounds were isolated as formate salts.

No.	Name	Data/SM
16	*N*-(4-Cyanophenyl)-2-(trifluoromethyl)benzenesulfonamide 	MS *m/z* 325 $[M-H]^-$; Rt = 2.027 minutes. LCMS Method B, Prep HPLC Method A with 43-73% organic gradient. 4-aminobenzonitrile.
19	*N*-[2-(Difluoromethoxy)phenyl]-2-(trifluoromethyl)benzenesulfonamide 	MS *m/z* 366 $[M-H]^-$; Rt = 2.301 minutes. LCMS Method B, Prep HPLC Method A with 53-83% organic gradient. 2-(difluoromethoxy)aniline.
20	*N*-[3-(Trifluoromethoxy)phenyl]-2-(trifluoromethyl)benzenesulfonamide 	MS *m/z* 386 $[M+H]^+$; Rt = 3.358 minutes. LCMS Method A, Prep HPLC Method C with 54-84% organic gradient. 3-trifluoromethoxyaniline.

No.	Name	Data/SM
21	*N*-[4-Fluoro-3-(trifluoromethyl)phenyl]-2-(trifluoromethyl)benzenesulfonamide 	MS *m/z* 386 $[M-H]^-$; Rt = 2.350 minutes. LCMS Method B, Prep HPLC Method B with 59-89% organic gradient. 3-trifluoromethyl-4-fluoroaniline.
27	*N*-(5-Chloro-2-cyanophenyl)-2-(trifluoromethyl)benzenesulfonamide 	MS *m/z* 359 $[M-H]^-$; Rt = 2.290 minutes. LCMS Method B, Prep HPLC Method C with 46-76% organic gradient. 2-amino-4-chlorobenzonitrile.
29	*N*-(2-Chloro-5-cyanophenyl)-2-(trifluoromethyl)benzenesulfonamide 	MS *m/z* 359 $[M-H]^-$; Rt = 2.159 minutes. LCMS Method B, Prep HPLC Method C with 46-76% organic gradient. 3-amino-4-chlorobenzonitrile.

Compound 28

Compound **28** *N*-(2-chloro-4-cyanophenyl)-2-(trifluoromethyl) benzenesulfonamide was prepared according to the method indicated for compound **22** using the appropriate sulfonyl chloride and amine and purified using the following Preparative HPLC conditions:

Preparative Method:

Column: Gemini C18 110A, 100 x 21.2 mm, 5 micron

Mobile Phase A: 0.1% formic acid in water, Mobile phase B: 0.1% formic acid in MeCN

Gradient: from 5% B to 95% B at 7 minutes and return to 5% B at 9.1 minutes.

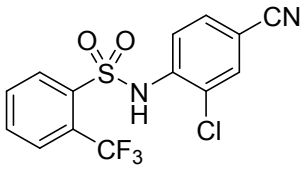
Flow rate: 18 mL/min, run time = 10 minutes.

LCMS method:

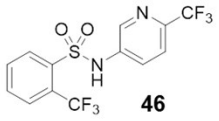
Column: RESTEK C18 30 x 2.1 mm, 3 micron

Mobile phase A: 0.05% formic acid in water; Mobile phase B: MeCN

Gradient: from 2-10% B at 1 minute, to 98% B at 2 min and returning to 2% B at 2.9-3.0 min. Flow rate: 1.5 mL/min.

No.	Name	SM	Data/SM
28	*N*-(2-chloro-4-cyanophenyl)-2-(trifluoromethyl) benzenesulfonamide 	2-(trifluoromethyl) benzenesulfonyl chloride and 3-chloro-4-aminobenzonitrile;	MS *m/z* 359 $[M-H]^-$; ^1H NMR (400MHz, $CDCl_3$): δ ppm 7.50 (dd, 1H), 7.56 (br s, 1H), 7.60 (d, 1H), 7.68-7.77 (m, 3H) 7.92 (d, 1H), 8.16 (d, 1H).

4. Selected HPLC, MS and NMR spectra



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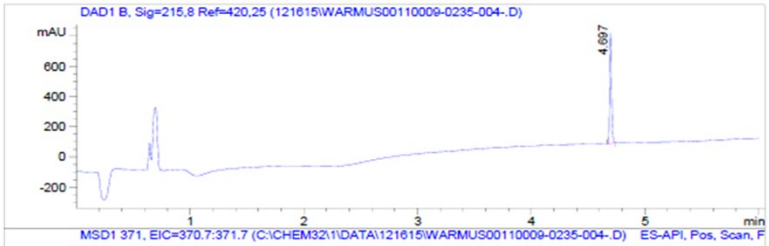
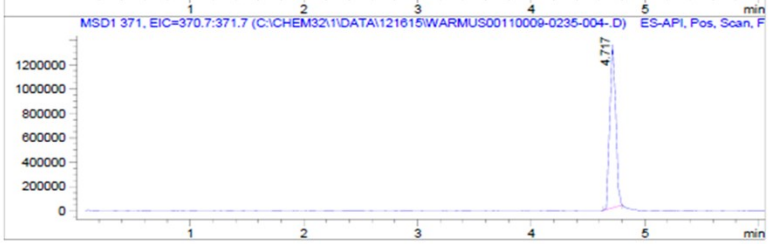




Column:

Kinetic C18 100mm x 3.0mm 2.6u

Gradient Conditions:

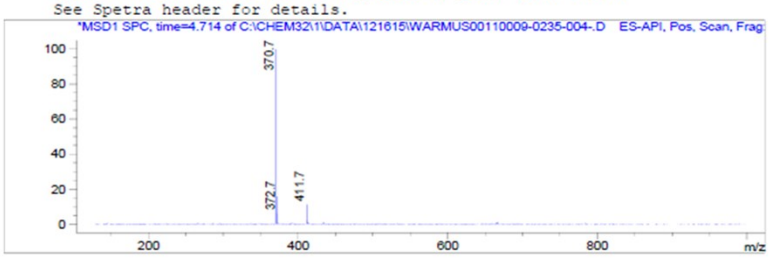
Mobile Phase A: 0.1% Formic Acid in Water
Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Time (min)	%A	%B
0.00	95	5
0.5	95	5
4	0	100
5.4	0	100
5.5	95	5

Detection: 215nm
APCI (+) 175-2000Daltons

Flow: 0.750mL/min

Spectra below are for the intergrated MS peaks from above.
See Spetra header for details.



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#	Meas. R	Area	Area %	Signal Desc.
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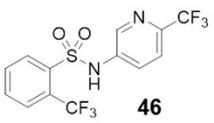

46


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PULPROG           zg30
TD               32768
SOLVENT           DMSO
NS                  16
DS                   2
SWH           6393.862 Hz
FIDRES        0.195125 Hz
AQ            2.5624576 sec
RG               190.31
DW               78.200 usec
DE                6.50 usec
TE                298.1 K
D1           0.75000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1        399.8023988 MHz
NUC1                1H
P1                14.00 usec
PLW1        19.95299911 W

F2 - Processing parameters
SI                65536
SF          399.8000000 MHz
WDW                  EM
SSB       0
LB                0.39 Hz
GB        0
PC                1.00
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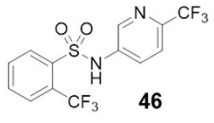

46


```
INSTRUM           spect
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PULPROG         zgpg30
TD               65536
SOLVENT           DMSO
NS                2048
DS                   4
SWH          24038.461 Hz
FIDRES        0.366798 Hz
AQ            1.3631488 sec
RG               190.31
DW               20.800 usec
DE                6.50 usec
TE                298.1 K
D1           0.89999998 sec
D11          0.03000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1        100.5398425 MHz
NUC1                13C
P1                10.00 usec
PLW1        54.95399857 W

======== CHANNEL f2 ========
SFO2        399.8015992 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2        19.95299911 W
PLW12        0.48280001 W
PLW13        0.39107001 W

F2 - Processing parameters
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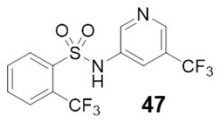
SF          100.5297900 MHz
WDW                  EM
SSB       0
LB                0.39 Hz
GB        0
PC                1.40
```



