Supporting information

Medicinal Chemistry Optimization of Antiplasmodial Imidazopyridazine Hits from High Throughput Screening of a SoftFocus Kinase Library: Part 2.

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A. Additional synthetic methods

1) Preparation of compound 26

1.1. Step 1:

N-Boc-piperazine (3g, 18 mmol, 5 eq) was added to a solution of 4-bromobenzene sulfonyl chloride (1g, 3.9 mmol, 1 eq) in THF (5 mL) at 0°C. The resulting mixture was stirred at RT overnight. Water was added and the compound was extracted in DCM. The organic phase was washed with saturated aqueous NaHCO₃ and dried over Na₂SO₄. The solvent was removed to afford the desired compound in quantitative yield.

¹H NMR (400 MHz, CDCl₃), δ (ppm): 7.68 (d, 1H; J = 8.8); 7.61 (d, 2H; J = 8.8); 3.53, 3.42 (m, 4H); 2.99, 2.92 (m, 4H); 1.41 (s, 9H)

1.2. Step 2:

The bromide from step 1.1 (1.58g, 3.9 mmol, 1 eq) was dissolved in DMF (3 mL) with bis-pinacolato diboron (1g, 4.29 mmol, 1.1 eq) and KOAc (1.1 g, 11.7 mmol, 3 eq). The resulting mixture was flushed under N₂ for 30 min. PdCl₂ (dppf) was then added and the mixture was heated to 60°C and stirred overnight at this temperature. DMF was removed and the residue was dissolved in EtOAc. After filtration on celite, EtOAc was evaporated

and the compound was purified on silica gel using Petroleum Ether and EtOAc (1:1). The boronic ester was obtained in 64% yield.

^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.69 (d, 1H; J = 8.8); 7.61 (d, 2H; J = 8.8); 3.53, 3.42 (m, 4H); 3.00, 2.93 (m, 4H); 1.41 (s, 9H); 1.26 (s, 12H)

1.3. Step 3:

The boronic ester from step 1.2 (130 mg, 0.284 mmol, 1.1 eq) was dissolved in DMF (2 mL) with compound **5-a** (90 mg, 0.258 mmol, 1 eq) and the palladium source. After 15 min flushing with N_2 , 270 μL of aqueous 1M K_2CO_3 solution was added. The resulting mixture was stirred overnight at 90°C . DCM and water were then added and the product was extracted in DCM. After evaporation of the solvent, purification on silica gel (DCM/MeOH 98:2, 95:5) gave the desired compound in 50% yield (77 mg).

^1H NMR (400 MHz, CDCl_3), δ (ppm): 8.54 (s, 1H); 8.36, 8.33 (m, 3H); 8.23 (s, 1H); 8.19 (d, 1H; J = 9.6); 8.10 (d, 1H; J = 8.0); 7.89 (d, 2H; J = 8.8); 7.80 (t, 1H; J = 8.0); 7.66 (d, 1H; J = 9.6); 3.54, 3.51 (m, 4H); 3.13 (s, 3H); 3.08, 3.03 (m, 4H); 1.38 (s, 9H)

1.4. Step 4:

Compound from step 1.3 (77 mg, 0.129 mmol, 1eq) was dissolved in DCM. HCl (4M in dioxane) (322 μ L, 10 eq) was added at RT. The solution was stirred overnight at RT. After addition of water, the solution was extracted in DCM. The solvent was removed. The residue was then dissolved in a mixture of DCM/MeOH (1:1) and stirred with Amberlyst A21 for 30 min. The resin was filtered off and rinsed with MeOH and the filtrate was concentrated. Purification on silica gel afforded compound **26** in 30% yield (20 mg).

^1H NMR (400 MHz, CDCl_3), δ (ppm): 8.55 (s, 1H); 8.40, 8.29 (m, 3H); 8.22 (s, 1H); 8.19 (d, 1H; $J = 9.6$); 8.10 (d, 1H; $J = 7.2$); 7.90 (d, 2H; $J = 8.8$); 7.79 (t, 1H; $J = 8.0$); 7.66 (d, 1H; $J = 9.6$); 3.14 (s, 3H); 3.11, 3.01 (m, 4H); 3.00, 2.89 (m, 4H); MS (EI⁺): $m/z = 496.9$ (exact Mass = 497.1191)

2) Preparation of compound 34

2.1. Step 1:

EDCI.HCl (5.2 g, 27.5 mmol, 2.5 eq) was added in small batches to a solution of 4-iodo benzoic acid (3 g, 12.1 mmol, 1.1 eq) and DMAP (3.3 g, 27.5 mmol, 2.5 eq) in DCM (30 mL). The resulting mixture was stirred for 10 min at which time the Boc-piperazine was added. The solution was stirred overnight at RT then washed with saturated aqueous NH_4Cl and NaHCO_3 . After evaporation of the solvent, purification on silica gel (DCM/MeOH 98:2) afforded 3.7 g of the desired product (74%).

