Electronic Supplementary Material (ESI) for MedChemComm.

The discovery and evaluation of diaryl ether heterocyclic sulfonamides as URAT1 inhibitors for the treatment of gout.

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Contents

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

br	broad
$CDCl_3$	Chloroform-d1
CI	Confidence Intervals (95% unless otherwise stated)
δ	Chemical shift
d	Doublet
DMSO	Dimethylsulfoxide
ELSD	Evaporative Light Scattering Detector
ESI	Electrospray ionisation
EtOAc	Ethyl acetate
Et_2O	Diethylether
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-to-charge ratio
N	Normal concentration
NMR	Nuclear Magnetic Resonance
R_t	Retention time
s	Singlet
t	Triplet
UV-TIC	Ultraviolet-total ion count

2. General Chemistry Experimental Prodecures

^{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 electrospray ionisation (ESI). 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.

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 3mm 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 1000uL

Method 2 basic conditions

Column Gemini NX C18, 5um 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

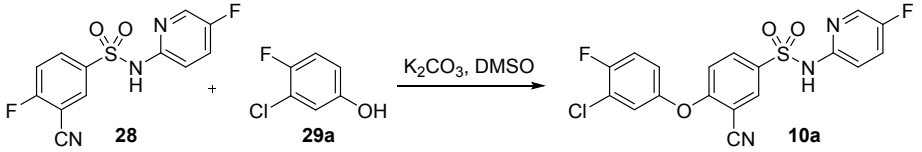
Gradient initial 0% B, 1 min – 5% B; 7 min – 98% B; 9 min – 98% B; 9.1 min – 5% B; 10 min – 5% B

Flow rate 18 mL/min

Injection volume 1000 μL

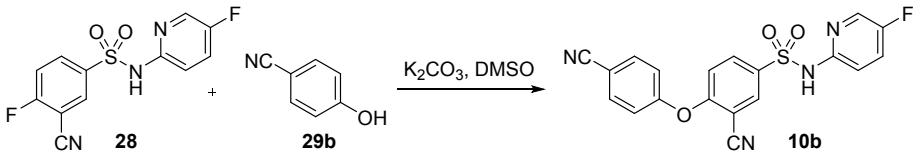
3. Chemistry experimental procedures and analytical data for intermediates and test compounds

4-(3-Chloro-4-fluorophenoxy)-3-cyano-*N*-(5-fluoropyridin-2-yl)benzenesulfonamide (10a)



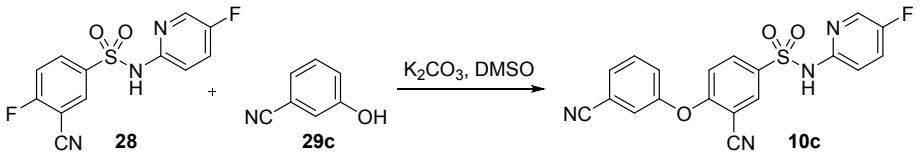
A suspension of 3-cyano-4-fluoro-*N*-(5-fluoropyridin-2-yl) benzene sulfonamide (**28**, 200.0 mg, 0.68 mmol), 3-chloro-4-fluorophenol (**29a**, 148.9 mg, 1.02 mmol) and K_2CO_3 (282 mg, 2.04 mmol) in DMSO (3 mL) was heated at 60 °C for 17 h. The reaction mixture was poured into water (80 mL) and product extracted into EtOAc (3 x 30 mL). The combined organics were concentrated and purified on silica gel using EtOAc:heptanes (1:1) to give the title compound (**10a**, 230 mg, 54%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23-7.40 (d, J = 2.8 Hz, 1H), 8.12 (d, J = 2.4 Hz, 1H), 7.90 (dd, J = 9.2, 2.4 Hz, 1H), 7.49 (m, 2H), 7.26-7.20 (m, 3H), 7.03-7.00 (m, 1H), 6.84 (d, J = 8.8 Hz, 1H); ^{19}F-NMR (376 MHz, $CDCl_3$) δ -117.00, -130.05: HPLC (syst 1, 4.5 min, acid) R_t 3.12 min, ELSD >95% purity; LRMS *m/z* 421.95 & 423.90 $[MH]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{18}H_{10}ClF_2N_3O_3S$ 422.0172, found 422.0162.

3-Cyano-4-(4-cyanophenoxy)-*N*-(5-fluoropyridin-2-yl)benzenesulfonamide (10b)



A suspension of 3-cyano-4-fluoro-*N*-(4-fluorophenyl) benzenesulfonamide (**28**, 11.8 mg, 0.040 mmol), 4-hydroxybenzonitrile (**29b**, 7.1 mg, 0.060 mmol) and K_2CO_3 (16.6 mg, 0.12 mmol) in DMSO (0.6 mL) was heated at 90 °C for 24 h then cooled to rt. The reaction mixture was filtered, concentrated and purified by preparative HPLC to give the title compound as a beige solid (**10b**, 15.4 mg, 97%). HPLC (syst 2, 4.5 min, acid) R_t 3.36 min, ELSD >95% purity; LRMS *m/z* 395 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{11}FN_4O_3S$ 395.0609, found 395.0606.

3-Cyano-4-(3-cyanophenoxy)-*N*-(5-fluoropyridin-2-yl)benzenesulfonamide (10c)

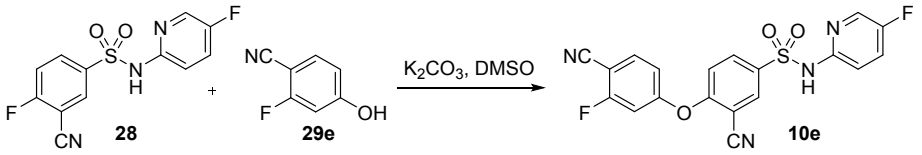


A suspension of 3-cyano-4-fluoro-*N*-(4-fluorophenyl) benzenesulfonamide (**28**, 11.8 mg, 0.040 mmol), 3-hydroxybenzonitrile (**29c**, 7.1 mg, 0.060 mmol) and K_2CO_3 (16.6 mg, 0.12 mmol) in DMSO (0.6 mL) was heated at 90 °C for 24 h then cooled to rt. The reaction mixture was filtered, concentrated and purified by preparative HPLC to give the title compound as a beige solid (**10c**, 11.2 mg, 71%). HPLC (syst 2, 4.5 min, acid) R_t 3.38 min, ELSD >95% purity; LRMS *m/z* 395 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{11}FN_4O_3S$ 395.0609, found 395.0611.

3-Cyano-4-(3-cyano-4-fluorophenoxy)-*N*-(4-fluorophenyl)benzenesulfonamide (10d)

A suspension of 3-cyano-4-fluoro-*N*-(4-fluorophenyl) benzenesulfonamide (**28**, 100 mg, 0.339 mmol), 2-fluoro-5-hydroxybenzonitrile (**29d**, 46.4 mg, 0.339 mmol), and K_2CO_3 (117 mg, 0.847 mmol) in DMSO (1.0 mL) was heated at 60 °C for 16 h then cooled to rt. The reaction mixture was filtered, concentrated and purified by preparative HPLC to give the title compound as a beige solid (**10d**, 32 mg, 23%). ^{1}H NMR (400 MHz, *d*6-DMSO) δ 11.19 (bs, 1H), 8.41 (d, *J* = 2.3 Hz, 1H), 8.21 (d, *J* = 3.1 Hz, 1H), 8.11-8.05 (m, 2H), 7.75-7.79 (m, 1H), 7.73-7.67 (m, 2H), 7.13-7.09 (m, 2H); HPLC (syst 2, 4.5 min, acid) R_t 2.87 min ELSD >95% purity; LRMS *m/z* 412.92 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{10}F_2N_4O_3S$ 413.0514, found 413.0512.

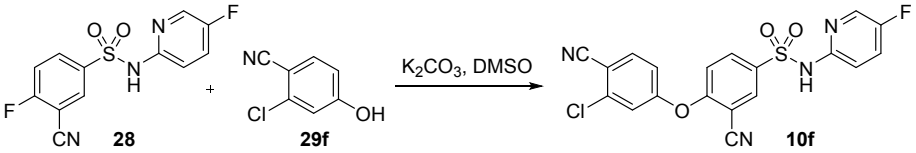
3-Cyano-4-(4-cyano-3-fluorophenoxy)-*N*-(5-fluoropyridin-2-yl)benzenesulfonamide (10e)



A suspension of 3-cyano-4-fluoro-*N*-(5-fluoropyridin-2-yl)benzene sulfonamide (**28**, 300 mg, 1.02 mmol), 2-fluoro-4-hydroxybenzonitrile (**29e**, 208 mg, 1.52 mmol) and K_2CO_3 (421 mg, 3.06 mmol) in DMSO (5 mL) was heated to 80 °C for 18 h. The reaction mixture was poured into water (80 mL) and product extracted with EtOAc (3 x 30 mL). The combined organics were concentrated

and purified by reverse phase chromatography (60 g C18, MeCN/water, 3:97 to 100:0, formic acid additive) to afford the desired product as a white solid (**10e**, 110 mg, 26%). ^{1}H NMR (400 MHz, *d*4-methanol) δ 8.40 (d, *J* = 2.2 Hz, 1H), 8.22 (dd, *J* = 8.9, 2.3 Hz, 1H), 8.13 (d, *J* = 3.1 Hz, 1H), 7.85 (dd, *J* =8.5, 7.6 Hz, 1H), 7.60-7.50 (td, *J* = 8.7, 2.9 Hz, 1H), 7.28-7.20 (m, 2H), 7.18-7.10 (m, 2H); ^{19}F NMR (376 MHz, *d*4-methanol) δ -115, -136: HPLC (syst 1, 4.5 min, acid) R_t 2.95 min, ELSD >95% purity; LRMS *m/z* 412.96 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{10}F_2N_4O_3S$ 413.0514, found 413.0506.

4-(3-Chloro-4-cyanophenoxy)-3-cyano-*N*-(5-fluoropyridin-2-yl)benzenesulfonamide (10f)



Smaller scale synthesis batch:

A suspension of 3-cyano-4-fluoro-*N*-(5-fluoropyridin-2-yl)benzene sulfonamide (**28**, 10.5 g, 35.6 mmol), 2-chloro-4-hydroxybenzonitrile (**29f**, 8.19 g, 53.3 mmol) and K_2CO_3 (14.74 g, 106.7 mmol) in DMSO (100 mL) was heated to 80 °C for 44 h. The reaction mixture was cooled to rt then poured into sat. aq. $NaHCO_3$ (200 mL) and EtOAc (1 L) was added. The organic phase was washed with sat. aq. $NaHCO_3$ (3 x 200 mL), water (2 x 200 mL) and brine (2 x 200 mL) then dried over Na_2SO_4, filtered and concentrated to give the crude product which was purified by reverse phase chromatography (400 g, C-18 column, eluting with 0-100% MeCN/water with 0.1% formic acid) to afford the title compound as a colourless solid (**10f**, 6.98 g, 46%).