47

Column:

Kinetic C18 100mm x 3.0mm 2.6u

Gradient Conditions:

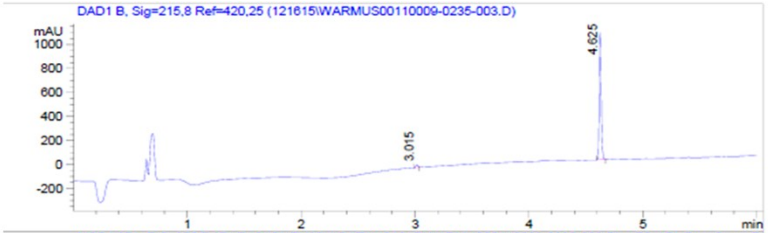
Mobile Phase A: 0.1% Formic Acid in Water

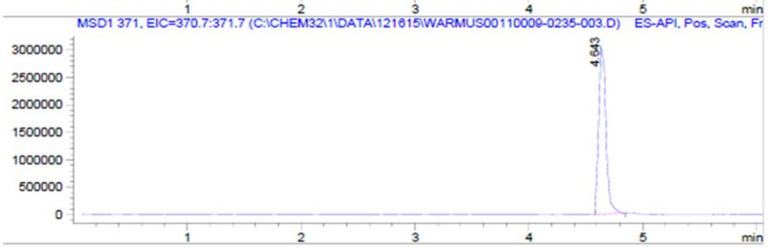
Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Time (min)	%A	%B
0.00	95	5
0.5	95	5
4	0	100
5.4	0	100
5.5	95	5

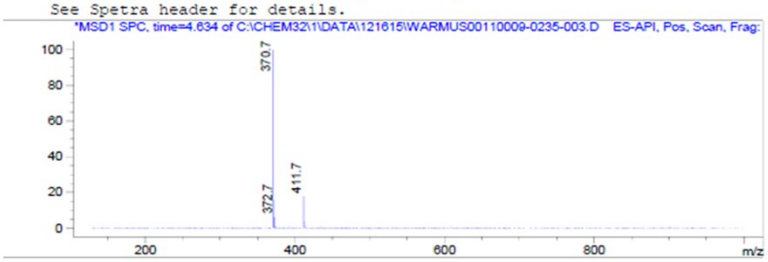
Detection: 215nm
APCI (+) 175-2000Daltons

Flow: 0.750mL/min





Spectra below are for the intergrated MS peaks from above.
See Spetra header for details.



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#	Meas. R	Area	Area %	Signal Desc.
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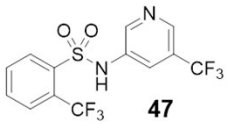

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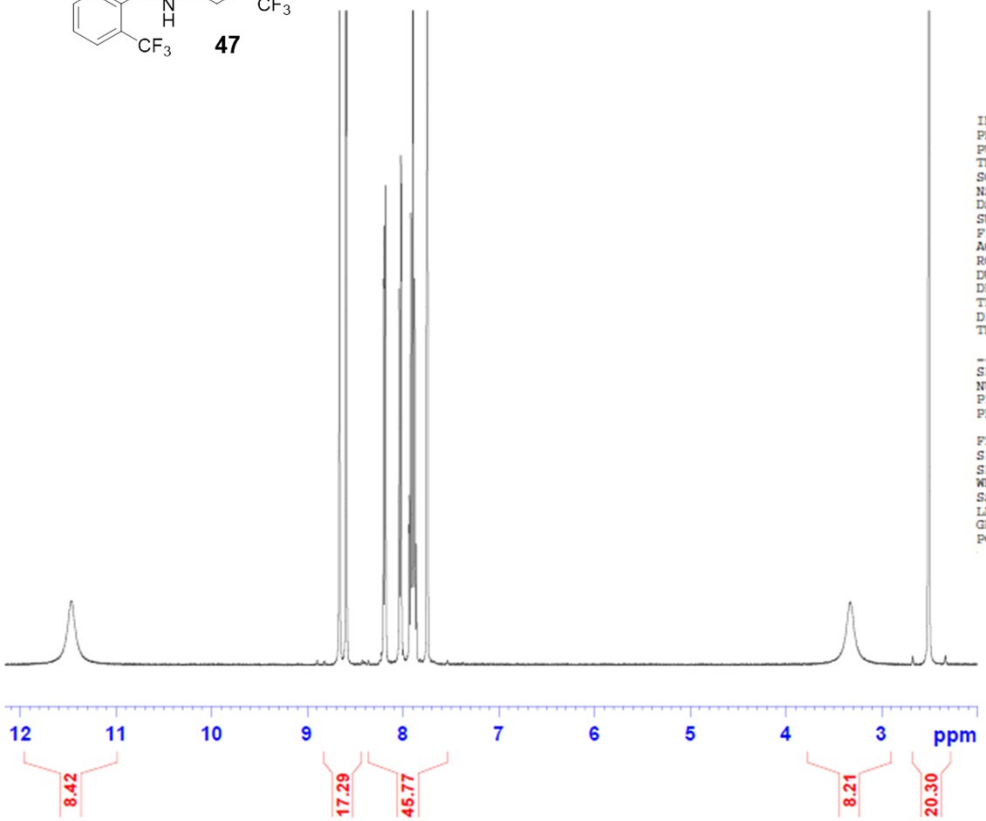


```
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                    16
DS                     2
SWH             6393.862 Hz
FIDRES          0.195125 Hz
AQ              2.5624576 sec
RG                190.31
DW                78.200 usec
DE                 6.50 usec
TE                298.1 K
D1             0.75000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         399.8023988 MHz
NUC1                   1H
P1                  14.00 usec
PLW1         19.95299911 W

F2 - Processing parameters
SI                 65536
SF           399.8000000 MHz
WDW                   EM
SSB       0
LB                 0.39 Hz
GB        0
PC                 1.00
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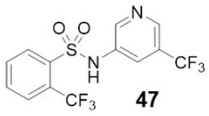

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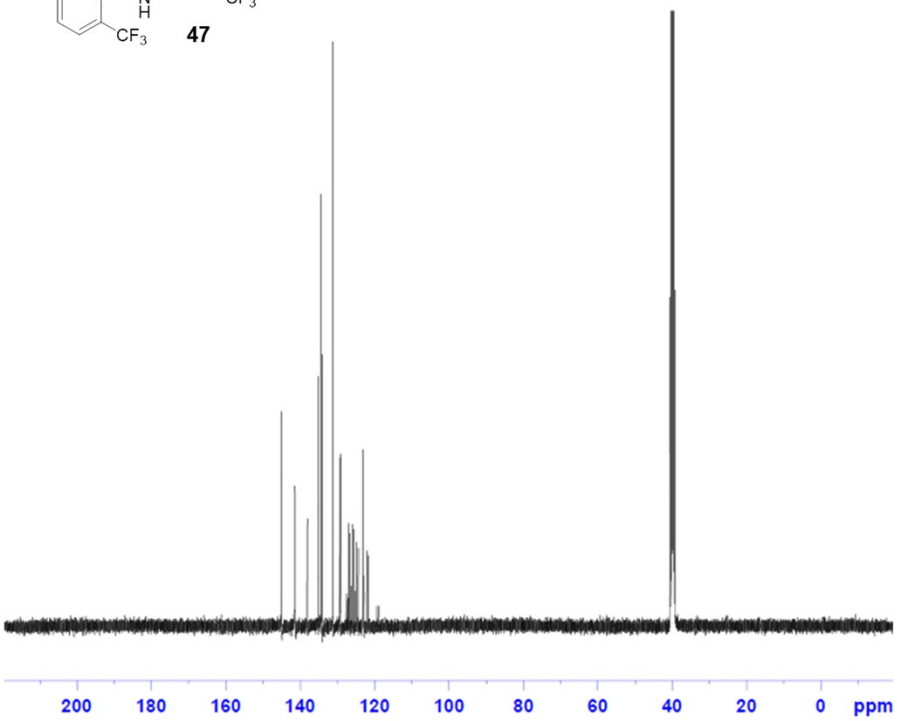