^1H NMR (300 MHz, CDCl_3), δ (ppm): 7.70 (d, 2H; $J = 8.8$); 7.09 (d, 3H; $J = 8.8$); 3.70, 3.10 (br m, 8H); 1.42 (s, 9H)

2.2. Step 2:

Iodo from step 2.1 (200 mg, 0.480 mmol, 1 eq) was dissolved in DMF (2 mL) with bis-pinacolato diboron (134 mg, 0.528 mmol, 1.1 eq) and KOAc (141 mg, 1.44 mmol, 3 eq). The resulting mixture was flushed with N_2 for 30 min, at which time $\text{PdCl}_2(\text{dppf})$ (20 mg, 0.024 mmol, 0.05 eq) was added. The resulting solution was heated to 60°C for 24h. DMF was removed *in vacuo*. The residue was dissolved in EtOAc and filtered on celite. The solvent was removed and the residue was used as is in the next step.

2.3. Step 3

6-chloro-3-iodo-imidazopyridazine (89 mg, 0.320 mmol, 1 eq), boronic ester from step 2.2 and diphenylphosphine palladium dichloride (11 mg, 0.016 mmol, 0.05 eq) were dissolved in DMF (2 mL) and flushed with N_2 for 15 min. 340 μL of 1M aqueous solution of potassium carbonate was then added. The resulting mixture was stirred at 80°C overnight. It was then dissolved in DCM and water and extracted. The organic layer was concentrated and the compound was purified on silica gel (DCM/MeOH 98:2) to afford the desired compound in 56% yield (53 mg).

2.4. Step 4

Compound from step 2.3 (53 mg, 0.180 mmol, 1 eq), 3-methylsulfonyl phenylboronic acid (29 mg, 0.144 mmol, 1.2 eq) and diphenylphosphine palladium dichloride (4 mg, 0.006 mmol, 0.05 eq) were dissolved in DMF (1 mL) and flushed with N₂ for 15 min. 130 μ L of 1M aqueous solution of potassium carbonate was then added. The resulting mixture was stirred at 90°C overnight. It was then dissolved in DCM and water and extracted. The organic layer was concentrated and the compound was purified on silica gel (DCM/MeOH 98:2) to afford the desired compound in 40% yield (40 mg).

¹H NMR (400 MHz, CDCl₃), δ (ppm): 8.57 (s, 1H); 8.34 (d, 1H; J = 8.4); 8.24, 8.14 (m, 4H); 8.09 (d, 1H; J = 8.0); 7.78 (dd, 1H; J = 8.0, J = 7.6); 7.63 (d, 1H; J = 9.6); 7.59 (d, 2H; J = 8.0); 3.88, 3.41 (br signal, 8H); 3.13 (s, 3H); 1.48 (s, 9H)

2.5. Step 5

A solution of compound from step 2.4 (40 mg, 0.071 mmol, 1 eq) and DCM (4M in dioxane) (177 μ L, 10 eq) in DCM was stirred at RT for 12 h. The solvents were removed and the residue was dissolved in DCM/MeOH (1:1). Amberlyst A21 resin was added and the solution was stirred for 30 min. The resin was filtered off, the filtrate was concentrated and the final compound was purified on silica gel (DCM/MeOH 95:5, 90:10) in 34% yield.

