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Trisubstituted Pyrimidines as Efficacious and Fast-acting Antimalarials

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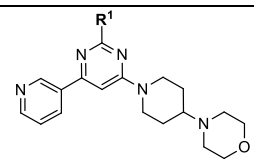
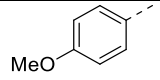
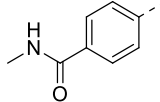
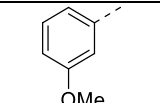
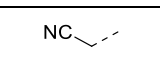
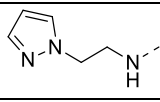
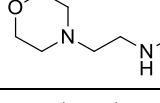
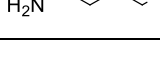
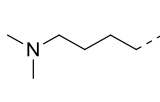
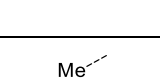
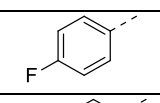
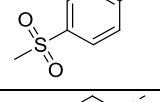
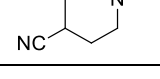
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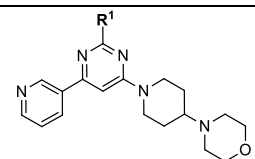

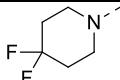
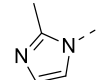
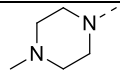
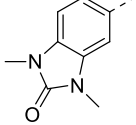
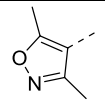
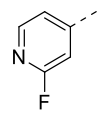
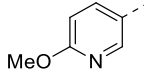
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Supplementary Table 1: Variations of R¹

	R ¹	Pf (3D7) EC ₅₀ (μM)	MRC5 EC ₅₀ (μM)	MWT	clogP	PSA	CYP inhibition IC ₅₀ (μM)
63		18	ND	431	3.2	64	ND
64		43	ND	458	2.4	84	ND
65		50	ND	431	3.2	64	ND
66		50	ND	364	1.8	78	ND
67		50	ND	434	2.4	84	ND
68		50	ND	453	2.1	80	ND
69		23	50*	382	1.4	80	ND
70		43	50*	410	2.9	58	1A2 , <10 2C9 , <10 2C19 <10 2D6 , <10 3A4 , <10
71		50	ND	339	2.1	54	ND
72		4.3	ND	419	3.4	54	ND
73		40	ND	479	2.5	89	ND
74		38	ND	433	2.6	81	ND

Supplementary Table 2: Variations of R¹

	R ¹	Pf (3D7) EC ₅₀ (μM)	MRC5 EC ₅₀ (μM)	MWT	clogP	PSA	CYP inhibition IC ₅₀ (μM)
12		0.3	36	402	2.3	67	1A2 , 93 2C9 , 1.9 2C19 , 6.3 2D6 , 0.7 3A4 , 0.1
75		4.1	ND	444	3.1	58	ND
76		10	ND	405	2.6	72	ND
77		16	ND	423	2.4	61	ND
78		16	ND	485	2.8	81	ND
79		50	ND	420	3.0	80	ND
80		12	ND	420	2.6	67	1A2 <10 2C9 <10 2C19 <10 2D6 <10 3A4 <10
81		18	ND	432	2.3	77	1A2 <10 2C9 <10 2C19 <10 2D6 <10 3A4 <10

Chemistry Experimental

4-(1-(2-(4-methoxyphenyl)-6-(pyridine-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine

(63) To a solution of 4-[1-(6-chloro-2-iodo-pyrimidin-4-yl)-4-piperidyl]morpholine (**61**) (0.15 g, 0.37 mmol) and 4-methoxyphenyl boronic acid (0.06 mg, 0.37) in DME (3 ml) in a 5 ml sealed microwave tube, Pd(PPh₃)₂Cl₂ (0.01 g, 0.02 mmol) and an aqueous 2M Na₂CO₃ solution (0.55 ml) were added. The reaction was heated at 120°C for 20 min under microwave irradiation. The reaction mixture was diluted with methanol (10 ml) and applied to a SCX column (5 g) and the product was eluted with 2M NH₃ in MeOH. Solvents were removed under reduced pressure and the product was further purified by column chromatography (12g silica cartridge) using A: DCM, B: 10% MeOH in DCM as eluents and the following gradient: 1 min hold at 100%A, 18 min ramp to 50% B and 3 min hold at 50% B. The fractions containing product were pooled together and solvents were removed to obtain 4-(1-(6-chloro-2-(4-methoxyphenyl)pyrimidin-4-yl)piperidin-4-yl)morpholine as an off-white solid (130 mg, 91% yield, 81% purity by LCMS). The product was used for the next step without further purification.