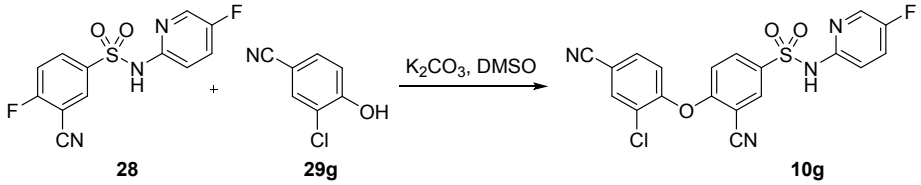
Scale up synthesis batch:

A mixture of 3-cyano-4-fluoro-*N*-(5-fluoropyridin-2-yl)benzenesulfonamide (**28**, 110 g, 0.38 mol), 2-chloro-4-hydroxybenzonitrile (**29f**, 85.8 g, 0.56 mol) and K_2CO_3 (154 g, 1.12 mol) in DMSO (1.1 L) was heated to 80 °C for 32 h, then stirred for an additional 16 h at rt. The reaction mixture was poured in to water (3 L). The combined organic layers were washed with water (1 L), citric acid solution (117 g, in 3.5 L, 3% w/w, 1.5 mol of citric acid for 1 mol of product) and water (1 L). The organic phase was concentrated in vacuo to give crude material (136 g). The compound was crystallised from EtOAc (1.6 L, 12 mL/g) to give material (110 g). This material was combined with 35 g of previously obtained material then partially dissolved in boiling ethyl acetate

(~1.5 mL, 10 mL/g ca.). The mixture was left to cool overnight to room temperature and filtered to afford the title compound as a white solid (**10f**, 113 g, 54%).

^{1}H NMR (400 MHz, d_6-DMSO) δ 11.34 (br. s., 1H), 8.44 (d, *J*=2.34 Hz, 1H), 8.22 (d, *J*=2.73 Hz, 1H), 8.16 (dd, *J*=2.30, 9.00 Hz, 1H, partially obscured), 8.13 (d, *J*=8.59 Hz, 1H), 7.83 (d, *J*=2.34 Hz, 1H), 7.71 (ddd, *J*=3.10, 8.60, 8.60 Hz, 1H), 7.47 (dd, *J*=2.34, 8.98 Hz, 1H), 7.32 (d, *J*=8.98 Hz, 1H), 7.13 (dd, *J*=3.90, 8.98 Hz, 1H); ^{13}C NMR (101 MHz, d_6-DMSO) δ 160.6, 158.6, 155.2 (d, J = 248.0 Hz), 148.0, 138.0, 137.2, 137.0, 135.6 (m), 134.6, 134.0, 126.8 (d, J = 22.7 Hz), 122.4, 120.4, 119.1, 116.1, 114.8, 114.6 (d, J = 4.40 Hz), 109.7, 104.2; ^{19}F NMR (376 MHz d_6-DMSO): δ -134.44: HPLC (syst 1, 4.5 min, acid) R_t 3.03 min, ELSD >95% purity; LRMS *m/z* 428.95 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{10}ClFN_4O_3S$ 429.0219, found 429.0221.

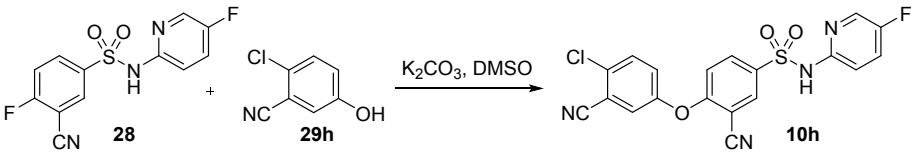
4-(2-Chloro-4-cyanophenoxy)-3-cyano-*N*-(5-fluoropyridin-2-yl)benzenesulfonamide (10g)



28 **29g** **10g**

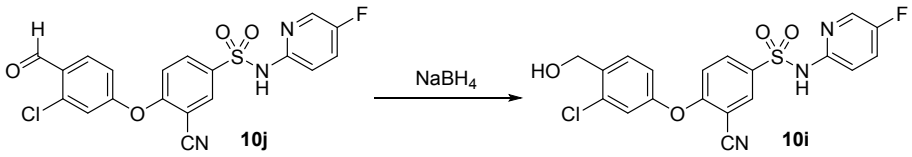
A suspension of 3-cyano-4-fluoro-*N*-(5-fluoropyridin-2-yl) benzene sulfonamide (**28**, 350 mg, 1.19 mmol), 3-chloro-4-hydroxybenzonitrile (**29g**, 275 mg, 1.79 mmol) and K_2CO_3 (493 mg, 3.57 mmol) in DMSO (5 mL) was heated to 80 °C for 16 h. The reaction was diluted with EtOAc (55 mL) and washed with sat. aq. $NaHCO_3$ (2 x 60 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated to afford a yellow solid (366 mg). The crude material was purified by flash chromatography (elution: 0-60% EtOAc/heptane, then 10% MeOH/EtOAc) and Biotage SP1 (30 g, C-18 column, eluting with 10-60% MeCN/water with 0.1% formic acid) to afford the title compound as a yellow solid (**10g**, 175 mg, 34%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.36 (bs, 1H), 8.44 (d, *J* = 2.4 Hz, 1H), 8.37 (d, *J* = 2.0 Hz, 1H), 8.20 (d, *J* = 3.0 Hz, 1H), 8.11 (dd, *J* = 9.0, 2.4 Hz, 1H), 8.00 (dd *J* = 8.5, 2.0 Hz, 1H), 7.63-7.70 (m, 2H), 7.17-7.12 (m, 2H); ^{19}F NMR (376 MHz, *d*6-DMSO) δ -134.57: HPLC (syst 2, 4.5 min, buffer) R_t 2.59 min, ELSD >95% purity; LRMS *m/z* 428.88 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{10}ClFN_4O_3S$ 429.0219, found 429.0212.

4-(4-Chloro-3-cyanophenoxy)-3-cyano-*N*-(5-fluoropyridin-2-yl)benzenesulfonamide (10h)



A suspension of 2-chloro-5-hydroxybenzonitrile (**28**, 52 mg, 0.339 mmol), 3-cyano-4-fluoro-*N*-(5-fluoropyridin-2-yl)benzene sulfonamide (**29h**, 100 mg, 0.339 mmol) and K_2CO_3 (117 mg, 0.847 mmol) in DMSO (1 mL) was heated at 60 °C for 17 h. Half of the reaction mixture was submitted to preparative HPLC to give the title compound as cream coloured solid (**10h**, 21 mg, 30%). ^{1}H NMR (400 MHz, *d*6-DMSO) δ 11.25 (br s, 1H), 8.38 (d, *J* = 2.3 Hz, 1H), 8.18 (d, *J* = 3.0 Hz, 1H), 8.06 (d, *J* = 2.8 Hz, 1H), 8.05 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.84 (d, *J* = 8.9 Hz, 1H), 7.70 (dd, *J* = 9.0, 2.9 Hz, 1H), 7.70-7.65 (m, 1H), 7.12 (d, *J* = 7.15 Hz, 1H), 7.05 (dd, *J* = 9.0, 2.9 Hz, 1H); ^{19}F NMR (376 MHz, *d*6-DMSO) δ -134.5: HPLC (syst 2, 4.5 min, acid) R_t 2.96 min, ELSD >95% purity; LRMS *m/z* 428.95, 430.90 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{10}ClFN_4O_3S$ 429.0219, found 429.0221.

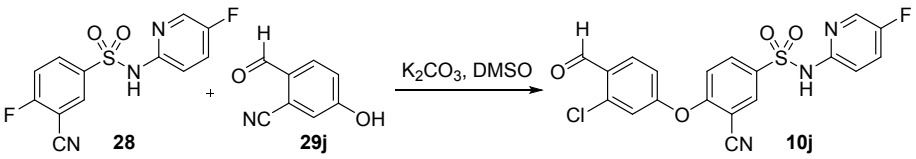
4-(3-Chloro-4-(hydroxymethyl)phenoxy)-3-cyano-*N*-(5-fluoropyridin-2-yl)benzene sulfonamide (10i)



To a suspension of 4-(3-chloro-4-formylphenoxy)-3-cyano-*N*-(5-fluoropyridin-2-yl) benzenesulfonamide (**10j**, 276.9 g, 0.64 mol) in methanol (5.5 L) at 0 °C was added $NaBH_4$ portion-wise over 25 min. The reaction was stirred at rt for 1.5 h, cooled to 0 °C then water (4.8 L) slowly added. The reaction mixture was warmed to rt and 1M HCl (2.5 L) was slowly added resulting in a suspension of the product. The mixture was left to stir at rt for 1 h and then the suspension was filtered and dried for 16 h at 40 °C under vacuum to give crude product. The crude product was dissolved in acetone (2.5 L) and then silica (380 g) was added to the vessel. The mixture was stirred at rt for 20 min and then filtered through a silica pad and washed with acetone (1.5 L). Water (10 L) was slowly added resulting in the precipitation of the product. The mixture was left to stir at rt for 1 h and then the suspension was filtered and the residue dried overnight at 40 °C to yield 4-(3-chloro-4-(hydroxymethyl)phenoxy)-3-cyano-N-(5-fluoropyridin-2-yl)benzenesulfonamide as a cream powder (**10i**, 268.2 g, 96%). ^{1}H NMR (400 MHz, *d*6-DMSO)

δ 11.31 (br. s., 1H), 8.39 (d, *J*=2.34 Hz, 1H), 8.22 (d, *J*=2.73 Hz, 1H), 8.12 (dd, J=2.34, 8.98 Hz, 1H), 7.70 (dd, J=8.60, 3.10 Hz, 1H, partially obscured), 7.66 (d, *J*=8.59 Hz, 1H), 7.48 (d, *J*=2.73 Hz, 1H), 7.30 (dd, *J*=2.34, 8.59 Hz, 1H), 7.12 (dd, *J*=3.51, 8.98 Hz, 1H), 7.05 (d, *J*=8.90 Hz, 1H), 5.50 (t, *J*=5.46 Hz, 1H), 4.59 (d, *J*=5.07 Hz, 2H); ^{13}C NMR (101MHz, *d*6-DMSO) δ 162.48, 156.42 (d, J = 247 Hz), 152.96, 147.99, 138.19, 135.63 (d, partially obscured), 135.54, 134.66, 133.88, 132.52, 130.17, 126.88 (d, J=19.1 Hz), 121.82, 120.02, 117.05, 115.11, 114.54 (d, J = 5.14Hz), 102.89, 60.38; ^{19}F NMR (376 MHz, $CDCl_3$): δ -134.5: HPLC (syst 1, 25 min, acid) R_t 14.09 min, ELSD 98.9% purity; LRMS *m/z* 434.02 $[M+H]^+$; HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{13}ClFN_3O_4S$ 434.0372, found 434.0369.