^1H NMR (300 MHz, $\text{dms}\text{-d}_6$), δ (ppm): 8.63 (t, 1H; $J = 1.8$); 8.49 (dd, 1H; $J = 0.9$, $J = 8.7$); 8.39 (s, 1H); 8.38 (d, 1H; $J = 9.6$); 8.32 (d, 2H; $J = 8.4$); 8.09 (dd, 1H; $J = 0.9$, $J = 8.7$); 8.03 (d, 1H; $J = 9.6$); 7.88 (t, 1H; $J = 7.8$); 7.56 (d, 2H; $J = 8.4$); 3.68, 3.41 (m, 4H); 3.32 (s, 3H); 2.86, 2.62 (m, 4H); MS (EI $^+$): $m/z = 461.2$ (exact Mass = 461.1522)

3) Preparation of *tert*-butyl (3-((3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)propyl)carbamate

Step 3.1: 3-Bromobenzenethiol (0.86 mL, 8.33 mmol, 1 eq) was dissolved in DMF and NaH (60% in oil) (367 mg, 9.17 mmol, 1.1 eq) was added in portions at RT. The mixture was stirred at RT for 5 min and Boc-bromopropylamine (2.38 g, 10 mmol, 1.2 eq) was slowly added. The resulting mixture was stirred at RT for 4 days. 50 mL of water were then added and the solution was extracted with EtOAc (3x30 mL). After the solvents were removed, the bromo-thioester was purified on silica gel with 10 to 20 % EtOAc in Hexanes in 68% yield.

^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.41 (t, 1H; $J = 1.8$); 7.25 (ddd, 1H; $J = 7.8$, 1.8, 1.1); 7.19 (ddd, 1H; $J = 7.8$, 1.8, 1.1); 7.09 (t, 1H; $J = 7.8$); 4.73 (br s, 1H); 3.20 (m, 2H); 2.91 (t, 2H; $J = 7.2$); 1.79 (m, 2H); 1.41 (s, 9H)

Step 3.2: The thioester from step 3.1 (1.96 mg, 5.66 mmol, 1 eq) was dissolved in DCM (15 mL) at 0°C. mCPBA (1.95 mg, 11.32 mmol, 2 eq) was added in portions. The resulting mixture was stirred at RT for 3 h and filtered. The filtrate was washed with

NaHCO₃, water and brine. It was dried over Na₂SO₄ and concentrated to give a white solid. The product was washed with hexane and dried off. 1.44 g were obtained (67%).

¹H NMR (400 MHz, CDCl₃), δ (ppm): 8.05 (t, 1H; J = 1.8); 7.84 (ddd, 1H; J = 7.8, 1.8, 1.1); 7.69 (ddd, 1H; J = 7.8, 1.8, 1.1); 7.45 (t, 1H; J = 7.8); 4.64 (br s, 1H); 3.24 (q, 2H; J = 6.3); 3.14 (m, 2H); 1.93 (m, 2H); 1.42 (s, 9H)

Step 3.3: The boronic ester was formed following the procedure described in step 2.2.

4) Preparation of *tert*-butyl (3-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)propyl)carbamate

The same procedure as for boronic ester *tert*-butyl (3-((3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)propyl)carbamate was followed, using 4-bromobenzenethiol as starting material.

Step 4.1: ¹H NMR (400 MHz, CDCl₃), δ (ppm): 7.39 (d, 2H; J = 8.7); 7.19 (d, 2H; J = 8.7); 4.59 (br s, 1H); 3.23 (q, 2H; J = 6.6); 2.91 (t, 2H; J = 7.2); 1.80 (m, 2H); 1.44 (s, 9H)

Step 4.2: ¹H NMR (400 MHz, CDCl₃), δ (ppm): 8.02 (d, 2H; J = 8.6); 7.79 (d, 2H; J = 8.6); 4.67 (br s, 1H); 3.26 (q, 2H; J = 6.6); 3.19 (m, 2H); 1.97 (m, 2H); 1.42 (s, 9H)

5) Boc-deprotection procedure for thio-ester derivatives:

Compounds **22** and **36** were obtained from the Boc-protected intermediates using a 20% solution of TFA/DCM (v:v). The resulting mixtures were stirred on ice for 1h, concentrated, dissolved in MeOH/Et₂O and filtered off to give the desired compounds in 79% (**22**) and 99% (**36**) yield. Analytical data are described in the table below.