```
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                  2048
DS                     4
SWH            24038.461 Hz
FIDRES          0.366798 Hz
AQ              1.3631488 sec
RG                190.31
DW                20.800 usec
DE                 6.50 usec
TE                298.1 K
D1             0.89999998 sec
D11            0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         100.5398425 MHz
NUC1                  13C
P1                  10.00 usec
PLW1         54.95399857 W

======== CHANNEL f2 ========
SFO2         399.8015992 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2               90.00 usec
PLW2         19.95299911 W
PLW12         0.48280001 W
PLW13         0.39107001 W

F2 - Processing parameters
SI                 65536
SF           100.5297900 MHz
WDW                   EM
SSB       0
LB                 0.39 Hz
GB        0
PC                 1.40
```

5. Polypharmacology profiles

Compound **46**:

Cerep *Full Safety Panel (10uM,*

Functional Agonism	% Resp	EC50 (nM)
Adenosine A1	12	
Adenosine A2a	3	
Adrenergic Alpha 1a	15	
Adrenergic Alpha 2a	-5	
Adrenergic Alpha 2b	-1	
Adrenergic Beta 1	-1	
Adrenergic Beta 2	-0	
Angiotensin 1	8	
Cannabinoid 1	33	
Cholecystokinin :	0	
Dopamine 1	2	
Dopamine 2s	-15	
Endothelin A	16	
Histamine 1	3	
Histamine 2	-0	
Histamine 3	16	
Muscarinic 1	9	
Muscarinic 2	-14	
Muscarinic 3	7	
Neurokinin 1	2	
Opioid Delta	-8	
Opioid Kappa	10	
Opioid Mu	13	
Serotonin 1a	-1	
Serotonin 1b	-3	
Serotonin 2a	-3	
Serotonin 2b	-2	
Serotonin 4e	-1	
Vasopressin 1a	25	
Corticotrophin Releasing Factor 1 (CR		
Melanocortin 2 (MC2R)		
Thyrotropin Releasing Hormone 1 (TRH		

Functional Antagonism	% Inh	IC50 (nM)	Kb (nM)
Adrenergic Alpha 1a	49		
Adrenergic Alpha 2b	27		
Adrenergic Beta 1	-24		
Adrenergic Beta 2	-21		
Angiotensin 1	37		
Cannabinoid 1	-4		
Dopamine 1	32		
Dopamine 2s	-3		
Histamine 1	25		
Muscarinic 1	52	1760	207
Muscarinic 2	8		
Muscarinic 3	19		
Opioid Mu			
CRF1			
MC2R			
TRH1			

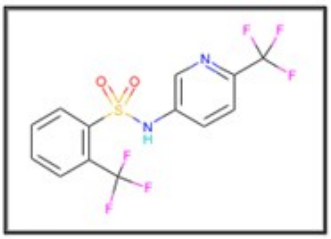
Ion Channel - Bindin	% Inh	IC50 (nM)	Ki (nM)
L-Type Calcium (Verapam	2		
L-Type Calcium (Nifedipin	-15		
L-Type Calcium (Diltiazer	-20		
GABAa (Cl Channel	13		
GABAa1 Receptor	-21		
GABAa (Benzodiazepine Site	0		
AMPA Receptor	4		
NMDA Recepto	-4		
NMDA Receptor (PCP Sit	0		
Nicotinic ACh Receptor (Musc	-7		
Nicotinic ACh Receptor (Neuron	-11		
Serotonin 3	-2		
Sodium (Site 2	11		

Transporters - Binding	% Inh	IC50 (nM)	Ki (nM)
Norepinephrine Transporte	-0		
Dopamine Transporte	20		
Serotonin Transporter	2		
Choline Transporter	-10		
GABA Transporter	-3		

Enzyme	% Inh	IC50 (nM)
Angiotensin Converting Enzyn	-5	
Acetylcholine Esteras	11	
Cyclooxygenase :	15	
Monoamine Oxidase	-3	
PDE3B	3	
PDE4D2	7	

Kinase	% Inh	IC50 (nM)
Abl Kinase	8	
Aurora A Kinase		
EGFR Kinase	9	
Lck Kinase	1	
p38 MAP Kinase	2	
Src Kinase	5	
VEGFR2 (KDR) Kinas	20	

NHR - Binding	% Inh	IC50 (nM)	Ki (nM)
Androgen Receptor (Binding	-2		
Glucocorticoid Receptor (Bindin	-2.5		
PPAR gamma (Bindin	-4.7		



Additional Data

Chemical Properties

Parent Molecular Weigt	370.27
cLOGP	3.25
TPSA	67.44

Promiscuitly Panel Data - Binding (10uM

	% Inh
Adenergic Alpha 1a	-11
Adrenergic Beta 2	-0
Cannabinoid 1	
Dopamine 1	-2
Histamine 1	-8
Muscarinic 1	-11
Opioid Mu	3

Other

L-Type Calcium (Functional) - IC50 (	
Nav 1.5 (Q Patch) - IC50 (n	
hERG (Functional) - IC50 (n	>100000.(
Dofetilide Binding - IC50 (n	>100000
THLE - IC50 (nM	153540

The M1 profile in CEREP suggested a partial effect. However, further follow up of the result using 12 h to shift the dose response curve of a reference agonist (Carbachol) showed no effect and the IC_{50} result could not be reproduced. Therefore the CEREP result is assumed to be a false positive.

Compound **47**:

Cerep *Full Safety Panel (10uM,*

Functional Agonism	% Resp	EC50 (nM)
Adenosine A1	-12	
Adenosine A2a	5	
Adrenergic Alpha 1a	9	
Adrenergic Alpha 2a	0	
Adrenergic Alpha 2b	-0	
Adrenergic Beta 1	1	
Adrenergic Beta 2	-0	
Angiotensin 1	5	
Cannabinoid 1	48	
Cholecy stokinin :	-1	
Dopamine 1	-1	
Dopamine 2s	-45	
Endothelin A	11	
Histamine 1	3	
Histamine 2	1	
Histamine 3	32	
Muscarinic 1	8	
Muscarinic 2	-15	
Muscarinic 3	7	
Neurokinin 1	3	
Opioid Delta	-15	
Opioid Kappa	32	
Opioid Mu	18	
Serotonin 1a	2	
Serotonin 1b	-4	
Serotonin 2a	-2	
Serotonin 2b	-1	
Serotonin 4e	-1	
Vasopressin 1a	21	
Corticotrophin Releasing Factor 1 (CR		
Melanocortin 2 (MC2R)		
Thy rotopin Releasing Hormone 1 (TRH		