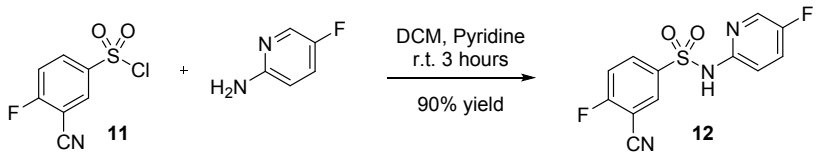
4-(3-Chloro-4-formylphenoxy)-3-cyano-*N*-(5-fluoropyridin-2-yl)benzenesulfonamide (10j)



A suspension of 3-cyano-4-fluoro-N-(5-fluoropyridin-2-yl) benzene sulfonamide (**28**, 108.2 g, 0.37 mol), K_2HPO_4 (191.3 g, 1.10 mol) and 2-chloro-4-hydroxybenzaldehyde (**29j**, 63.1 g, 0.40 mmol) in DMSO (760 mL) was heated to 100 °C and left to stir for 2.5 h then cooled to rt. The reaction mixture was poured into water (3.0 L) resulting in some precipitation of product. EtOAc (3.0 L) was added and the aq. layer acidified to pH 3 using conc. HCl. The aq. layer was removed and the resulting suspension filtered and solid washed with EtOAc (200 mL) and 1M HCl (20 mL) to give crude product. The organic filtrates were retained and washed with 1M HCl (700 mL) and sat. brine (2 × 700 mL) and dried over $MgSO_4$, filtered and concentrated to yield more crude product. Both batches of crude product were combined and slurried in EtOAc (750 mL) at reflux for 45 min. The mixture was cooled to rt, filtered and the solid washed with EtOAc (2× 100 mL). The product was died overnight under vacuum at 40 °C to yield 4-(3-chloro-4-formylphenoxy)-3-cyano-N-(5-fluoropyridin-2-yl)benzenesulfonamide (**10j**, 121.1 g, 77%) as a cream powder. ^{1}H NMR (400 MHz, *d*6-DMSO) δ 11.34 (brs, 1H), 10.28 (s, 1H), 8.42 (d, *J* = 2.3 Hz, 1H), 8.20 (d, *J* = 3.1 Hz, 1H), 8.14 (dd, *J* = 9.0 Hz, 2.4 Hz, 1H), 7.95 (d, *J* = 8.6 Hz, 1H), 7.69 (td, *J* = 8.6 Hz, 3 Hz, 1H), 7.63 (d, *J* = 2.4 Hz, 1H), 7.38 (dd, *J* = 8.2, 2.5 Hz, 1H), 7.28 (d, *J* = 8.9 Hz, 1H), 7.09

(dd, *J* = 9.2, 3.7 Hz, 1H); ^{19}F NMR (376 MHz, *d*6-DMSO) δ -134.4; HPLC (syst 1, 4.5 min, acid) R_t 3.58 min, ELSD >95% purity; LRMS *m/z* 432.09 $[M+H]^+$.

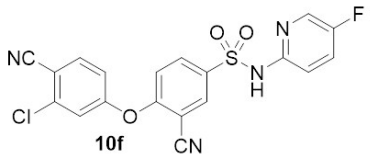
3-Cyano-4-fluoro-N-(5-fluoropyridin-2-yl)benzenesulfonamide (28)



Solid 3-Cyano-4-fluorobenzene-1-sulfonyl chloride (**27**, 60.3 g, 274 mmol) was added to a solution of 5-fluoropyridin-2-amine (40.0 g, 357 mmol) and pyridine (67 mL, 823 mmol) in DCM (1 L) at rt then stirred for 3 h. The solvent was removed under vacuum and the residue stirred in dilute HCl (2N, 850 mL) for 16 h. The precipitate was removed by filtration and the residue washed with water (200 mL) and dried under high vacuum overnight. The crude material was triturated with TBME (500 mL) to give the title compound (**28**, 70.3 g, 87%) as an orange solid. ^{1}H NMR (400 MHz, *d*6-DMSO) δ 11.45 (bs, 1H), 8.44 (dd, *J* = 6.0, 2.4 Hz, 1H), 8.26-8.24 (m, 1H), 8.18 (d, *J* = 3.2 Hz, 1H), 7.72-7.66 (m, 2H), 7.11-7.08 (m, 1H); ^{19}F-NMR (376 MHz, *d*6-DMSO) δ -101.50, -134.20: HPLC (syst 2, 4.5 min, acid) R_t 2.55 min, ELSD >95% purity; LRMS *m/z* 296.06 $[M+H]^+$.

The full syntheses of compounds **11-17** & **23-26** and relevant intermediates can be found in published patent application WO2014170792. The full syntheses of compounds **18-22** and relevant intermediates can be found in published patent application WO2014170793.

4. Selected HPLC, MS and NMR spectra



10f

Column:

Kinetic C18 100mm x 3.0mm 2.6u

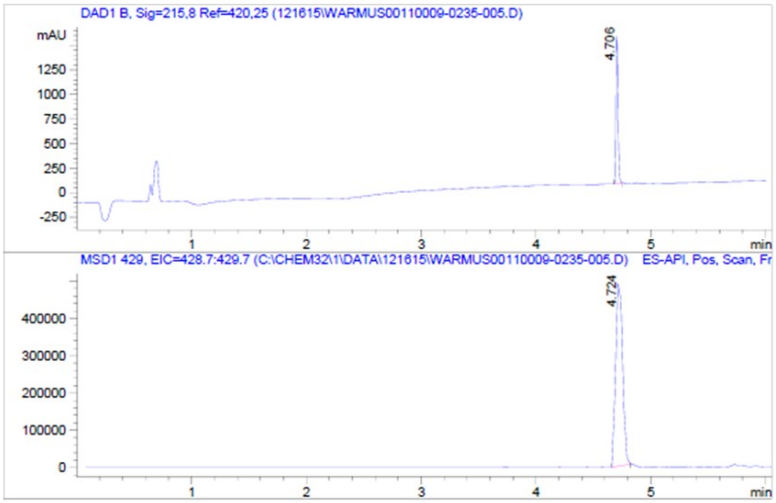
Gradient Conditions:

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

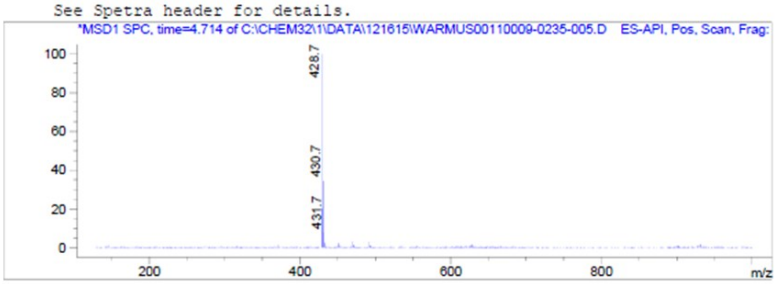
Time (min)	%A	%B
0.00	95	5
0.5	95	5
4	0	100
5.4	0	100
5.5	95	5

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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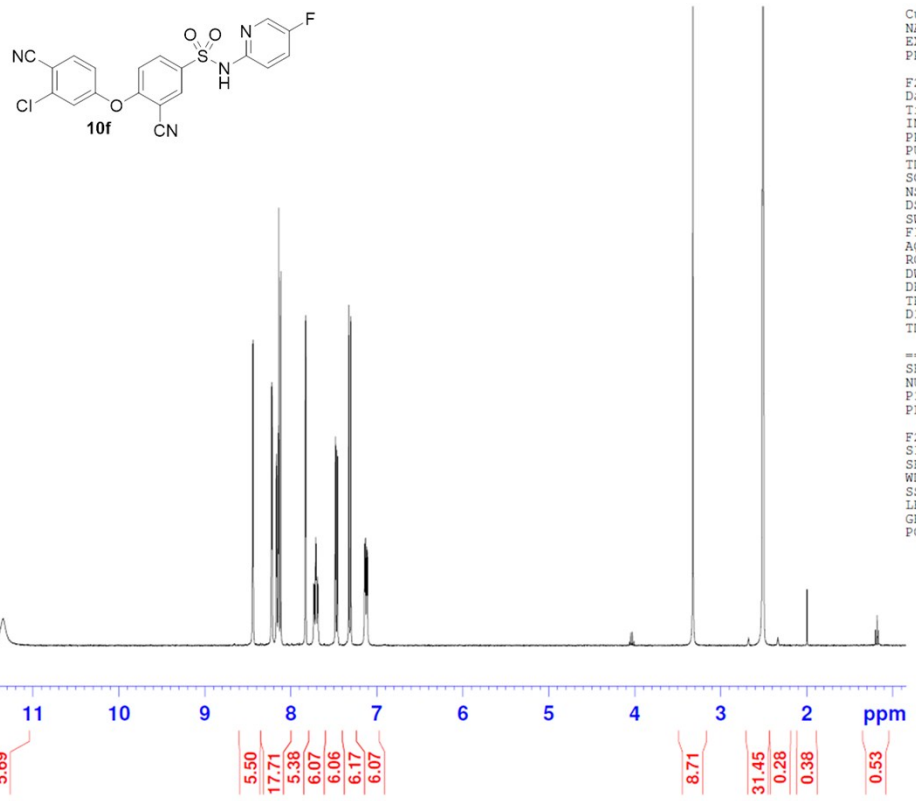


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PROCNO                   1

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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                 298.2 K
D1              0.75000000 sec
TD0                     1

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NUC1                   1H
P1                 14.00 usec
PLW1          19.95299911 W