B. Additional spectrometric data (¹H NMR and Mass):

1) Using Pyridine rings to improve solubility

Compound	Structure	NMR ¹ H δ (ppm)	MS (EI) ⁺ m/z [*]
6		(300 MHz, CDCl ₃), 9.55 (d, 1H; J = 2.4); 9.30 (d, 1H; J = 2.1); 8.78 (dd, 1H; J = 2.1, J = 2.4); 8.35 (d, 2H; J = 8.4); 8.30 (s, 1H); 8.27 (d, 1H; J = 9.6); 8.12 (d, 1H; J = 8.4); 7.66 (d, 1H; J = 9.6); 3.22 (s, 3H); 3.14 (s, 3H)	428.1
7		(300 MHz, CDCl ₃), 9.47 (d, 1H; J = 2.1); 9.06 (s, 1H); 8.52-8.50 (m, 1H); 8.37 (d, 2H; J = 8.4); 8.29(s, 1H); 8.25 (d, 1H, J = 9.3); 8.11 (d, 2H, J = 8.4); 7.65 (d, 1H, J = 9.3); 3.14 (s, 3H)	418.1
8		(300 MHz, CDCl ₃), 8.97 (d, 1H; J = 5.1); 8.36 (d, 2H; J = 8.4); 8.30-8.25 (m, 3H); 8.14-8.11 (m, 3H); 7.68 (d, 1H, J = 9.3); 3.14 (s, 3H)	418.1
9		(400 MHz, dmsO-d ₆), 8.56 (d, 2H; J = 7.2); 8.50 (d, 2H; J = 6.8); 8.35 (d, 1H; J = 8.4); 8.14, 8.06 (m, 3H); 7.90 (d, 1H; J = 9.2); 7.64 (s, 1H); 5.64 (s, 1H); 3.28 (s, 3H)	365.1
10		(300 MHz, CDCl ₃), 9.53 (d, 1H; J = 1.8); 8.75 (dd, 1H; J = 1.8; J = 8.4); 8.51 (d, 1H); 8.29 (d, 1H; J = 8.7); 8.27 (s, 1H); 8.26, 8.20 (m, 2H); 8.12 (d, 1H; J = 7.2); 7.79 (d, 1H; J = 7.8); 7.71 (d, 1H; J = 9.3); 3.30 (s, 3H); 3.15 (s, 3H)	428.0
11		(400 MHz, CDCl ₃), 9.47 (s, 1H); 9.43 (s, 1H); 9.14 (s, 1H); 8.66 (s, 1H); 8.37 (s, 1H); 8.33 (d, 1H; J = 7.6); 8.28 (d, 1H; J = 9.2); 8.14 (s, 1H);	428.0

		J = 8.0); 7.82 (dd, 1H; J = 7.6, J = 8.0); 7.76 (d, 1H; J = 9.2); 3.27 (s, 3H); 3.22 (s, 3H)	
12		(400 MHz, dms _o -d ₆), 8.77 (d, 1H; J = 2.8); 8.60 (t, 1H; J = 1.8); 8.44 (dd, 1H; J = 2.0, 8.0); 8.32 (d, 1H; J = 9.6); 8.16, 8.09 (m, 1H); 8.11 (s, 1H); 7.93 (d, 1H; J = 8.4); 7.91 (d, 1H; J = 9.6); 7.88 (t, 1H; J = 8.0); 6.63 (d, 1H); 6.25 (s, 2H); 3.33 (s, 3H)	364.9
13		(400 MHz, CDCl ₃), 9.51 (s, 1H); 9.44 (s, 1H); 9.27 (s, 1H); 8.77, 8.70 (m, 2H); 8.32 (s, 1H); 8.29 (d, 1H; J = 9.6); 8.23 (d, 2H; J = 8.4); 7.73 (d, 1H; J = 9.6); 3.28 (s, 3H); 3.21 (s, 3H)	429.1
14		(400 MHz, CDCl ₃), 9.49 (d, 1H; J = 2.0); 9.46, 9.44 (m, 2H); 9.32 (d, 1H; J = 2.0); 9.15 (d, 1H; J = 2.0); 8.94 (d, 1H; J = 2.0); 8.37 (s, 1H); 8.41 (s, 1H); 8.33 (d, 1H; J = 9.2); 7.78 (d, 1H; J = 9.2); 3.31 (s, 3H); 3.26 (s, 3H)	428.9
15		(400 MHz, CDCl ₃), 9.57 (d, 1H; J = 2.0); 9.32 (d, 1H; J = 2.0); 8.79 (t, 1H; J = 2.0); 8.43 (d, 1H; J = 9.2); 8.34, 8.30 (m, 3H); 8.09 (d, 2H; J = 8.8); 7.78 (d, 1H; J = 9.2); 3.23 (s, 3H); 2.59, 2.51 (m, 1H); 1.46, 1.40 (m, 2H); 1.15, 1.06 (m, 2H)	453.7