Functional Antagonism	% Inh	IC50 (nM)	Kb (nM)
Adrenergic Alpha 1a	25		
Adrenergic Alpha 2b	13		
Adrenergic Beta 1	-3		
Adrenergic Beta 2	27		
Angiotensin 1	24		
Cannabinoid 1	-10		
Dopamine 1	0		
Dopamine 2s	-15		
Histamine 1	7		
Muscarinic 1	31		
Muscarinic 2	4		
Muscarinic 3	13		
Opioid Mu			
CRF1			
MC2R			
TRH1			

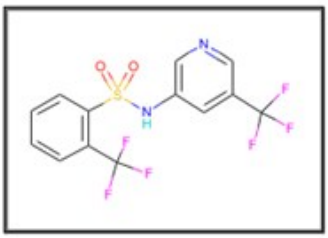
Ion Channel - Bindin	% Inh	IC50 (nM)	KI (nM)
L-Ty pe Calcium (Verapam	8		
L-Ty pe Calcium (Nifedipin	15		
L-Ty pe Calcium (Diltiazer	-55		
GABAa (Cl Channel	12		
GABAa1 Receptor	-8		
GABAa (Benzodiazepine Site	-14		
AMPA Receptor	-19		
NMDA Recepto	0		
NMDA Receptor (PCP Sit	1		
Nicotinic ACh Receptor (Musc	-7		
Nicotinic ACh Receptor (Neuron	1		
Serotonin 3	-4		
Sodium (Site 2	2		

Transporters - Binding	% Inh	IC50 (nM)	KI (nM)
Norepinephrine Transporte	-3		
Dopamine Transporte	3		
Serotonin Transporter	0		
Choline Transporte	1		
GABA Transporter	-3		

Enzyme	% Inh	IC50 (nM)
Angiotensin Converting Enzy n	-21	
Acety lcholine Esteras	3	
Cy clooxy genase :	7	
Monoamine Oxidase	-0	
PDE3B	4	
PDE4D2	-0	

Kinase	% Inh	IC50 (nM)
Abl Kinase	21	
Aurora A Kinase		
EGFR Kinase	4	
Lck Kinase	-2	
p38 MAP Kinase	20	
Src Kinase	-6	
VEGFR2 (KDR) Kinas	16	

NHR - Binding	% Inh	IC50 (nM)	KI (nM)
Androgen Receptor (Binding	-4		
Glucocorticoid Receptor (Bindin	0.6		
PPAR gamma (Bindin	20.7		



Additional Data

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Parent Molecular Weigl	370.27
cLOGP	3.25
TPSA	67.44

Promiscuitly Panel Data - Binding (10uM

	% Inh
Adenergic Alpha 1a	-4
Adrenergic Beta 2	-5
Cannabinoid 1	
Dopamine 1	-3
Histamine 1	15
Muscarinic 1	-10
Opioid Mu	-7

Other

L-Ty pe Calcium (Functional) - IC50 (	
Nav 1.5 (Q Patch) - IC50 (n	>100000.(
hERG (Functional) - IC50 (n	>100000.(
Dofetilide Binding - IC50 (n	>100000
THLE - IC50 (nM	228019

6. General biology experimental procedures

Biological Assay

a. Generation of a custom clonal cell line for URAT1 transporter activity assay

The nucleotide sequence for the long isoform of URAT1 (NM_144585) was C-terminally fused to that of enhanced green fluorescent protein (eGFP) (hereinafter referred to as URAT1(L)GFP). The combined sequence was codon-optimised and custom synthesized. The synthesized sequence was generated in pDONR221 Gateway entry vector (Invitrogen Life Technologies) prior to cloning in pLenti6.3/V5 Gateway destination vector (Invitrogen Life Technologies). A schematic of the URAT1(L)GFP construct is set forth in Figure 1A. The nucleotide and amino acid sequence of the URAT1(L)GFP construct is set out in Figure 1B, which also shows alignment of the nucleotide sequence with NM_144585.

Lentiviral particles were generated according to ViraPower HiPerform expression system procedure (Invitrogen Life Technologies) and used to transduce CHO cells. Blasticidin selection enabled the generation of a stable clonal pool of cells, confirmed by expression of GFP and V5 epitope. The clonal pools were sorted using fluorescence-activated cell sorting (FACS) on the basis of GFP expression with the gating set at the top 50% of expression into single cells which were subsequently expanded to generate clonal lines. One clone was identified with the best assay performance as determined by maximal separation between complete inhibition of uric acid transport (with 10 μM benzbromarone) and no inhibition (DMSO). This cell line was used for all screening activities and is referred to as CHO-URAT1(L)GFP#8 or CHO#8.

b. URAT-1 Inhibitor activity

The potency of the compounds of formula (I) as inhibitors of the URAT-1 transporter was determined as follows.

CHO#8 cells were cultured in cell line maintenance flasks in medium consisting of Dulbecco's modified Eagle medium (DMEM) with high glucose and sodium pyruvate (4.5 g of glucose per litre, Invitrogen Life Technologies), supplemented with heat-inactivated foetal bovine serum (FBC, 10 % v/v), 1x NEAA (non-essential amino acids) and blasticidin (10 μg/ml). Cultures were grown in 175 cm^2 tissue culture flasks in a humidified incubator at approximately 37 °C in approximately 95% air/5% CO_2. Near confluent CHO#8 cell cultures were harvested by trypsinisation, re-suspended in culture medium and the process was repeated once or twice weekly to provide sufficient cells for use.

Assay ready flasks were generated by the same method, except the cells were not cultured in blasticidin.