To a stirred solution of 4-(1-(6-chloro-2-(4-methoxyphenyl)pyrimidin-4-yl)piperidin-4-yl)morpholine (0.130 g, 0.33 mmol) and 3-pyridylboronic acid (0.08 g, 0.66 mmol) in DME (3 mL), Pd(PPh₃)₄ (0.015 g, 0.01 mmol) and an aqueous solution of potassium phosphate (2M, 0.5 ml) were added. The reaction was heated at 120°C for 20 min under microwave irradiation. The reaction crude was diluted with methanol (5 ml) and applied to a SCX column (1 g) and the product was eluted with 2M NH₃ in MeOH. The product was further purified by preparative HPLC under basic conditions. The fractions containing product were pooled together and solvents were removed to obtain **63** as off-white solid (78 mg, 54% yield). Purity by LCMS (UV chromatogram, 190-450 nm) 98%. ¹H-NMR (500 MHz, CDCl₃) δ 9.25 (dd, 1H, *J*=0.7, 2.2 Hz), 8.66 (dd, 1H, *J*=1.7, 4.8 Hz), 8.47-8.44 (m, 2H), 8.42-8.40 (m, 1H), 7.40-7.38 (m, 1H), 6.98-6.95 (m, 2H), 6.77 (s, 1H), 4.62-4.61 (m, 2H), 3.85 (s, 3H), 3.71-3.69 (m, 4H), 3.01-2.96 (m, 2H), 2.56-2.46 (m, 5H), 1.98-1.95 (m, 2H), 1.57-1.49 (m, 2H); LRMS (ES⁺) *m/z* 351 [M+H]⁺.

N-methyl-4-(4-(4-morpholinopiperidin-1-yl)-6-(pyridin-3-yl)pyrimidin-2-yl)benzamide (**64**)

Prepared in an analogous 3-step procedure to that of compound **63**: To a stirred solution of 1-(4-chloro-6-(4-morpholinopiperidin-1-yl)pyrimidin-2-yl)-*N*-methylbenzamide (0.10 g, 0.24 mmol) and 3-pyridylboronic acid (0.06 g, 0.48 mmol) in DMF (3 mL), Pd(PPh₃)₄ (0.015 g, 0.01 mmol) and an aqueous solution of potassium carbonate (2M, 0.5 ml) were added. The reaction was heated at 120°C for 20 min under microwave irradiation. The reaction crude was diluted with methanol (10 ml) and applied to a SCX column (2 g) and the product was eluted with 2M NH₃ in MeOH. The product was further purified by preparative HPLC under basic conditions. The fractions containing product were pooled together and solvents were removed to obtain **64** as off-white solid (72 mg, 65% yield). Purity by LCMS (UV chromatogram, 190-450 nm) 98%. ¹H-NMR (500 MHz, CDCl₃) δ 9.28-9.27 (m, 1H), 8.71 (dd, 1H, *J*=1.7, 4.8 Hz), 8.58-8.56 (m, 2H), 8.46-8.43 (, 1H), 7.87-7.85 (m, 2H), 6.88 (s, 1H), 6.23 (bs, 1H), 4.67-4.65

(m, 2H), 3.74-3.72 (m, 4H), 3.10-3.05 (m, 5H), 2.60-2.52 (m, 5H), 2.03-2.013 (m, 2H), 1.63-1.55 (m, 2H); LRMS (ES⁺) m/z 459 [M+H]⁺.

4-(1-(2-(3-methoxyphenyl)-6-(pyridine-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine

(65) Prepared in an analogous 3-step procedure to that of compound **63**: To a solution of 4-[1-(6-chloro-2-iodo-pyrimidin-4-yl)-4-piperidyl]morpholine (**62**) (0.15 g, 0.37 mmol) and 4-methoxyphenyl boronic acid (0.06 mg, 0.37) in DME (3 ml) in a 5 ml sealed microwave tube, Pd(PPh₃)₂Cl₂ (0.01 g, 0.02 mmol) and an aqueous 2M Na₂CO₃ solution (0.55 ml) were added. The reaction was heated at 120°C for 20 min under microwave irradiation. The reaction mixture was diluted with methanol (10 ml) and applied to a SCX column (5 g) and the product was eluted with 2M NH₃ in MeOH. Solvents were removed under reduced pressure and the product was further purified by column chromatography (12g silica cartridge) using A: DCM, B: 10% MeOH in DCM as eluents and the following gradient: 1 min hold at 100%A, 18 min ramp to 50% B and 3 min hold at 50% B. The fractions containing product were pooled together and solvents were removed to obtain 4-(1-(6-chloro-2-(4-methoxyphenyl)pyrimidin-4-yl)piperidin-4-yl)morpholine as an off-white solid (120 mg, 84% yield, 74% purity by LCMS). The product was used for the next step without further purification.

To a stirred solution of 4-(1-(6-chloro-2-(4-methoxyphenyl)pyrimidin-4-yl)piperidin-4-yl)morpholine (0.12 g, 0.31 mmol) and 3-pyridylboronic acid (0.076 g, 0.62 mmol) in DME (3 mL), Pd(PPh₃)₄ (0.015 g, 0.01 mmol) and an aqueous solution of potassium phosphate (2M, 0.5 ml) were added. The reaction was heated at 120°C for 20 min under microwave irradiation. The reaction crude was diluted with methanol (5 ml) and applied to a SCX column (1 g) and the product was eluted with 2M NH₃ in MeOH. The product was further purified by preparative HPLC under basic conditions. The fractions containing product were pooled together and solvents were removed to obtain **65** as off-white solid (30 mg, 23% yield). Purity by LCMS (UV chromatogram, 190-450 nm) 98%. ¹H-NMR (500 MHz, CDCl₃) δ 9.27 (dd, 1H, *J*=0.7, 2.2 Hz), 8.68 (dd, 1H, *J*=1.7, 4.8 Hz), 8.44-8.42 (m, 2H), 8.13-8.11 (m, 1H), 8.09-8.08 (m, 1H), 7.42-7.37 (m, 2H), 7.01 (ddd, 1H, *J*=0.9, 2.7, 8.2Hz), 6.84 (s, 1H), 4.65-4.63 (m, 2H), 3.90 (s, 3H), 3.73-3.71 (m, 4H), 3.05-3.00 (m, 2H), 2.28-2.54 (m, 4H), 2.53-2.48 (m, 1H), 2.00-1.97 (m, 2H), 1.60-1.52(m, 2H); LRMS (ES⁺) m/z 432 [M+H]⁺.

4-(4-morpholinopiperidin-1-yl)-6-(pyridin-3-yl)pyrimidine-2-carbonitrile (66) To a stirred solution of 6-chloro-4-(4-morpholino-1-piperidyl)-1,6-dihydropyrimidine-2-carbonitrile (0.128 g, 0.41 mmol) and 3-pyridylboronic acid (0.103 g, 0.83 mmol) in DME (4 mL), an aqueous solution of sodium carbonate (2M, 0.26 g, 1.24 mmol) and PdCl₂(PPh₃)₂ (0.014 g, 0.02 mmol) were added. The reaction was heated at 120°C for 20 min under microwave irradiation. The reaction crude was diluted with methanol (5 ml) and applied to a SCX column (5 g) and the product was eluted with 2M NH₃ in MeOH. The product was further purified by preparative HPLC under acidic conditions. The fractions containing product were pooled together and solvents were removed to obtain xx as off-white solid (47 mg, 31% yield). Purity by LCMS (UV chromatogram, 190-450 nm) 98%. ¹H-NMR (500 MHz, CDCl₃) δ 9.14-9.13 (m, 1H), 8.72 (dd, 1H, *J*=1.7, 4.8 Hz), 8.36-8.34 (m, 1H), 7.45 (ddd, 1H, *J*=0.8, 4.8, 8.0

Hz), 6.97 (s, 1H), 4.59-4.56 (m, 2H), 3.76-3.74 (m, 4H), 3.10-3.05 (m, 2H), 2.63-2.57 (m, 5H), 2.04-2.02 (m, 2H), 1.60-1.52 (m, 2H); LRMS (ES⁺) *m/z* 351 [M+H]⁺.