2) Using water-solubilizing groups to improve solubility:

Compound	Structure	NMR ¹ H δ (ppm)	MS (EI) ⁺ m/z
17		(300 MHz, CDCl ₃), 8.46 (t, 1H; J = 1.8); 8.40 (d, 2H; J = 8.7); 8.17 (dd, 1H; J = 1.8, J = 6.0); 8.16 (s, 1H); 8.13 (d, 1H;	436.0

		J = 9.6); 8.07 (d, 2H; J = 8.7); 7.96 (dd, 1H; J = 1.2, J = 7.8); 7.79 (d, 1H; J = 9.7); 7.62 (t, 1H; J = 7.8); 3.75 (t, 2H; J = 6.0); 3.56 (t, 2H; J = 6.0); 3.12 (s, 3H)	
18		(300 MHz, dms _o -d ₆), 8.59, 8.55 (m, 3H); 8.50 (s, 1H); 8.39 (d, 1H; J = 9.6); 8.33 (dd, 1H; J = 1.8, J = 7.8); 8.10 (d, 2H; J = 8.7); 8.10, 8.05 (m, 1H); 8.03 (d, 1H; J = 9.6); 7.70 (t, 1H; J = 7.8); 3.28 (s, 3H)	400.5
19		(300 MHz, dms _o -d ₆), 8.61 (t, 1H; J = 2.0); 8.56, 8.50 (m, 4H); 8.43 (d, 1H; J = 9.6); 8.11 (d, 2H; J = 8.8); 8.07, 8.04 (m, 2H); 7.83 (dd, 1H; J = 7.6, J = 8.4); 3.29 (s, 3H)	374.0
20		(400 MHz, CDCl ₃), 8.78 (s, 1H); 8.58 (d, 2H; J = 8.4); 8.55 (s, 1H); 8.45 (d, 2H; J = 9.6); 8.38 (d, 1H; J = 8.0); 8.24 (d, 1H; J = 8.0); 8.10 (d, 2H; J = 8.4); 8.04 (d, 1H; J = 9.6); 7.85 (t, 1H; J = 8.0)	(ESI) ⁻ : 416.0 (M-H)
21		(400 MHz, dms _o -d ₆), 8.61 (s, 1H); 8.59, 8.54 (m, 4H); 8.52 (d, 1H; J = 7.8); 8.44 (d, 1H; J = 9.6); 8.11, 8.07 (m, 4H); 7.89 (t, 1H; J = 7.8); 4.92 (s, 1H); 3.77 (t, 2H; J = 6.4); 3.60 (t, 2H; J = 6.4); 3.35 (s, 3H)	456.9
24		(400 MHz, CDCl ₃), 8.55 (d, 3H; J = 8.4); 8.50 (s, 1H); 8.38 (d, 1H; J = 9.6); 8.34 (d, 1H; J = 1.6); 8.30 (d, 1H; J = 8.0); 8.10 (d, 2H; J = 8.4); 8.04 (d, 1H; J = 9.6); 7.82 (d, 1H; J = 8.0); 7.70 (t, 1H; J = 8.0); 5.81 (d, 1H; J = 7.6); 4.62, 4.50 (br s, 2H); 4.38, 4.26 (br s, 1H); 4.23, 4.12 (br s, 1H); 3.92, 3.78 (br s,	448.1