Assay ready frozen cells were generated by freezing 40,000,000 cells in 1 mL of FBS (without blasticidin) containing 10% DMSO per vial. One vial was sufficient for 5 assay plates. Each vial was thawed rapidly to 37 °C, washed and re-suspended in pre-warmed culture medium for seeding onto assay plates.

CHO#8 cells were seeded onto Cytostar^{TM} 96-well plates at a density of 5 x 10^5 cells per well. The cells were cultured for 1 day at approximately 37 ºC in a humidified incubator containing approximately 5% CO_2 in air. After approximately 24 hours culture, cells were used for uptake experiments.

On the day of assay, culture medium was removed from the wells and the cells were washed once with 50 μL of chloride-containing buffer (136.7 mM NaCl, 5.36 mM KCl, 0.952 mM $CaCl_2$, 0.441 mM KH_2PO_4, 0.812 mM $MgSO_4$, 5.6 mM D-glucose, 0.383 mM $Na_2HPO_4.2H_2O$, 10 mM HEPES, pH 7.4 with NaOH). The cells were pre-incubated with another 50 μL of chloride-containing buffer for one hour at approximately 37 ºC in a humidified incubator containing approximately 5% CO_2 in air.

Assay compound plates were prepared by diluting the compounds of formula (I) with chloride-free buffer (125 mM Na-gluconate, 4.8 mM K-gluconate, 1.3 mM Ca-gluconate, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5.6 mM D-glucose, 25 mM HEPES, pH 7.4 with NaOH) in 100% DMSO to a final concentration of 1% DMSO. [^{14}C]-Uric acid working stock was made by addition of radiolabeled compound to a final concentration of 120 nM in chloride-free buffer. In all wells, the final assay concentration of solvent (DMSO) was 0.25%; the final assay concentration of [^{14}C]-uric acid was 30nM in chloride-free buffer and the final compound of formula (I) concentrations ranged from 0 to 10 μM. The vehicle comparator was DMSO (i.e. no inhibition of uric acid transport) and the pharmacological blockade (i.e. 100% inhibition of uric acid transport) was defined by benzbromarone at 10 μM final assay concentration.

After pre-incubation, cells were washed with 50 μL of chloride-free buffer and another 50 μL of chloride-free buffer was added. Thereafter, 25 μL of compound of formula (I) was added from the prepared compound plate and the cells were pre-incubated for 15 minutes prior to the addition of 25 mL of [^{14}C] uric acid. The plate was incubated at room temperature and protected from light for three hours prior to measuring proximity-induced scintillation on a Wallac microbeta at 1 minute/well.

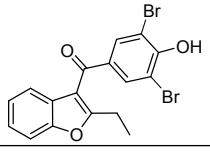
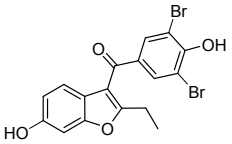
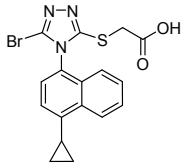
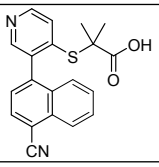
The accumulation of [^{14}C]-uric acid into CHO#8 cells was calculated and the IC_{50} (μM) values, defined as the concentration of inhibitor required for 50% inhibition of transport, were determined from a 4 parameter logistic fit to generate sigmoid curves from dose response data.

c. URAT-1 Assay Standards

A number of literature reported compounds were run initially in our uric acid radiolabel uptake assay to help validate the assay. Initial tests revealed good correlation with reported data for these compounds across a range of IC_{50} values (see Table 1 below for values obtained and Figure 2 for

benzbromarone data). A benzbromarone dose response curve was included on every assay plate for every assay run to assess assay reproducibility over time (both between and within assay run variability). For benzbromarone, 5-6 independent IC_{50} determinations provided a geometric mean with less than 2-fold error, based on 95% confidence intervals.

Table 1. URAT1 assay standards

Compound	Structure	Pfizer IC_{50}	Reported IC_{50}
Benzbromarone		22 nM n= 965 21-23 nM (95% CI)	26 nM (+/-3 nM)[1]
6-OH Benzbromarone		34 nM (n= 6) 18-64 nM (95% CI)	138 nM (+/- 88 nM)[1]
Lesinurad (RDEA-594)		6704 nM (n= 5) 1976-22747 nM (95% CI)	3360 nM[2]
Verinurad (RDEA-3170)		23 nM (n= 46) 20-26 nM (95% CI)	24 nM[3]

[1]M. F. Wempe et al., J. Med. Chem., 2011, 54, 2701-2713.
[2]J. N. Tan et al., Ardea, Abstracts Arthritis & Rheumatism, 2013, Vol 65. http://www.blackwellpublishing.com/acrmeeting/abstract.asp?MeetingID=799&id=109090
[3]J. Miner et al., Annals Rheumatic Disease, 2014, 71(Suppl 3):446.

Figure 1A

Schematic showing organization of the URAT1(L)GFP construct (N to C terminal direction).

Figure 1B

Sequence alignment of the codon optimized URAT1(L)GFP construct with the wild type human URAT1 sequence deposited as NM_144585.

Alignment row 1 is the sequence from accession NM_144585.