***N*-(2-(1*H*-pyrazol-1-yl)ethyl)-4-(4-morpholinopiperidin-1-yl)-6-(pyridine-3-yl)**

pyrimidin-2-amine (67) Prepared in an analogous 3-step procedure to that of compound **74**: A solution of 4-[1-(6-chloro-2-iodo-pyrimidin-4-yl)-4-piperidyl]morpholine (**13**) (0.15 g, 0.37 mmol), 2-(1*H*-pyrazol-1-yl)ethanamine (0.04 ml, 0.40 mmol) and DIPEA (0.13 ml, 0.40 mmol) in NMP (2 ml) was heated at 200°C for 15 min under microwaved irradiation. Reaction crude was diluted with MeOH (5 ml) and applied to a SCX cartridge (5g) and the product was diluted with a solution of 2N NH₃ in methanol. Solvents were removed under reduced pressure and the product was further purified by column chromatography (12g silica cartridge) using A: DCM, B: 10% MeOH in DCM as eluents and the following gradient: 1 min hold at 100%A, 18 min ramp to 50% B and 3 min hold at 50% B. The fractions containing product were pooled together and solvents were removed to obtain *N*-(2-(1*H*-pyrazol-1-yl)ethyl)-4-chloro-6-(4-morpholinopiperidin-1-yl)pyrimidin-2-amine as an off-white solid (62 mg, 43%, 88% purity by LCMS). The product was used for the next step without further purification. To a stirred solution of *N*-(2-(1*H*-pyrazol-1-yl)ethyl)-4-chloro-6-(4-morpholinopiperidin-1-yl)pyrimidin-2-amine (0.06 g, 0.16 mmol) and 3-pyridylboronic acid (0.04 g, 0.32 mmol) in DMF (3 mL), Pd(PPh₃)₄ (0.015 g, 0.01 mmol) and an aqueous solution of potassium carbonate (2M, 0.5 ml) were added. The reaction was heated at 120°C for 20 min under microwave irradiation. The reaction crude was diluted with methanol (10 ml) and applied to a SCX column (2 g) and the product was eluted with 2M NH₃ in MeOH. The product was further purified by preparative HPLC under basic conditions. The fractions containing product were pooled together and solvents were removed to obtain **67** as off-white solid (28 mg, 41% yield). Purity by LCMS (UV chromatogram, 190-450 nm) 98%. ¹H-NMR (500 MHz, CDCl₃) δ 9.11 (broad peak, 1H), 8.63 (dd, 1H, *J* = 1.7, 4.8 Hz), 8.23-8.21 (m, 1H), 7.53 (d, 1H, *J* = 1.7 Hz), 7.38 (d, 1H, *J* = 2.0 Hz), 7.34 (ddd, 1H, *J* = 0.6, 4.8, 8.0 Hz), 6.35 (s, 1H), 6.22 (dd, 1H, *J* = 2.1, 2.1 Hz), 5.12 (bs, 1H), 4.48-4.45 (m, 2H), 4.38 (t, 1H, *J* = 5.8 Hz), 3.89 (t, 1H, *J* = 6.0 Hz), 3.72-3.70 (m, 4H), 2.93-2.88 (m, 2H), 2.57-2.55 (m, 4H), 2.50-2.44 (m, 1H), 1.94-1.92 (m, 2H), 1.53-1.45 (m, 2H); LRMS (ES⁺) *m/z* 435 [M+H]⁺.

***N*-(2-morpholinoethyl)-4-(4-morpholinopiperidin-1-yl)-6-(pyridine-3-yl)pyrimidin-2-**

amine (68) Prepared in an analogous 3-step procedure to that of compound **74**: A solution of 4-[1-(6-chloro-2-iodo-pyrimidin-4-yl)-4-piperidyl]morpholine (**13**) (0.15 g, 0.37 mmol), 2-morpholinoethanamine (0.05 ml, 0.40 mmol) and DIPEA (0.13 ml, 0.40 mmol) in NMP (2 ml) was heated at 200°C for 15 min under microwaved irradiation. Reaction crude was diluted with MeOH (5 ml) and applied to a SCX cartridge (5g) and the product was diluted with a solution of 2N NH₃ in methanol. Solvents were removed under reduced pressure and the product was further purified by column chromatography (12g silica cartridge) using A: DCM, B: 10% MeOH in DCM as eluents and the following gradient: 1 min hold at 100%A, 18 min ramp to 50% B and 3 min hold at 50% B. The fractions containing product were pooled together and solvents were removed to obtain 4-chloro-*N*-(2-morpholinoethyl)-6-(4-morpholinopiperidin-1-yl)pyrimidin-2-amine as an off-white solid (105 mg, 70%, 69% purity by LCMS). The product was used for the next step without further purification. To a stirred solution of 4-chloro-