		1H); 3.19 (s, 3H)	
25		(400 MHz, CDCl ₃), 8.66 (s, 1H); 8.55, 8.49 (m, 3H); 8.44 (d, 1H); 8.17, 8.06 (m, 2H); 8.00, 7.87 (m, 3H); 7.72, 7.64 (m, 1H); 3.45, 3.40 (m, 2H); 3.26 (s, 3H); 2.93, 2.83 (m, 2H)	(ESI) ⁺ : 473.1 (M+H) ⁺ : 495.0 (M+Na) ⁺
27		(300 MHz, CD ₃ OD/ CDCl ₃), 8.66 (t, 1H; J = 1.8); 8.43 (dd, 1H; J = 1.8, J = 8.1); 8.37 (d, 2H; J = 8.7); 8.25 (s, 1H); 8.23 (d, 1H; J = 9.6); 8.12 (m, 1H; J = 7.8); 8.07 (d, 2H; J = 8.7); 7.91 (d, 1H; J = 9.6); 7.84 (t, 1H; J = 8.0); 3.20 (s, 3H)	428.0
28		(400 MHz, CDCl ₃ / CD ₃ OD), 8.63 (t, 1H; J = 1.8); 8.38 (dd, 1H; J = 0.9, J = 8.1); 8.27 (d, 2H; J = 8.4); 8.19 (d, 1H; J = 9.6); 8.18 (s, 1H); 8.09 (dd, 1H; J = 0.9, J = 8.1); 8.03 (d, 2H; J = 8.4); 7.84, 7.79 (m, 2H); 3.18 (s, 3H)	392.0
29		(400 MHz, dmso-d ₆), 9.03 (s, 2H); 8.58 (s, 1H); 8.44 (d, 1H; J = 7.6); 8.34 (d, 1H; J = 9.6); 8.24 (s, 1H); 8.10 (d, 1H; J = 7.2); 7.95 (d, 1H; J = 9.6); 7.88 (t, 1H; J = 7.6); 6.96 (s, 2H); 3.32 (s, 3H)	365.9
30		(400 MHz, dmso-d ₆), 8.67 (t, 1H; J = 1.6); 8.12 (dd, 1H; J = 1.6, J = 8.4); 8.44 (s, 1H); 8.42 (d, 2H; J = 8.8); 8.40 (d, 1H; J = 9.6); 8.19 (d, 2H; J = 8.8); 8.12 (t, 1H; J = 8.8); 8.04 (d, 1H; J = 9.6); 7.90 (t, 1H; J = 7.6); 3.36 (s, 3H)	417.0
31		(400 MHz, dmso-d ₆), 9.09 (s, 1H); 8.65 (s, 1H); 8.60 (d, 1H; J = 8.0); 8.51 (s, 1H); 8.46 (d, 1H; J = 9.6); 8.43 (d, 1H; J = 8.0); 8.16, 8.07	417.0

		(m, 3H); 7.89 (dd, 1H; J = 8.0); 8.04 (dd, 1H; J = 8.0); 3.33 (s, 3H); 3.16 (s, 3H)	
33		(400 MHz, DMSO-d ₆), 8.66 (t, 1H; J = 2.0); 8.52 (d, 1H; J = 7.6); 8.45 (s, 1H); 8.43, 8.37 (m, 3H); 8.14, 8.08 (m, 3H); 8.06 (d, 1H; J = 9.6); 7.90 (t, 1H; J = 8.0); 3.34 (s, 3H)	393.0
36		(300 MHz, DMSO-d ₆), 8.67, 8.63 (m, 2H); 8.60 (d, 2H; J = 8.9); 8.54 (d, 1H; J = 7.9); 8.47 (d, 1H; J = 9.6); 8.17, 8.10 (m, 2H); 8.06 (d, 2H; J = 8.9); 7.91 (t, 1H; J = 7.9); 7.68 (s, 2H); 3.51 (t, 2H; J = 7.1); 3.36 (s, 3H); 2.90 (t, 2H; J = 7.1); 1.90 (t, 2H; J = 7.1)	470.6
37		(400 MHz, dmsO-d ₆), 8.43 (s, 1H); 8.40 (t, 1H; J = 1.6); 8.38, 8.34 (m, 3H); 8.29 (dd, 1H; J = 1.6, J = 7.2); 8.05 (d, 2H; J = 8.4); 7.98 (d, 1H; J = 9.6); 7.87 (dd, 1H; J = 1.6, J = 8.4); 7.80 (t, 1H; J = 8.0); 3.28 (s, 3H); 2.85 (s, 3H)	376.0
38		(300 MHz, CDCl ₃), 8.29, 8.19 (m, 4H); 8.18, 8.13 (m, 2H); 7.79 (d, 1H; J = 7.2); 7.70 (t, 1H; J = 7.5); 7.63, 7.56 (m, 3H); 4.02 (br s, 1H); 3.45, 3.31 (m, 2H); 2.07, 1.90 (m, 2H); 1.69, 1.50 (m, 4H)	466.2
39		(400 MHz, dmsO-d ₆), 8.35 (d, 2H; J = 8.4); 8.27, 8.17 (m, 3H); 8.16 (d, 1H; J = 9.6); 7.83, 7.75 (m, 3H); 7.70 (t, 1H; J = 8.0); 7.62 (d, 1H; J = 9.6); 2.81 (s, 3H)	401.1
41		(400 MHz, CDCl ₃), 9.49 (s, 1H); 9.34 (s, 1H); 9.15 (s, 1H); 8.59 (s, 1H); 8.35, 8.25 (m, 3H); 8.08 (d, 1H; J = 8.0); 7.85, 7.75 (m, 2H); 3.26 (s, 3H); 2.77, 2.66 (m,	454.0