Alignment row 2 is the sequence of the construct in the Gateway destination vector pLenti6.3V5/DEST (encoding URAT1(L)GFP) with the nucleotide alignment indicated with NM_144585 above and the nucleotide numbering below.

Alignment row 3 is the amino acid translation with sequence annotation indicated in italics below.

GGGCCCCTCTTCTGGGCCCCTTGAGTAGGTTCC**ATG**GCATTTTCTGAACTCCTGGAC
CTC

||||| || | || ||||||||

ACAAGTTTGTACAAAAAAGCAGGCTTCGCCACC**ATG**GCCTTCAGCGAGCTGCTGGA
CCTG

---------+---------+---------+---------+---------+---------+60

M__A__F__S__E__L__L__D__L__

attB1-5' ***URAT1 Initiation codon***

GTGGGTGGCCTGGGCAGGTTCCAGGTTCTCCAGACGATGGCTCTGATGGTCTCCATC
ATG

||||| |||||||||| |||||||| || ||||| ||||| |||||||| |||||||||

GTGGGAGGCCTGGGCAGATTCCAGGTGCTGCAGACCATGGCCCTGATGGTGTCCATC
ATG

---------+---------+---------+---------+---------+---------+120

V__G__G__L__G__R__F__Q__V__L__Q__T__M__A__L__M__V__S__I__M__

TGGCTGTGTACCCAGAGCATGCTGGAGAACTTCTCGGCCGCCGTGCCCAGCCACCGC
TGC

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TGGCTGTGCACCCAGAGCATGCTGGAAAACTTCTCTGCCGCCGTGCCCAGCCACAGA
TGC

---------+---------+---------+---------+---------+---------+180

W__L__C__T__Q__S__M__L__E__N__F__S__A__A__V__P__S__H__R__C__

TGGGCACCCCTCCTGGACAACAGCACGGCTCAGGCCAGCATCCTAGGGAGCTTGAG
TCCT

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TGGGCCCCTCTGCTGGACAACAGCACCGCCCAGGCCAGCATCCTGGGCAGCCTGTCT
CCA

---------+---------+---------+---------+---------+---------+240

W__A__P__L__L__D__N__S__T__A__Q__A__S__I__L__G__S__L__S__P__

GAGGCCCTCCTGGCTATTTCCATCCCGCCGGGCCCCAACCAGAGGCCCCACCAGTGC
CGC

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GAGGCCCTGCTGGCCATCAGCATCCCCCCTGGCCCCAACCAGAGGCCCCACCAGTG
CAGA

---------+---------+---------+---------+---------+---------+300

E__A__L__L__A__I__S__I__P__P__G__P__N__Q__R__P__H__Q__C__R__

CGCTTCCGCCAGCCACAGTGGCAGCTCTTGGACCCCAATGCCACGGCCACCAGCTGG
AGC

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CGGTTCCGGCAGCCTCAGTGGCAGCTGCTGGATCCCAACGCCACCGCCACCTCTTGG
AGC

---------+---------+---------+---------+---------+---------+360

R__F__R__Q__P__Q__W__Q__L__L__D__P__N__A__T__A__T__S__W__S__

GAGGCCGACACGGAGCCGTGTGTGGATGGCTGGGTCTATGACCGCAGCATCTTCAC
CTCC

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GAGGCCGACACCGAGCCCTGTGTGGACGGCTGGGTGTACGACCGGTCCATCTTCAC
CAGC

---------+---------+---------+---------+---------+---------+420

E__A__D__T__E__P__C__V__D__G__W__V__Y__D__R__S__I__F__T__S__

ACAATCGTGGCCAAGTGGAACCTCGTGTGTGACTCTCATGCTCTGAAGCCCATGGCC
CAG

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ACCATCGTGGCCAAGTGGAACCTGGTGTGCGACAGTCACGCCCTGAAGCCCATGGC
CCAG

---------+---------+---------+---------+---------+---------+480

T__I__V__A__K__W__N__L__V__C__D__S__H__A__L__K__P__M__A__Q__

TCCATCTACCTGGCTGGGATTCTGGTGGGAGCTGCTGCGTGCGGCCCTGCCTCAGAC
AGG

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AGCATCTACCTGGCCGGCATTCTGGTGGGAGCCGCCGCTTGTGGCCCTGCCAGCGAT
AGA

---------+---------+---------+---------+---------+---------+540

S__I__Y__L__A__G__I__L__V__G__A__A__A__C__G__P__A__S__D__R__

TTTGGGCGCAGGCTGGTGCTAACCTGGAGCTACCTTCAGATGGCTGTGATGGGTACG
GCA

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TTCGGCAGACGGCTGGTGCTGACCTGGTCCTACCTGCAGATGGCCGTGATGGGCACC
GCC

---------+---------+---------+---------+---------+---------+600

F__G__R__R__L__V__L__T__W__S__Y__L__Q__M__A__V__M__G__T__A__

GCTGCCTTCGCCCCTGCCTTCCCCGTGTACTGCCTGTTCCGCTTCCTGTTGGCCTTTG
CC

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GCAGCCTTTGCCCCTGCCTTCCCTGTGTACTGCCTGTTCCGGTTCCTGCTGGCCTTCG
CC

---------+---------+---------+---------+---------+---------+660

A__A__F__A__P__A__F__P__V__Y__C__L__F__R__F__L__L__A__F__A__

GTGGCAGGCGTCATGATGAACACGGGCACTCTCCTGATGGAGTGGACGGCGGCACG
GGCC

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GTGGCCGGCGTGATGATGAACACCGGCACCCTGCTGATGGAATGGACCGCCGCCAG
AGCC

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V__A__G__V__M__M__N__T__G__T__L__L__M__E__W__T__A__A__R__A__

CGACCCTTGGTGATGACCTTGAACTCTCTGGGCTTCAGCTTCGGCCATGGCCTGACA
GCT

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AGACCCCTGGTGATGACCCTGAACAGCCTGGGCTTCAGCTTCGGACATGGCCTCACA
GCC

---------+---------+---------+---------+---------+---------+780

R__P__L__V__M__T__L__N__S__L__G__F__S__F__G__H__G__L__T__A__

GCAGTGGCCTACGGTGTGCGGGACTGGACACTGCTGCAGCTGGTGGTCTCGGTCCCC
TTC

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GCTGTGGCTTATGGCGTGCGGGACTGGACACTGCTGCAGCTGGTGGTGTCCGTGCCC