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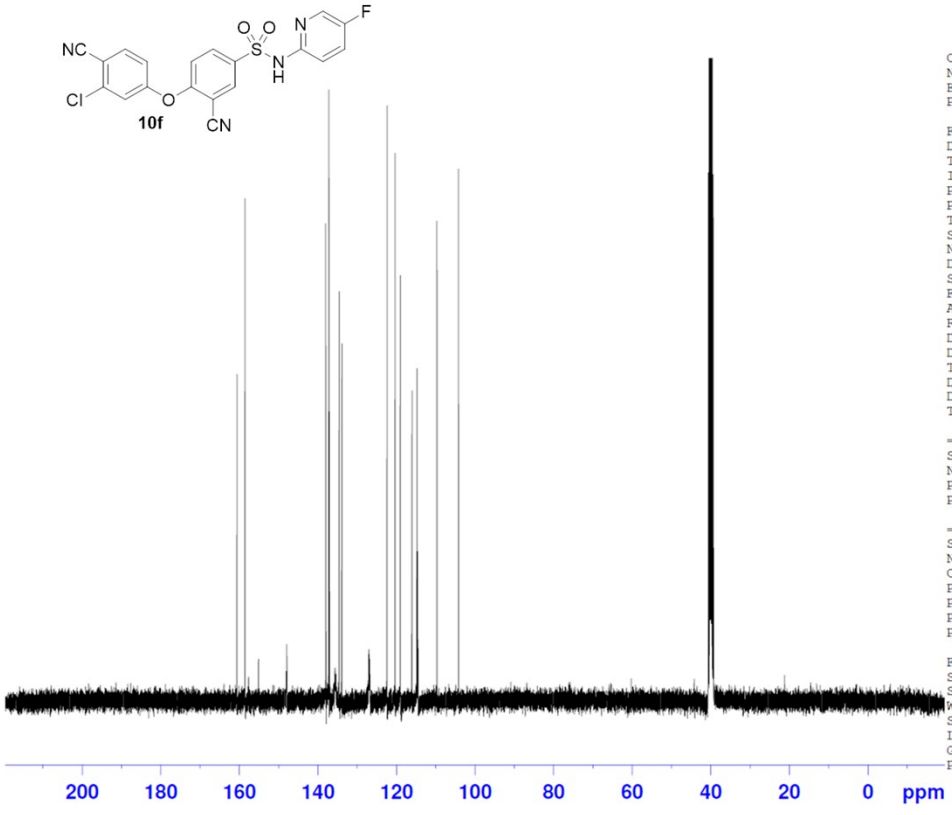


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PROCNO                   1

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Time                20.23
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                 298.2 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
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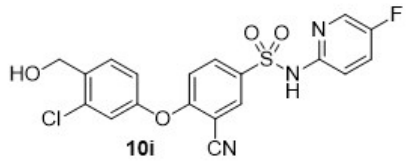
```



10i

Column:

Kinetic C18 100mm x 3.0mm 2.6u

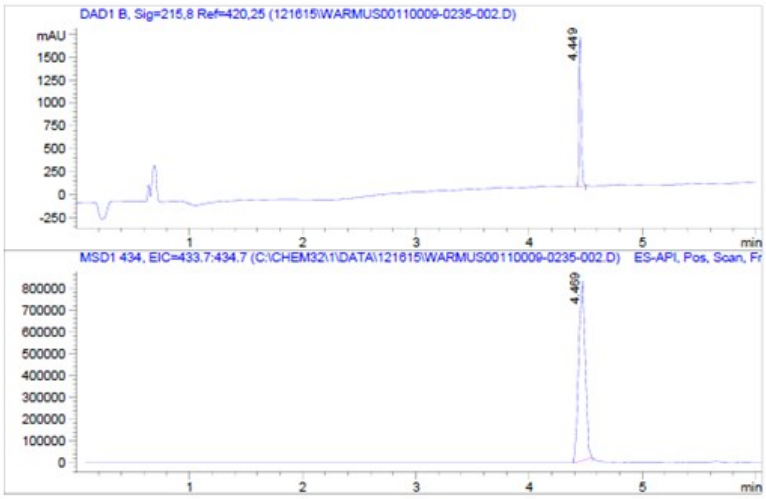
Gradient Conditions:

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

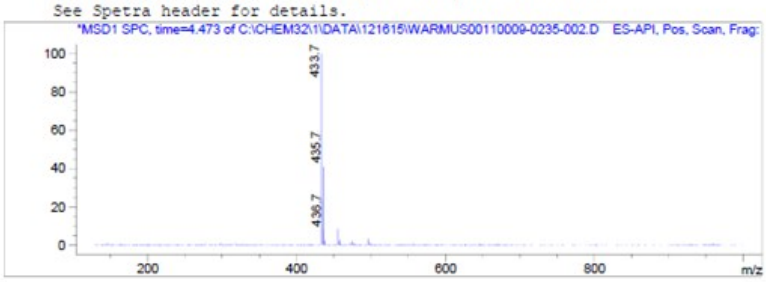
Time (min)	%A	%B
0.00	95	5
0.5	95	5
4	0	100
5.4	0	100
5.5	95	5

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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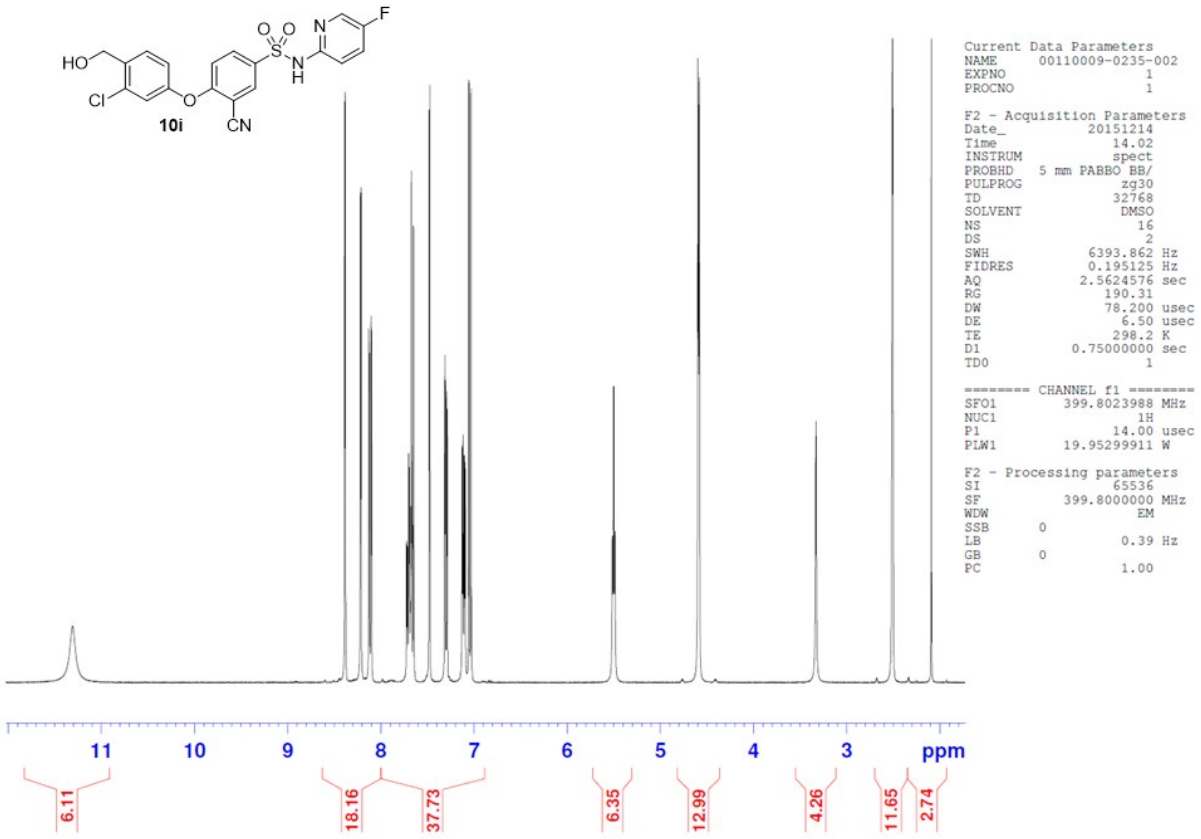


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PROCNO                   1

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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                  298.2 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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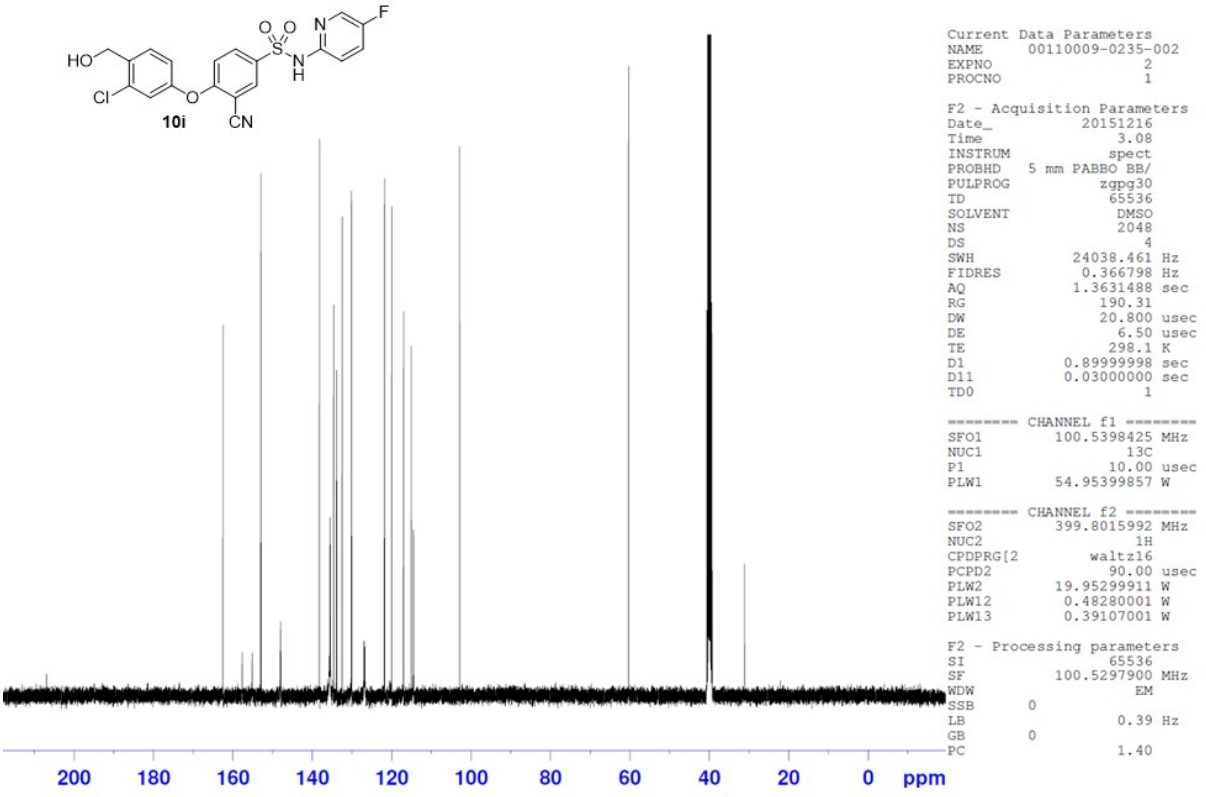


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PROCNO                   1

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Time                  3.08
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
SI                   65536
SF            100.5297900 MHz
WDW                     EM
SSB       0
LB                  0.39 Hz
GB        0
PC                  1.40
```