		1H); 1.49, 1.38 (m, 2H); 1.16, 1.05 (m, 2H)	
42		(400 MHz, CDCl ₃), 9.53 (s, 1H); 8.74 (dd, 1H; J = 2.0, J = 8.0); 8.47 (s, 1H); 8.35, 8.25 (m, 3H); 8.10 (dd, 1H; J = 0.8, J = 8.0); 7.85, 7.75 (m, 2H); 3.31 (s, 3H); 2.60, 2.50 (m, 1H); 1.49, 1.41 (m, 2H); 1.17, 1.08 (m, 2H)	454.0
44		(400 MHz, dms ^o -d ₆), 8.60 (s, 1H); 8.50 (d, 1H; J = 7.8); 8.47 (s, 1H); 8.40 (d, 1H; J = 9.6); 8.37 (d, 2H; J = 8.1); 8.09 (d, 1H; J = 7.8); 8.06 (d, 2H; J = 8.1); 8.04 (d, 1H; J = 9.6); 7.90 (t, 1H; J = 7.8); 7.39 (s, 2H); 3.01 (m, 1H); 1.25, 1.20 (m, 2H); 1.13, 1.10 (m, 2H)	418.5
46		(300 MHz, CDCl ₃), 8.51 (t, 1H; J = 2.0); 8.40 (d, 2H; J = 8.4); 8.32 (d, 1H; J = 8.4); 8.26 (s, 1H); 8.20 (d, 1H; J = 9.6); 8.13, 8.04 (m, 3H); 7.79 (t, 1H; J = 7.8); 7.66 (d, 1H; J = 9.6); 3.13 (s, 3H); 2.60, 2.48 (m, 1H); 1.48, 1.40 (m, 2H); 1.16, 1.07 (m, 2H)	452.9

* Unless otherwise specified

C. HPLC Methods

Using a XBridge C18 column, 1 μ L injection volume, flow 1.2 mL/min; gradient: 25 – 100% B in 9 min (hold 5 min), 100 – 25% in 1 min (hold 6 min) (Mobile phase A: 10 mM buffer (Ammonium acetate/acetic acid) in H₂O and Mobile phase B: 10 mM buffer (Ammonium acetate/acetic acid) in CH₃OH with PDA – maximum chromatogram (210 – 400 nm).

D. Cytotoxicity data

Table ST1.

Compd	IC ₅₀ (μM)		SI	
	CHO	Vero	CHO	Vero
1	>234	-	>30000	-
8	-	>239	-	5975
22	>212	-	>75000	-
33	>254	-	>7055	-
36	>212	-	>2120	-
44	5.57	-	2932	-
45	2.90	-	2632	-

E. Effect of the introduction of water solubilizing groups on solubility and Pf IC₅₀

Compound in DMSO was spiked into either pH 6.5 phosphate buffer or 0.01M HCl (approx pH 2.0) with the final DMSO concentration being 1%. Reserpine and hydrocortisone are run as controls in all assays. Samples were then analysed via Nephelometry to determine a solubility range as described by Bevan and Lloyd.²

Table ST2.

Compd	Structure	Solubility		IC ₅₀ (nM) ^{a,b}	
		pH 2	pH 6.5	K1	NF54
16		188	157	-	24
17		207	98	-	18
18		25-50	3.1-6.3	99	102

19		12.5-25	1.6-3.1	96	107
20		<5	143	-	>2395
21		93	8.6	-	2.2
22		212	203	-	3.0
23		>200	178	-	4
24		<5	<5	4.1	4.2
25		<5	<5	-	5.1
26		166	162	8.8	14
27		12.5-25	1.6-3.1	11	12
28		112	<5	-	14
29		181	7	25	38
30		<5	179	-	377
31		6.2	169	-	>2396

32		207	98	-	6.5
33		<5	34	-	36
34		192	<5	-	13
35		191	172	21	18
36		>200	180	-	10
37		191	171	-	103
38		13	<5	-	1470
39		87	68	-	29
40		205	192	30	35