TTC

---------+---------+---------+---------+---------+---------+840

A__V__A__Y__G__V__R__D__W__T__L__L__Q__L__V__V__S__V__P__F__

TTCCTCTGCTTTTTGTACTCCTGGTGGCTGGCAGAGTCGGCACGATGGCTCCTCACCA
CA

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TTCCTGTGCTTCCTGTACAGCTGGTGGCTCGCTGAGAGCGCCCGGTGGCTGCTGACC
ACA

---------+---------+---------+---------+---------+---------+900

F__L__C__F__L__Y__S__W__W__L__A__E__S__A__R__W__L__L__T__T__

GGCAGGCTGGATTGGGGCCTGCAGGAGCTGTGGAGGGTGGCTGCCATCAACGGAAA
GGGG

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GGCAGACTGGACTGGGGCCTGCAGGAACTGTGGCGGGTCGCCGCCATCAATGGCAA
GGGC

---------+---------+---------+---------+---------+---------+960

G__R__L__D__W__G__L__Q__E__L__W__R__V__A__A__I__N__G__K__G__

GCAGTGCAGGACACCCTGACCCCTGAGGTCTTGCTTTCAGCCATGCGGGAGGAGCT
GAGC

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GCCGTGCAGGACACCCTGACCCCTGAGGTGCTGCTGAGCGCCATGCGCGAGGAACT
GAGC

---------+---------+---------+---------+---------+---------+1020

A__V__Q__D__T__L__T__P__E__V__L__L__S__A__M__R__E__E__L__S__

ATGGGCCAGCCTCCTGCCAGCCTGGGCACCCTGCTCCGCATGCCCGGACTGCGCTTC
CGG

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ATGGGCCAGCCTCCAGCCAGCCTGGGCACACTGCTGAGAATGCCCGGCCTGCGGTT
CCGG

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M__G__Q__P__P__A__S__L__G__T__L__L__R__M__P__G__L__R__F__R__

ACCTGTATCTCCACGTTGTGCTGGTTCGCCTTTGGCTTCACCTTCTTCGGCCTGGCCC
TG

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ACCTGCATCAGCACCCTGTGTTGGTTCGCCTTCGGCTTCACCTTCTTCGGCCTGGCCC
TG

---------+---------+---------+---------+---------+---------+1140

T__C__I__S__T__L__C__W__F__A__F__G__F__T__F__F__G__L__A__L__

GACCTGCAGGCCCTGGGCAGCAACATCTTCCTGCTCCAAATGTTCATTGGTGTCGTG
GAC

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GACCTCCAGGCCCTGGGCAGCAACATCTTCCTGCTGCAGATGTTCATCGGCGTGGTG
GAC

---------+---------+---------+---------+---------+---------+1200

D__L__Q__A__L__G__S__N__I__F__L__L__Q__M__F__I__G__V__V__D__

ATCCCAGCCAAGATGGGCGCCCTGCTGCTGCTGAGCCACCTGGGCCGCCGCCCCAC
GCTG

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ATCCCCGCCAAGATGGGCGCCCTGCTGCTGCTGTCTCACCTGGGCAGAAGGCCTACC
CTG

---------+---------+---------+---------+---------+---------+1260

I__P__A__K__M__G__A__L__L__L__L__S__H__L__G__R__R__P__T__L__

GCCGCATCCCTGTTGCTGGCAGGGCTCTGCATTCTGGCCAACACGCTGGTGCCCCAC
GAA

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GCCGCCTCTCTGCTGCTGGCCGGACTGTGCATCCTGGCCAACACCCTGGTGCCCCAC
GAG

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A__A__S__L__L__L__A__G__L__C__I__L__A__N__T__L__V__P__H__E__

ATGGGGGCTCTGCGCTCAGCCTTGGCCGTGCTGGGGCTGGGCGGGGTGGGGGCTGC
CTTC

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ATGGGAGCCCTGAGATCTGCCCTGGCCGTCCTGGGACTGGGAGGCGTGGGAGCTGC
CTTC

---------+---------+---------+---------+---------+---------+1380

M__G__A__L__R__S__A__L__A__V__L__G__L__G__G__V__G__A__A__F__

ACCTGCATCACCATCTACAGCAGCGAGCTCTTCCCCACTGTGCTCAGGATGACGGCA
GTG

||||| |||||||||||||||||||| |||||||| ||||| ||||||| || |||

ACCTGTATCACCATCTACAGCAGCGAGCTGTTCCCCACCGTGCTGCGGATGACAGCC
GTG

---------+---------+---------+---------+---------+---------+1440

T__C__I__T__I__Y__S__S__E__L__F__P__T__V__L__R__M__T__A__V__

GGCTTGGGCCAGATGGCAGCCCGTGGAGGAGCCATCCTGGGGCCTCTGGTCCGGCT
GCTG

||| |||| |||||||| ||| | || |||||||||||||| |||||||| || ||||||

GGCCTGGGACAGATGGCCGCCAGAGGCGGAGCCATCCTGGGACCTCTGGTGCGCCT
GCTG

---------+---------+---------+---------+---------+---------+1500

G__L__G__Q__M__A__A__R__G__G__A__I__L__G__P__L__V__R__L__L__

GGTGTCCATGGCCCCTGGCTGCCCTTGCTGGTGTATGGGACGGTGCCAGTGCTGAGT
GGC

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GGAGTGCACGGACCTTGGCTCCCTCTGCTGGTGTACGGCACCGTGCCTGTGCTGTCT
GGA

---------+---------+---------+---------+---------+---------+1560

G__V__H__G__P__W__L__P__L__L__V__Y__G__T__V__P__V__L__S__G__

CTGGCCGCACTGCTTCTGCCCGAGACCCAGAGCTTGCCGCTGCCCGACACCATCCAA
GAT

||||| || ||||| |||||||||| |||||| |||| |||||||||||||||| ||

CTGGCTGCTCTGCTGCTGCCCGAGACACAGAGCCTGCCCCTGCCCGACACCATCCAG
GAC

---------+---------+---------+---------+---------+---------+1620

L__A__A__L__L__L__P__E__T__Q__S__L__P__L__P__D__T__I__Q__D__

GTGCAGAACCAGGCAGTAAAGAAGGCAACACATGGCACGCTGGGGAACTCTGTCCT
AAAA

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GTGCAGAACCAGGCCGTGAAGAAGGCCACCCACGGCACCCTGGGCAACAGCGTGCT
GAAG

---------+---------+---------+---------+---------+---------+1680

V__Q__N__Q__A__V__K__K__A__T__H__G__T__L__G__N__S__V__L__K__

TCCACACAGTTC