5. Polypharmacology profiles

Compound **10f**

Cerep *Full Safety Panel (10uM,*

Functional Agonism	% Resp	EC50 (nM)
Adenosine A1	0	
Adenosine A2a	12	
Adrenergic Alpha 1a	12	
Adrenergic Alpha 2a	8	
Adrenergic Alpha 2b	-4	
Adrenergic Beta 1	-0	
Adrenergic Beta 2	-1	
Angiotensin 1	5	
Cannabinoid 1	22	
Cholecystokinin :	-1	
Dopamine 1	0	
Dopamine 2s	-11	
Endothelin A	7	
Histamine 1	-1	
Histamine 2	-0	
Histamine 3	38	
Muscarinic 1	12	
Muscarinic 2	11	
Muscarinic 3	12	
Neurokinin 1	-0	
Opioid Delta	-12	
Opioid Kappa	18	
Opioid Mu	17	
Serotonin 1a	11	
Serotonin 1b	35	
Serotonin 2a	-2	
Serotonin 2b	-3	
Serotonin 4e	-1	
Vasopressin 1a	37	
Corticotrophin Releasing Factor 1 (CR		
Melanocortin 2 (MC2R)		
Thyrotropin Releasing Hormone 1 (TRH		

Functional Antagonism	% Inh	IC50 (nM)	Kb (nM)
Adrenergic Alpha 1a	58	7020	1400
Adrenergic Alpha 2b	50	90600	11800
Adrenergic Beta 1	17		
Adrenergic Beta 2	-4		
Angiotensin 1	35		
Cannabinoid 1	-4		
Dopamine 1	16		
Dopamine 2s	0		
Histamine 1	39		
Muscarinic 1	66	4160	489
Muscarinic 2	0		
Muscarinic 3	74	4930	448
Opioid Mu			
CRF1			
MC2R			
TRH1			

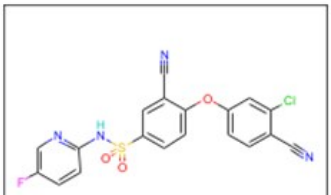
Ion Channel - Bindin	% Inh	IC50 (nM)	Ki (nM)
L-Type Calcium (Verapam	5		
L-Type Calcium (Nifedipin	30		
L-Type Calcium (Diltiazer	9		
GABAa (Cl Channel	67	5800	4810
GABAa1 Receptor	10		
GABAa (Benzodiazepine Site	-7		
AMPA Receptor	12		
NMDA Recepto	0		
NMDA Receptor (PCP Si	-4		
Nicotinic ACh Receptor (Musc	-0		
Nicotinic ACh Receptor (Neuron	3		
Serotonin 3	2		
Sodium (Site 2	8		

Transporters - Binding	% Inh	IC50 (nM)	Ki (nM)
Norepinephrine Transporte	1		
Dopamine Transporte	8		
Serotonin Transporter	3		
Choline Transporter	-11		
GABA Transporter	5		

Enzyme	% Inh	IC50 (nM)
Angiotensin Converting Enzyn	-8	
Acetylcholine Esterase	7	
Cyclooxygenase :	15	
Monoamine Oxidase	3	
PDE3B	9	
PDE4D2	0	

Kinase	% Inh	IC50 (nM)
Abl Kinase	12	
Aurora A Kinase		
EGFR Kinase	3	
Lck Kinase	-4	
p38 MAP Kinase	-4	
Src Kinase	10	
VEGFR2 (KDR) Kinas	15	

NHR - Binding	% Inh	IC50 (nM)	Ki (nM)
Androgen Receptor (Binding	2		
Glucocorticoid Receptor (Bindin	14.4		



Additional Data

Chemical Properties

Parent Molecular Weigh	428.83
cLOGP	3.74
TPSA	124.25

Promiscuity Panel Data - Binding (10uM

	% Inh
Adenergic Alpha 1a	-0
Adrenergic Beta 2	7
Cannabinoid 1	
Dopamine 1	-4
Histamine 1	7
Muscarinic 1	-0
Opioid Mu	4

Other

L-Type Calcium (Functional) - IC50 (	
Nav1.5 (Q Patch) - IC50 (n	
hERG (Functional) - IC50 (n	67382.0
Dofetilide Binding - IC50 (n	>100000

Compound **10i**

Cerep *Full Safety Panel (10uM)*

Functional Agonism	% Resp	EC50 (nM)
Adenosine A1	2	
Adenosine A2a	0	
Adrenergic Alpha 1a	1	
Adrenergic Alpha 2a	-2	
Adrenergic Alpha 2b	-0	
Adrenergic Beta 1	1	
Adrenergic Beta 2	1	
Angiotensin 1	0	
Cannabinoid 1	14	
Cholecystokinin :	2	
Dopamine 1	1	
Dopamine 2s	-8	
Endothelin A	4	
Histamine 1	-0	
Histamine 2	1	
Histamine 3	8	
Muscarinic 1	4	
Muscarinic 2	-7	
Muscarinic 3	0	
Neurokinin 1	-1	
Opioid Delta	-16	
Opioid Kappa	15	
Opioid Mu	-12	
Serotonin 1a	11	
Serotonin 1b	4	
Serotonin 2a	1	
Serotonin 2b	-3	
Serotonin 4e	-0	
Vasopressin 1a	18	
Corticotrophin Releasing Factor 1 (CR		
Melanocortin 2 (MC2R)		
Thyrotropin Releasing Hormone 1 (TRH		

Functional Antagonism	% Inh	IC50 (nM)	Kb (nM)
Adrenergic Alpha 1a	14		
Adrenergic Alpha 2b	14		
Adrenergic Beta 1	-8		
Adrenergic Beta 2	-6		
Angiotensin 1	23		
Cannabinoid 1	-4		
Dopamine 1	-8		
Dopamine 2s	19		
Histamine 1	5		
Muscarinic 1	28		
Muscarinic 2	3		
Muscarinic 3	7		
Opioid Mu			
CRF1			
MC2R			
TRH1			

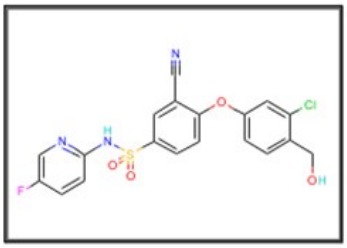
Ion Channel - Bindin	% Inh	IC50 (nM)	Ki (nM)
L-Type Calcium (Verapam	-4		
L-Type Calcium (Nifedipin	7		
L-Type Calcium (Diltiazer	-11		
GABAa (Cl Channel	28		
GABAa1 Receptor	-17		
GABAa (Benzodiazepine Site	-47		
AMPA Receptor	3		
NMDA Recepto	8		
NMDA Receptor (PCP Si	-2		
Nicotinic ACh Receptor (Musc	-13		
Nicotinic ACh Receptor (Neuron	2		
Serotonin 3	-8		
Sodium (Site 2	5		

Transporters - Binding	% Inh	IC50 (nM)	Ki (nM)
Norepinephrine Transporte	-14		
Dopamine Transporte	9		
Serotonin Transporter	-1		
Choline Transporter	-34		
GABA Transporter	-6		

Enzyme	% Inh	IC50 (nM)
Angiotensin Converting Enzym	-2	
Acetylcholine Esterase	5	
Cyclooxygenase :	15	
Monoamine Oxidase	-3	
PDE3B	11	
PDE4D2	-11	

Kinase	% Inh	IC50 (nM)
Abl Kinase	-5	
Aurora A Kinase		
EGFR Kinase	2	
Lck Kinase	-22	
p38 MAP Kinase	-0	
Src Kinase	18	
VEGFR2 (KDR) Kinas	-8	

NHR - Binding	% Inh	IC50 (nM)	Ki (nM)
Androgen Receptor (Binding	-8		
Glucocorticoid Receptor (Bindin	4.6		



Additional Data

Chemical Properties

Parent Molecular Weight	433.84
cLOGP	3.40
TPSA	120.69

Promiscuity Panel Data - Binding (10uM

	% Inh
Adenergic Alpha 1a	-12
Adrenergic Beta 2	-8
Cannabinoid 1	
Dopamine 1	-1
Histamine 1	4
Muscarinic 1	-11
Opioid Mu	1

Other

L-Type Calcium (Functional) - IC50 (nM	
Nav1.5 (Q Patch) - IC50 (nM	82815.992
hERG (Functional) - IC50 (nM	>100000.0
Dofetilide Binding - IC50 (nM	>100000

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 h 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 30 nM 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 min 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.

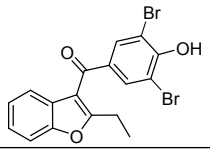
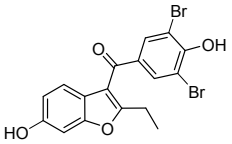
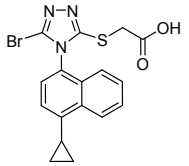
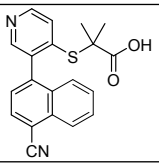
The accumulation of [^{14}C]-uric acid into CHO#8 cells was calculated and the IC_{50} (μM) values, defined as the concentration of inhibitor required for 50% inhibition of transport, were determined from a 4 parameter logistic fit to generate sigmoid curves from dose response data.