F. Metabolic stability

Compounds were incubated at 37°C at 1 μ M concentration in human, rat or mouse liver microsomes (BD Gentest, Discovery Labware Inc., Woburn, MA) suspended in 0.1 M phosphate buffer (pH 7.4) at a final protein concentration of 0.4 mg per mL. Metabolic reactions were initiated by the addition of an NADPH-regenerating system (1 mg/mL NADP, 1 mg/mL glucose-6-phosphate, 1 U/mL glucose-6-phosphate dehydrogenase) and $MgCl_2$ (0.67 mg/mL) and were quenched at various time points up to 60 min by the addition of ice-cold acetonitrile. Quenched samples were

centrifuged and the relative loss of parent compound over the course of the incubation was monitored by LC-MS using either a Micromass single quadrupole, triple quadrupole or TOF mass spectrometer (Waters Corporation, Milford, MA). Concentration versus time data for each compound were fitted to an exponential decay function to determine the first order rate constant for substrate depletion which was then used to calculate the degradation half-life, an *in vitro* intrinsic clearance value and subsequently a predicted *in vivo* intrinsic clearance value according to the methods of Obach.⁴ *In vivo* CL_{int} values were converted to a predicted *in vivo* hepatic extraction ratio (E_H) using the following equation: $E_H = CL_{int} / Q + CL_{int}$ where Q is liver blood flow which was assumed to be 20.7 mL/min/kg, 55.2 mL/min/kg and 90 mL/min/kg for humans, rats and mice, respectively.¹

Table ST3. % remaining after 30 minutes in liver microsomes (human/rat/mouse)

Compd	% remaining after 30'		
	h	r	m
16	64	27	18
17	69	70	34
20	99	100	100
21	87	93	100
22	91	100	100
23	75	75	65
24	97	82	59
25	80	93	88
26	12	37	3
28	99	87	55
29	99	79	74
30	96	97	98
32	69	70	34

33	10	95	96
34	85	80	52
35	86	96	92
36	99	85	98
37	84	54	9
38	72	88	50
39	32	48	46
40	71	35	39
41	88	88	96
42	100	97	100
43	95	71	100
44	100	96	76
45	5	11	8

Table ST4. *In vitro* metabolic stability in liver microsomes (human/rat)

Compounds were incubated at 37°C at 1 μ M concentration in human, rat or mouse liver microsomes (BD Gentest, Discovery Labware Inc., Woburn, MA) suspended in 0.1 M phosphate buffer (pH 7.4) at a final protein concentration of 0.4 mg per mL. Metabolic reactions were initiated by the addition of an NADPH-regenerating system (1 mg/mL NADP, 1 mg/mL glucose-6-phosphate, 1 U/mL glucose-6-phosphate dehydrogenase) and MgCl_2 (0.67 mg/mL) and were quenched at various time points up to 60 min by the addition of ice-cold acetonitrile. Quenched samples were centrifuged and the relative loss of parent compound over the course of the incubation was monitored by LC-MS using either a Micromass single quadrupole, triple quadrupole or TOF mass spectrometer (Waters Corporation, Milford, MA). Concentration versus time data for each compound were fitted to an exponential decay function to determine the first order rate constant for substrate depletion which was then used to calculate the degradation half-life, an *in vitro* intrinsic clearance value and subsequently a predicted *in vivo* intrinsic clearance value according to the methods of Obach.³ *In vivo* CL_{int} values were converted to a predicted *in*

vivo hepatic extraction ratio (E_H) using the following equation: $E_H = CL_{int} / Q + CL_{int}$ where Q is liver blood flow which was assumed to be 20.7 mL/min/kg, 55.2 mL/min/kg and 90 mL/min/kg for humans, rats and mice, respectively.¹

Compd	E_H values	
	h	r
18	<0.28	<0.19
19	<0.28	<0.19
27	0.32	0.23

G. Additional references

- (1) Davies, B. and Morris, T. Physiological parameters in laboratory animals and humans, *Pharm. Res.* 1993, 10, 1093-1095.
- (2) Bevan, C. D. and Lloyd, R. S. A High-Throughput Screening Method for the Determination of Aqueous Drug Solubility Using Laser Nephelometry in Microtiter Plates, *Anal. Chem.* **2000**, 72, 1781-1787.
- (4) Obach, R.S. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: An examination of in vitro half-life approach and non-specific binding to microsomes. *Drug Metab. Dispos.* **1999**, 27, 1350-1359.