||||| ||||||

TCCACCCAGTTCATGGTGTCCAAGGGGGAGGAACTGTTTACCGGCGTGGTGCCCATC
CTG

---------+---------+---------+---------+---------+---------+1740

S__T__Q__F__M__V__S__K__G__E__E__L__F__T__G__V__V__P__I__L__

End of URAT1 sequence

Initiation for eGFP

GTGGAACTGGACGGCGACGTGAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGA
AGGC

---------+---------+---------+---------+---------+---------+1800

V__E__L__D__G__D__V__N__G__H__K__F__S__V__S__G__E__G__E__G__

GACGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCC
CGTG

---------+---------+---------+---------+---------+---------+1860

D__A__T__Y__G__K__L__T__L__K__F__I__C__T__T__G__K__L__P__V__

CCTTGGCCCACCCTGGTGACAACCTTCACCTACGGCGTGCAGTGCTTCGCCAGATAC
CCC

---------+---------+---------+---------+---------+---------+1920

P__W__P__T__L__V__T__T__F__T__Y__G__V__Q__C__F__A__R__Y__P__

GACCACATGAAGCAGCACGATTTCTTCAAGTCCGCCATGCCCGAGGGCTACGTGCA
GGAA

---------+---------+---------+---------+---------+---------+1980

D__H__M__K__Q__H__D__F__F__K__S__A__M__P__E__G__Y__V__Q__E__

CGGACCATCTTCTTCAAGGACGACGGCAACTACAAGACCAGAGCCGAAGTGAAGTT
CGAG

---------+---------+---------+---------+---------+---------+2040

R__T__I__F__F__K__D__D__G__N__Y__K__T__R__A__E__V__K__F__E__

GGCGATACCCTGGTGAACCGGATCGAGCTGAAGGGCATCGACTTCAAAGAGGACGG
CAAT

---------+---------+---------+---------+---------+---------+2100

G__D__T__L__V__N__R__I__E__L__K__G__I__D__F__K__E__D__G__N__

ATCCTGGGCCACAAGCTGGAGTACAACTACAACAGCCACAAGGTGTACATCACCGC
CGAC

---------+---------+---------+---------+---------+---------+2160

I__L__G__H__K__L__E__Y__N__Y__N__S__H__K__V__Y__I__T__A__D__

AAGCAGAAAAACGGCATCAAAGTGAACTTCAAGACCCGGCACAACATCGAGGACG
GAAGC

---------+---------+---------+---------+---------+---------+2220

K__Q__K__N__G__I__K__V__N__F__K__T__R__H__N__I__E__D__G__S__

GTGCAGCTGGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCT
GCTG

---------+---------+---------+---------+---------+---------+2280

V__Q__L__A__D__H__Y__Q__Q__N__T__P__I__G__D__G__P__V__L__L__

CCTGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAGGACCCCAACGAGAA
GCGG

---------+---------+---------+---------+---------+---------+2340

P__D__N__H__Y__L__S__T__Q__S__A__L__S__K__D__P__N__E__K__R__

GACCACATGGTGCTGCTGGAATTCGTGACCGCCGCTGGCATCACACTGGGCATGGA
CGAG

---------+---------+---------+---------+---------+---------+2400

D__H__M__V__L__L__E__F__V__T__A__A__G__I__T__L__G__M__D__E__

attB2-3'

CTGTACAAGTACCCAGCTTTCTTGTACAAAGTGGTTGATATCCAGCACAGTGGCGGC
CGC

---------+---------+---------+---------+---------+---------+2460

L__Y__K__I__P__A__F__L__Y__K__V__V__N__I__Q__H__S__G__G__R__

End of Egfp

TCGAGTCTAGAGGGCCCGCGGTTCGAAGGTAAGCCTATCCCTAACCCTCTCCTCGGT
CTC

---------+---------+---------+---------+---------+---------+2520

S__S__L__E__G__P__R__F__E__G__K__P__I__P__N__P__L__L__G__L__

V5 EPITOPE

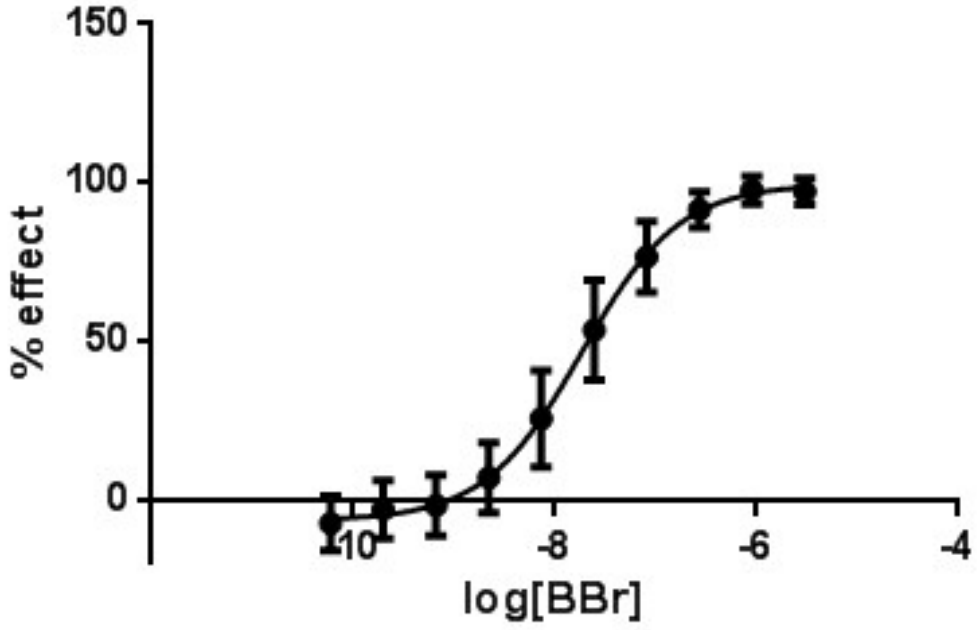
GATTCTACGCGTACCGGTTAGTAATGA

---------+---------+-------2547

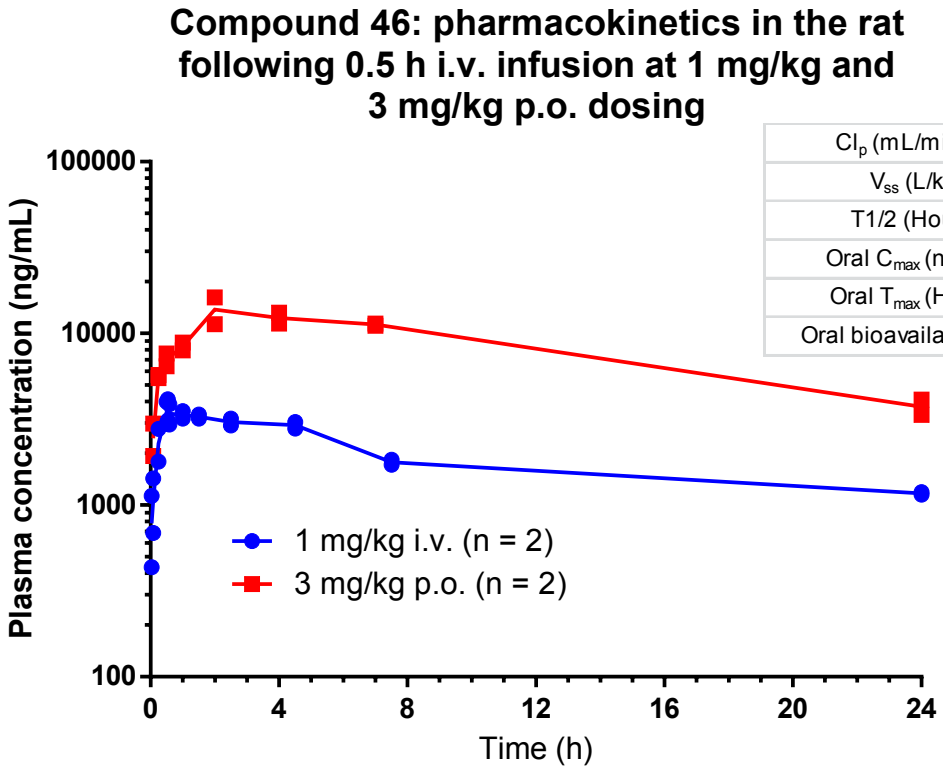
D__S__T__R__T__G__*__*__*__

STOP

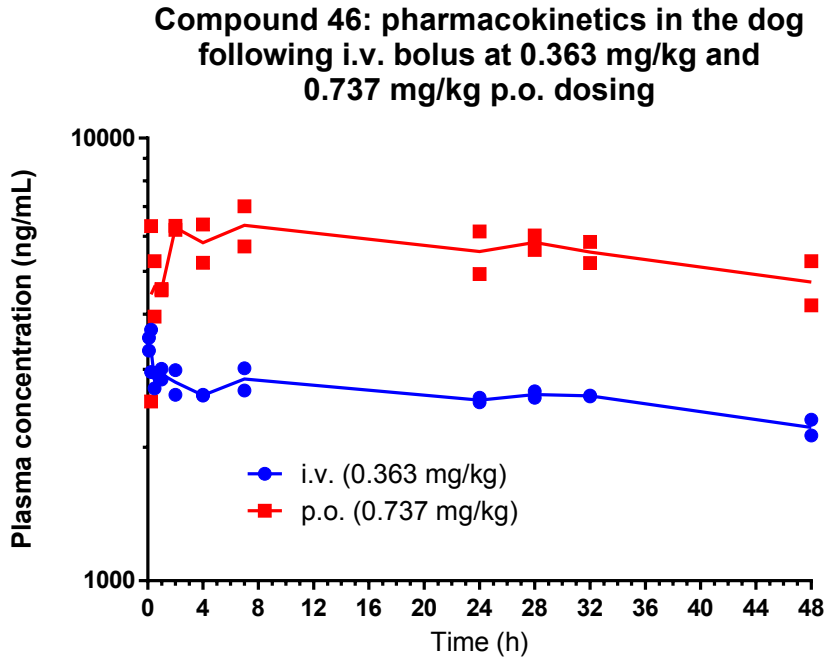
Figure 2 IC_{50} plot showing benzbromarone-mediated inhibition of [^{14}C] uric acid uptake by hURAT1 overexpressing cell line. This plot represents the average percentage inhibition of the concentration responsive data for benzbromarone (over 900 data points per concentration). It should be noted that pharmacological blockade of uptake is equivalent to uptake in parental cell lines lacking human URAT1 expression. Error bars are standard deviation.



7. Pharmacokinetic profile of compound 46 in rat & dog



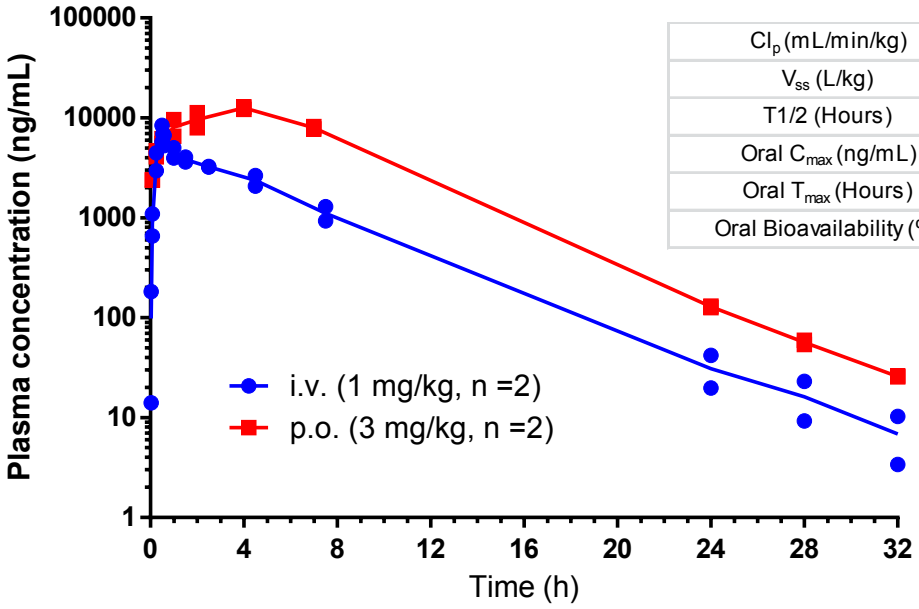
Cl_p (mL/min/kg)	0.183
V_{ss} (L/kg)	0.4
T1/2 (Hours)	27.3
Oral C_{max} (ng/mL)	14700
Oral T_{max} (Hours)	3
Oral bioavailability (%)	98



Cl_p (mL/min/kg)	0.0136
V_{ss} (L/kg)	0.118
T1/2 (Hours)	103
Oral C_{max} (ng/mL)	6700
Oral T_{max} (Hours)	5.5
Oral bioavailability (%)	104

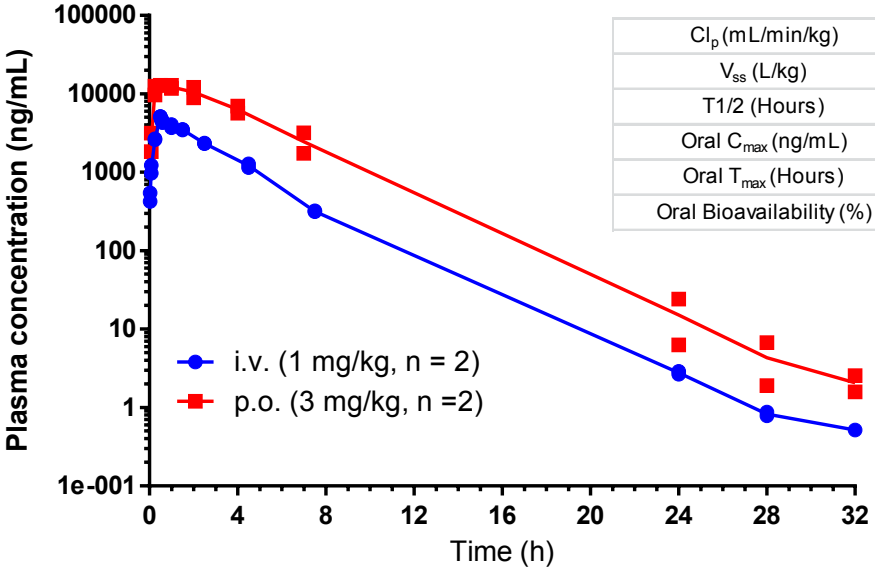
8. Pharmacokinetic profile of compound 47 in rat & dog

Compound 47: pharmacokinetics in the rat following 0.5 h i.v. infusion at 1 mg/kg and 3 mg/kg p.o. dosing



Cl_p (mL/min/kg)	0.55
V_{ss} (L/kg)	0.14
T1/2 (Hours)	3.28
Oral C_{max} (ng/mL)	12600
Oral T_{max} (Hours)	4
Oral Bioavailability (%)	150

Compound 47: pharmacokinetics in the dog following 0.5 h i.v. infusion at 1 mg/kg and 3 mg/kg p.o. dosing



Cl_p (mL/min/kg)	1.00
V_{ss} (L/kg)	0.18
T1/2 (Hours)	2.4
Oral C_{max} (ng/mL)	12900
Oral T_{max} (Hours)	0.5
Oral Bioavailability (%)	145