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.

```
GGGCCCCTCTTCTGGGCCCCTTGAGTAGGTTCCATGGCATTTTCTGAACTCCTGGACCTC
                              ||||| ||    |  || ||||||||

ACAAGTTTGTACAAAAAAGCAGGCTTCGCCACCATGGCCTTCAGCGAGCTGCTGGACCTG

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

                                    M__A__F__S__E__L__L__D__L__

attB1-5'                            URAT1 Initiation codon


GTGGGTGGCCTGGGCAGGTTCCAGGTTCTCCAGACGATGGCTCTGATGGTCTCCATCATG

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

GTGGGAGGCCTGGGCAGATTCCAGGTGCTGCAGACCATGGCCCTGATGGTGTCCATCATG
```

```
---------+---------+---------+---------+---------+---------+120
```

V__G__G__L__G__R__F__Q__V__L__Q__T__M__A__L__M__V__S__I__M__

```
TGGCTGTGTACCCAGAGCATGCTGGAGAACTTCTCGGCCGCCGTGCCCAGCCACCGCTGC
|||||||| |||||||||||||||| |||||||| |||||||||||||||||| | |||
TGGCTGTGCACCCAGAGCATGCTGGAAAACTTCTCTGCCGCCGTGCCCAGCCACAGATGC
---------+---------+---------+---------+---------+---------+180
```

W__L__C__T__Q__S__M__L__E__N__F__S__A__A__V__P__S__H__R__C__

```
TGGGCACCCCTCCTGGACAACAGCACGGCTCAGGCCAGCATCCTAGGGAGCTTGAGTCCT
||||| || || |||||||||||||| || |||||||||||||| || ||| ||  |||
TGGGCCCCTCTGCTGGACAACAGCACCGCCCAGGCCAGCATCCTGGGCAGCCTGTCTCCA
---------+---------+---------+---------+---------+---------+240
```

W__A__P__L__L__D__N__S__T__A__Q__A__S__I__L__G__S__L__S__P__

```
GAGGCCCTCCTGGCTATTTCCATCCCGCCGGGCCCCAACCAGAGGCCCCACCAGTGCCGC
|||||||| ||||| ||   |||||| || |||||||||||||||||||||||||| |
GAGGCCCTGCTGGCCATCAGCATCCCCCCTGGCCCCAACCAGAGGCCCCACCAGTGCAGA
```

```
---------+---------+---------+---------+---------+---------+300
```

E__A__L__L__A__I__S__I__P__P__G__P__N__Q__R__P__H__Q__C__R__

```
CGCTTCCGCCAGCCACAGTGGCAGCTCTTGGACCCCAATGCCACGGCCACCAGCTGGAGC
|| ||||| ||||| ||||||||||  |||| ||||| ||||| ||||||   ||||||
CGGTTCCGGCAGCCTCAGTGGCAGCTGCTGGATCCCAACGCCACCGCCACCTCTTGGAGC
---------+---------+---------+---------+---------+---------+360
```

R__F__R__Q__P__Q__W__Q__L__L__D__P__N__A__T__A__T__S__W__S__

```
GAGGCCGACACGGAGCCGTGTGTGGATGGCTGGGTCTATGACCGCAGCATCTTCACCTCC
|||||||||| ||||| |||||||| |||||||| || |||||   |||||||||  |
GAGGCCGACACCGAGCCCTGTGTGGACGGCTGGGTGTACGACCGGTCCATCTTCACCAGC
---------+---------+---------+---------+---------+---------+420
```

E__A__D__T__E__P__C__V__D__G__W__V__Y__D__R__S__I__F__T__S__

```
ACAATCGTGGCCAAGTGGAACCTCGTGTGTGACTCTCATGCTCTGAAGCCCATGGCCCAG
|| |||||||||||||||||| ||||| |||  ||| || ||||||||||||||||
ACCATCGTGGCCAAGTGGAACCTGGTGTGCGACAGTCACGCCCTGAAGCCCATGGCCCAG
```

```
---------+---------+---------+---------+---------+---------+480
```

T__I__V__A__K__W__N__L__V__C__D__S__H__A__L__K__P__M__A__Q__

```
TCCATCTACCTGGCTGGGATTCTGGTGGGAGCTGCTGCGTGCGGCCCTGCCTCAGACAGG
  |||||||||||| || |||||||||||||| || || || |||||||||   || ||
AGCATCTACCTGGCCGGCATTCTGGTGGGAGCCGCCGCTTGTGGCCCTGCCAGCGATAGA
---------+---------+---------+---------+---------+---------+540
```

S__I__Y__L__A__G__I__L__V__G__A__A__A__C__G__P__A__S__D__R__

```
TTTGGGCGCAGGCTGGTGCTAACCTGGAGCTACCTTCAGATGGCTGTGATGGGTACGGCA
|| ||  |  |||||||||| ||||||  |||||| |||||||| |||||||| || ||
TTCGGCAGACGGCTGGTGCTGACCTGGTCCTACCTGCAGATGGCCGTGATGGGCACCGCC
---------+---------+---------+---------+---------+---------+600
```

F__G__R__R__L__V__L__T__W__S__Y__L__Q__M__A__V__M__G__T__A__

```
GCTGCCTTCGCCCCTGCCTTCCCCGTGTACTGCCTGTTCCGCTTCCTGTTGGCCTTTGCC
|| ||||| |||||||||||||| |||||||||||||||| |||||| ||||||| |||
GCAGCCTTTGCCCCTGCCTTCCCTGTGTACTGCCTGTTCCGGTTCCTGCTGGCCTTCGCC
```

```
---------+---------+---------+---------+---------+---------+660
```

A__A__F__A__P__A__F__P__V__Y__C__L__F__R__F__L__L__A__F__A__

```
GTGGCAGGCGTCATGATGAACACGGGCACTCTCCTGATGGAGTGGACGGCGGCACGGGCC
|||||  |||||  ||||||||||  |||||  ||  ||||||||  |||||  ||  ||  |  |||
GTGGCCGGCGTGATGATGAACACCGGCACCCTGCTGATGGAATGGACCGCCGCCAGAGCC
---------+---------+---------+---------+---------+---------+720
```

V__A__G__V__M__M__N__T__G__T__L__L__M__E__W__T__A__A__R__A__

```
CGACCCTTGGTGATGACCTTGAACTCTCTGGGCTTCAGCTTCGGCCATGGCCTGACAGCT
 |||||  ||||||||||  |||||   ||||||||||||||||  ||||||||  |||||
AGACCCCTGGTGATGACCCTGAACAGCCTGGGCTTCAGCTTCGGACATGGCCTCACAGCC
---------+---------+---------+---------+---------+---------+780
```

R__P__L__V__M__T__L__N__S__L__G__F__S__F__G__H__G__L__T__A__

```
GCAGTGGCCTACGGTGTGCGGGACTGGACACTGCTGCAGCTGGTGGTCTCGGTCCCCTTC
||  |||||  ||  ||  ||||||||||||||||||||||||||||||  ||  ||  ||||||
GCTGTGGCTTATGGCGTGCGGGACTGGACACTGCTGCAGCTGGTGGTGTCCGTGCCCTTC
```

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

A__V__A__Y__G__V__R__D__W__T__L__L__Q__L__V__V__S__V__P__F__

TTCCTCTGCTTTTTGTACTCCTGGTGGCTGGCAGAGTCGGCACGATGGCTCCTCACCACA

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

TTCCTGTGCTTCCTGTACAGCTGGTGGCTCGCTGAGAGCGCCCGGTGGCTGCTGACCACA

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

F__L__C__F__L__Y__S__W__W__L__A__E__S__A__R__W__L__L__T__T__

GGCAGGCTGGATTGGGGCCTGCAGGAGCTGTGGAGGGTGGCTGCCATCAACGGAAAGGGG

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

GGCAGACTGGACTGGGGCCTGCAGGAACTGTGGCGGGTCGCCGCCATCAATGGCAAGGGC

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

G__R__L__D__W__G__L__Q__E__L__W__R__V__A__A__I__N__G__K__G__

GCAGTGCAGGACACCCTGACCCCTGAGGTCTTGCTTTCAGCCATGCGGGAGGAGCTGAGC

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

GCCGTGCAGGACACCCTGACCCCTGAGGTGCTGCTGAGCGCCATGCGCGAGGAACTGAGC

```
---------+---------+---------+---------+---------+---------+1020
```

A__V__Q__D__T__L__T__P__E__V__L__L__S__A__M__R__E__E__L__S__

```
ATGGGCCAGCCTCCTGCCAGCCTGGGCACCCTGCTCCGCATGCCCGGACTGCGCTTCCGG
|||||||||||||| |||||||||||||| |||||  | |||||||| ||||| ||||||
ATGGGCCAGCCTCCAGCCAGCCTGGGCACACTGCTGAGAATGCCCGGCCTGCGGTTCCGG
---------+---------+---------+---------+---------+---------+1080
```

M__G__Q__P__P__A__S__L__G__T__L__L__R__M__P__G__L__R__F__R__

```
ACCTGTATCTCCACGTTGTGCTGGTTCGCCTTTGGCTTCACCTTCTTCGGCCTGGCCCTG
|||||  |||   |||   ||||  ||||||||||  |||||||||||||||||||||||||
ACCTGCATCAGCACCCTGTGTTGGTTCGCCTTCGGCTTCACCTTCTTCGGCCTGGCCCTG
---------+---------+---------+---------+---------+---------+1140
```

T__C__I__S__T__L__C__W__F__A__F__G__F__T__F__F__G__L__A__L__

```
GACCTGCAGGCCCTGGGCAGCAACATCTTCCTGCTCCAAATGTTCATTGGTGTCGTGGAC
|||||  |||||||||||||||||||||||||||  ||  ||||||||  ||  ||  ||||||
GACCTCCAGGCCCTGGGCAGCAACATCTTCCTGCTGCAGATGTTCATCGGCGTGGTGGAC
```

```
---------+---------+---------+---------+---------+---------+1200
```

D__L__Q__A__L__G__S__N__I__F__L__L__Q__M__F__I__G__V__V__D__

```
ATCCCAGCCAAGATGGGCGCCCTGCTGCTGCTGAGCCACCTGGGCCGCCGCCCCACGCTG
|||||  ||||||||||||||||||||||||||   |||||||||  |  | ||  ||  |||
ATCCCCGCCAAGATGGGCGCCCTGCTGCTGCTGTCTCACCTGGGCAGAAGGCCTACCCTG
---------+---------+---------+---------+---------+---------+1260
```

I__P__A__K__M__G__A__L__L__L__L__S__H__L__G__R__R__P__T__L__

```
GCCGCATCCCTGTTGCTGGCAGGGCTCTGCATTCTGGCCAACACGCTGGTGCCCCACGAA
|||||  ||  |||  |||||||  ||  ||  |||||  ||||||||||  ||||||||||||||
GCCGCCTCTCTGCTGCTGGCCGGACTGTGCATCCTGGCCAACACCCTGGTGCCCCACGAG
---------+---------+---------+---------+---------+---------+1320
```

A__A__S__L__L__L__A__G__L__C__I__L__A__N__T__L__V__P__H__E__

```
ATGGGGGCTCTGCGCTCAGCCTTGGCCGTGCTGGGGCTGGGCGGGGTGGGGGCTGCCTTC
|||||  ||  |||  |  ||  |||  |||||||  |||||  |||||  ||  |||||  |||||||||
ATGGGAGCCCTGAGATCTGCCCTGGCCGTCCTGGGACTGGGAGGCGTGGGAGCTGCCTTC
```

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

M__G__A__L__R__S__A__L__A__V__L__G__L__G__G__V__G__A__A__F__

ACCTGCATCACCATCTACAGCAGCGAGCTCTTCCCCACTGTGCTCAGGATGACGGCAGTG

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

ACCTGTATCACCATCTACAGCAGCGAGCTGTTCCCCACCGTGCTGCGGATGACAGCCGTG

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

T__C__I__T__I__Y__S__S__E__L__F__P__T__V__L__R__M__T__A__V__

GGCTTGGGCCAGATGGCAGCCCGTGGAGGAGCCATCCTGGGGCCTCTGGTCCGGCTGCTG

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

GGCCTGGGACAGATGGCCGCCAGAGGCGGAGCCATCCTGGGACCTCTGGTGCGCCTGCTG

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

G__L__G__Q__M__A__A__R__G__G__A__I__L__G__P__L__V__R__L__L__

GGTGTCCATGGCCCCTGGCTGCCCTTGCTGGTGTATGGGACGGTGCCAGTGCTGAGTGGC

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

GGAGTGCACGGACCTTGGCTCCCTCTGCTGGTGTACGGCACCGTGCCTGTGCTGTCTGGA

```
---------+---------+---------+---------+---------+---------+1560
```

G__V__H__G__P__W__L__P__L__L__V__Y__G__T__V__P__V__L__S__G__

```
CTGGCCGCACTGCTTCTGCCCGAGACCCAGAGCTTGCCGCTGCCCGACACCATCCAAGAT
|||||  ||  |||||  ||||||||||| ||||||  ||||  |||||||||||||||||  ||
CTGGCTGCTCTGCTGCTGCCCGAGACACAGAGCCTGCCCCTGCCCGACACCATCCAGGAC
---------+---------+---------+---------+---------+---------+1620
```

L__A__A__L__L__L__P__E__T__Q__S__L__P__L__P__D__T__I__Q__D__

```
GTGCAGAACCAGGCAGTAAAGAAGGCAACACATGGCACGCTGGGGAACTCTGTCCTAAAA
||||||||||||||  ||  ||||||||  ||  ||  |||||  |||||  |||    ||  ||  ||
GTGCAGAACCAGGCCGTGAAGAAGGCCACCCACGGCACCCTGGGCAACAGCGTGCTGAAG
---------+---------+---------+---------+---------+---------+1680
```

V__Q__N__Q__A__V__K__K__A__T__H__G__T__L__G__N__S__V__L__K__

```
TCCACACAGTTC
|||||  ||||||
TCCACCCAGTTCATGGTGTCCAAGGGGGAGGAACTGTTTACCGGCGTGGTGCCCATCCTG
```

---------+---------+---------+---------+---------+---------+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

GTGGAACTGGACGGCGACGTGAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAAGGC

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

V__E__L__D__G__D__V__N__G__H__K__F__S__V__S__G__E__G__E__G__

GACGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTG

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

D__A__T__Y__G__K__L__T__L__K__F__I__C__T__T__G__K__L__P__V__

CCTTGGCCCACCCTGGTGACAACCTTCACCTACGGCGTGCAGTGCTTCGCCAGATACCCC

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

P__W__P__T__L__V__T__T__F__T__Y__G__V__Q__C__F__A__R__Y__P__

GACCACATGAAGCAGCACGATTTCTTCAAGTCCGCCATGCCCGAGGGCTACGTGCAGGAA

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

D__H__M__K__Q__H__D__F__F__K__S__A__M__P__E__G__Y__V__Q__E__

CGGACCATCTTCTTCAAGGACGACGGCAACTACAAGACCAGAGCCGAAGTGAAGTTCGAG

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

R__T__I__F__F__K__D__D__G__N__Y__K__T__R__A__E__V__K__F__E__

GGCGATACCCTGGTGAACCGGATCGAGCTGAAGGGCATCGACTTCAAAGAGGACGGCAAT

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

G__D__T__L__V__N__R__I__E__L__K__G__I__D__F__K__E__D__G__N__

ATCCTGGGCCACAAGCTGGAGTACAACTACAACAGCCACAAGGTGTACATCACCGCCGAC

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

I__L__G__H__K__L__E__Y__N__Y__N__S__H__K__V__Y__I__T__A__D__

AAGCAGAAAAACGGCATCAAAGTGAACTTCAAGACCCGGCACAACATCGAGGACGGAAGC

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

K__Q__K__N__G__I__K__V__N__F__K__T__R__H__N__I__E__D__G__S__

GTGCAGCTGGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTG

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

V__Q__L__A__D__H__Y__Q__Q__N__T__P__I__G__D__G__P__V__L__L__

CCTGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAGGACCCCAACGAGAAGCGG

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

P__D__N__H__Y__L__S__T__Q__S__A__L__S__K__D__P__N__E__K__R__

GACCACATGGTGCTGCTGGAATTCGTGACCGCCGCTGGCATCACACTGGGCATGGACGAG

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

D__H__M__V__L__L__E__F__V__T__A__A__G__I__T__L__G__M__D__E__

attB2-3'

CTGTACAAGTACCCAGCTTTCTTGTACAAAGTGGTTGATATCCAGCACAGTGGCGGCCGC

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

L__Y__K__I__P__A__F__L__Y__K__V__V__N__I__Q__H__S__G__G__R__

End of EGFP

TCGAGTCTAGAGGGCCCGCGGTTCGAAGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTC

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

S__S__L__E__G__P__R__F__E__G__K__P__I__P__N__P__L__L__G__L__

V5 EPITOPE

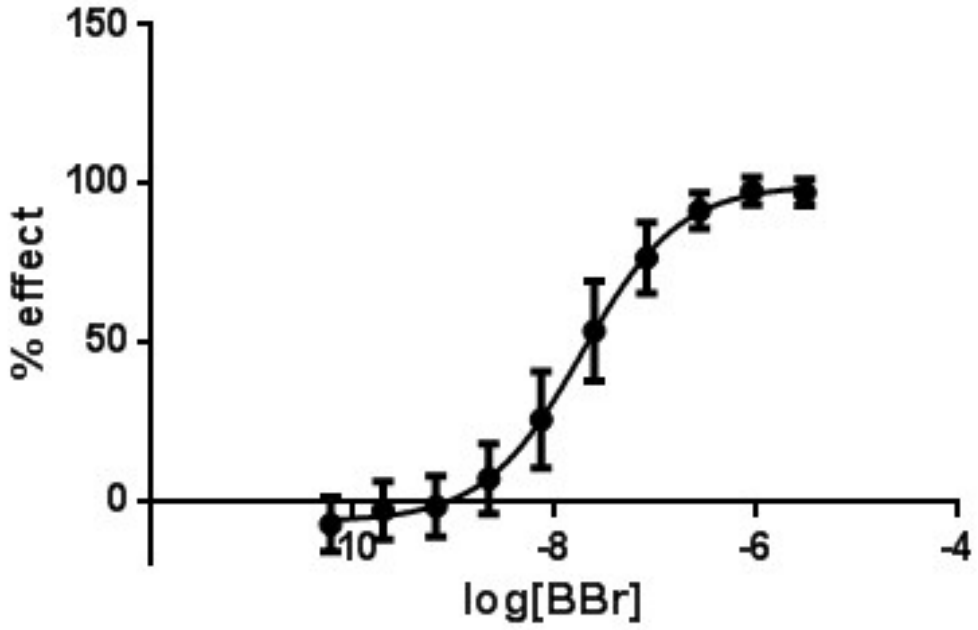
GATTCTACGCGTACCGGTTAGTAATGA

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

D__S__T__R__T__G__*__*__*__

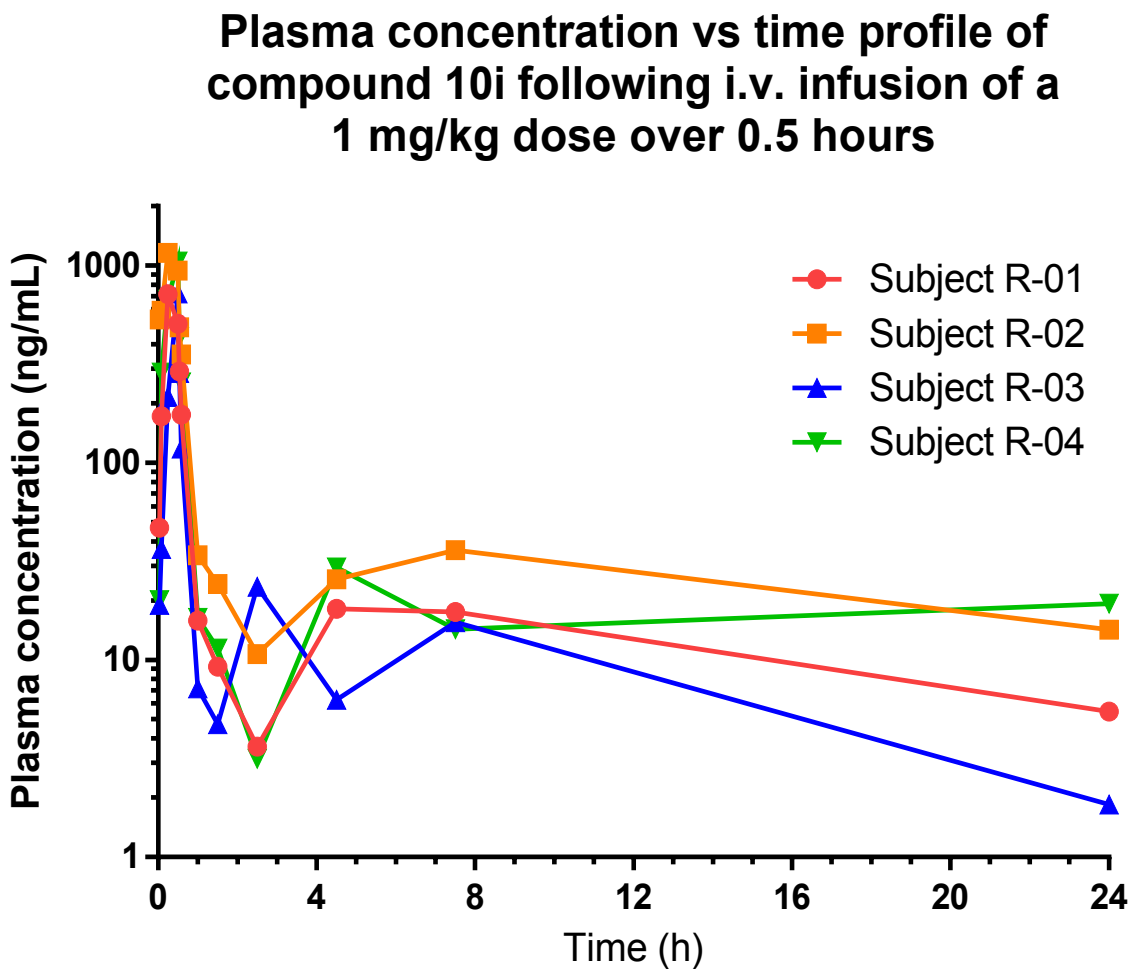
STOP

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.

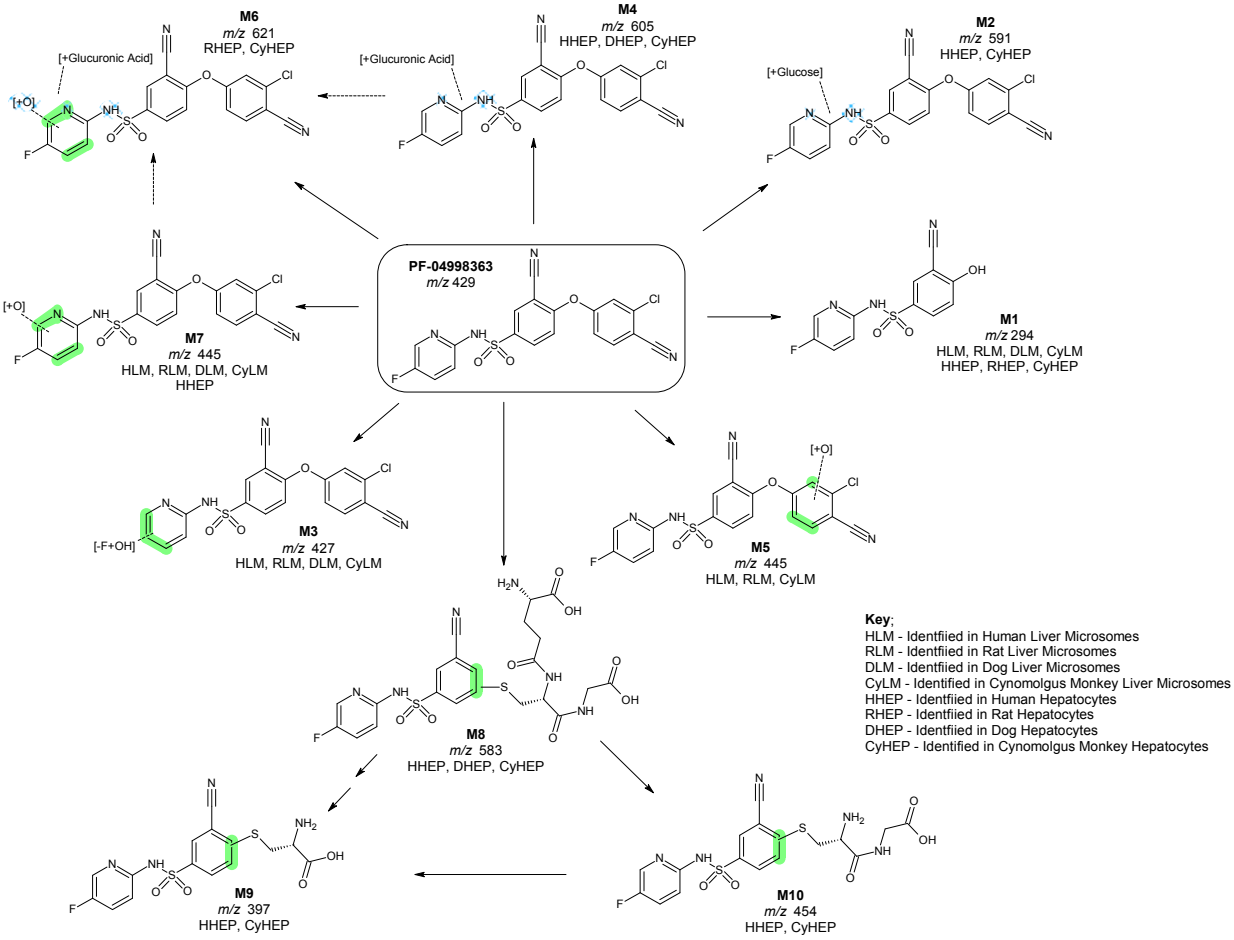


7. Pharmacokinetic profile of compound 10i in the rat

All experiments involving animals were conducted in our AAALAC-accredited facilities and were reviewed and approved by the Pfizer Institutional Animal Care and Use Committee.



8. Proposed Metabolites of compound 10f Identified in Human, Rat, Dog and Cynomolgus Monkey Liver Microsomes and Hepatocytes



Metabolites of 10f in Human, Rat, Dog and Cynomolgus Monkey Liver Microsomes

M#	*m/z*	Abundance compared to unchanged drug (%; MS response)			
		Human	Rat	Dog	Cynomolgus Monkey
10f	429	100	100	100	100
M1	294	2.0	0.4	<0.1	0.8
M2	591	ND	ND	ND	ND
M3	427	0.9	0.6	0.2	2
M4	605	ND	ND	ND	ND

M5	445	10.2	<0.1	ND	5
M6	621	ND	ND	ND	ND
M7	445	3.6	3.2	2.1	10
M8	583	ND	ND	ND	ND
M9	397	ND	ND	ND	ND
M10	454	ND	ND	ND	ND

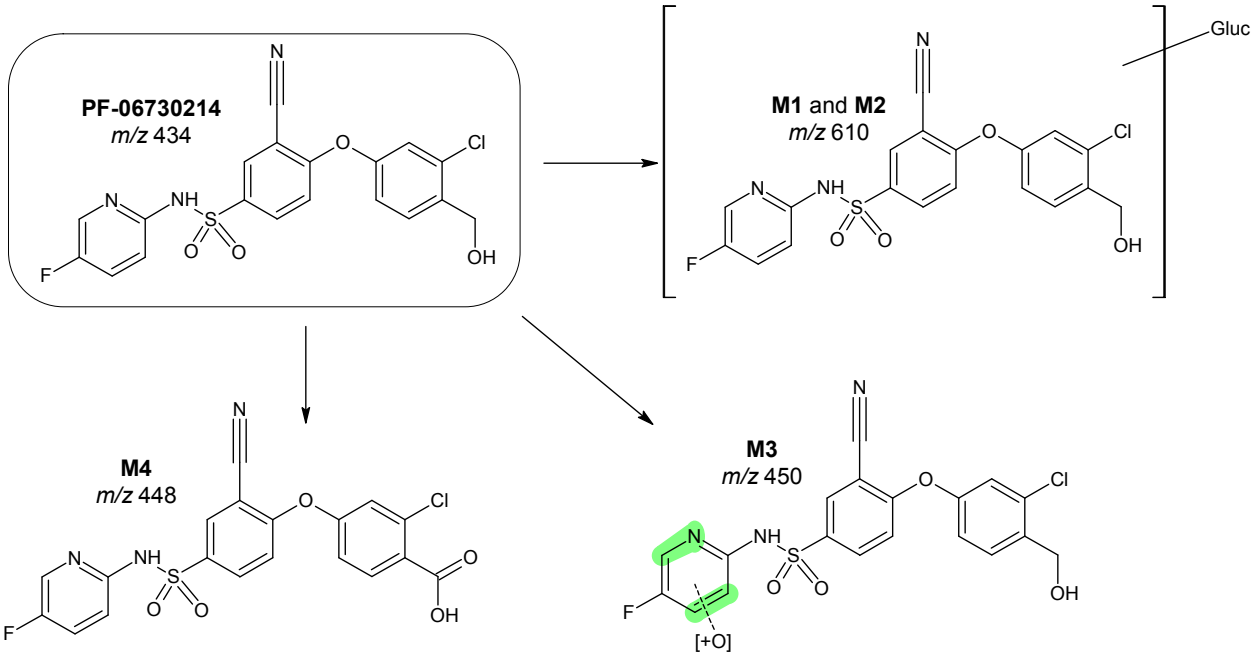
Not Detected (ND)

Metabolites of 10f Identified in Human, Rat, Dog and Cynomolgus Monkey Hepatocytes

M#	*m/z*	Abundance compared to unchanged drug (%; MS response)			
		Human	Rat	Dog	Cynomolgus Monkey
10f	429	100	100	100	100
M1	294	0.7	0.1	ND	0.6
M2	591	0.2	ND	ND	0.7
M3	427	ND	ND	ND	ND
M4	605	1	ND	0.2	0.4
M5	445	ND	ND	ND	ND
M6	621	ND	1.3	ND	0.4
M7	445	<0.1	ND	ND	ND
M8	583	2.7	ND	0.1	19
M9	397	0.9	ND	ND	5
M10	454	0.4	ND	ND	2

Not Detected (ND)

9. Proposed Metabolites of compound 10i Identified in Human, Rat, Dog and Cynomolgus Monkey Liver Microsomes and Hepatocytes



Metabolites of 10i Identified in Human, Rat, Dog and Cynomolgus Monkey Liver Microsomes

M#	*m/z*	Human	Rat	Dog	Cynomolgus Monkey
10i	434	+++	+++	+++	+++
M1	610	ND	ND	ND	ND
M2	610	ND	ND	ND	ND
M3	450	t	t	t	+
M4	448	+	+	t	t

+++	Observed as a major peak in the UV profile
++	Observed as a minor peak in the UV profile
+	Observed as a trace peak in the UV profile
t	Detected only by mass spectrometry
ND	Not Detected

Metabolites of 10i Identified in Human, Rat, Dog and Cynomolgus Monkey Hepatocytes

	M#	*m/z*	Human	Rat	Dog	Cynomolgus Monkey
10i		434	+++	+++	+++	+++
M1		610	t	ND	ND	+
M2		610	t	+++	ND	++
M3		450	ND	ND	ND	ND
M4		448	++	+	t	++

+++	Observed as a major peak in the UV profile
++	Observed as a minor peak in the UV profile
+	Observed as a trace peak in the UV profile
t	Detected only by mass spectrometry
ND	Not Detected