N-(2-morpholinoethyl)-6-(4-morpholinopiperidin-1-yl)pyrimidin-2-amine (0.105 g, 0.25 mmol) and 3-pyridylboronic acid (0.06 g, 0.50 mmol) in DMF (3 mL), Pd(PPh₃)₄ (0.015 g, 0.01 mmol) and an aqueous solution of potassium carbonate (2M, 0.5 ml) were added. The reaction was heated at 120°C for 20 min under microwave irradiation. The reaction crude was diluted with methanol (10 ml) and applied to a SCX column (2 g) and the product was eluted with 2M NH₃ in MeOH. The product was further purified by preparative HPLC under basic conditions. The fractions containing product were pooled together and solvents were removed to obtain **68** as off-white solid (28 mg, 24% yield). Purity by LCMS (UV chromatogram, 190-450 nm) 98%. ¹H-NMR (500 MHz, CDCl₃) δ 9.11-9.10 (m, 1H), 8.63 (dd, 1H, *J* = 1.6, 4.8 Hz), 8.23-8.22 (m, 1H), 7.34 (ddd, 1H, *J* = 0.6, 4.8, 7.9 Hz), 6.31 (s, 1H), 5.36 (bs, 1H), 4.49-4.47 (m, 2H), 3.73-3.71 (m, 8H), 3.57-3.54 (m, 2H), 2.93-2.87 (m, 2H), 2.60-2.56 (m, 6H), 2.49-2.44 (m, 5H), 1.94-1.91 (m, 2H), 1.54-1.46 (m, 2H); LRMS (ES⁺) *m/z* 454 [M+H]⁺.

3-(4-(4-morpholinopiperidin-1-yl)-6-(pyridin-3-yl)pyrimidin-2-yl)propan-1-amine (**69**)

Prepared in an analogous 4-step procedure to that of compound **51**: A mixture of *tert*-butyl N-[3-[4-chloro-6-(4-morpholino-1-piperidyl)pyrimidin-2-yl]propyl]carbamate (166 mg, 0.38 mmol) in DMF (4 mL) was prepared at rt and to it added 3-pyridyl boronic acid (93 mg, 0.76 mmol), potassium phosphate (160 mg, 0.76 mmol) in water (0.5 mL) and Pd tetrakis (22 mg, 0.02 mmol). Mixture then heated in a mw at 110°C for 1h. The mixture was then diluted with ethyl acetate (20 mL) and filtered through a celite column, filtrate then partitioned and organics washed with 5% LiCl aq. (3 x 10 mL), brine (10 mL), dried over MgSO₄ and concentrated under reduced pressure. Mixture then purified by column (0-10% methanol/dichloromethane) to afford *tert*-butyl(3-(4-(4-morpholinopiperidin-1-yl)-6-(pyridin-3-yl)pyrimidin-2-yl)propyl)carbamate (171 mg, 0.35 mmol, 94%) as a yellow oil. Purity by LCMS (UV chromatogram, 190-450 nm) > 95%. ¹H-NMR (500 MHz, CDCl₃) δ 9.11 (d, 1H, *J* = 1.7 Hz), 8.67 (dd, 1H, *J* = 1.6, 4.9 Hz), 8.29 (dt, 1H, *J* = 1.9, 8.0 Hz), 7.38 (dd, 1H, *J* = 4.9, 7.9 Hz), 6.73 (s, 1H), 4.94 (brs, 1H), 4.53 (brd, 2H, *J* = 12.6 Hz), 3.73 (t, 4H, *J* = 4.6 Hz), 3.25-3.23 (m, 2H), 2.97 (t, 2H, *J* = 7.3 Hz), 2.86 (t, 2H, *J* = 7.3 Hz), 2.57 (t, 4H, *J* = 4.7 Hz), 2.53-2.47 (m, 1H), 2.03 (t, 2H, *J* = 7.1 Hz), 1.97 (brd, 2H, *J* = 14.3 Hz), 1.56-1.48 (m, 2H), 1.43 (s, 9H); LRMS (ES⁺) *m/z* 483 [M+H]⁺.

A mixture of *tert*-butyl(3-(4-(4-morpholinopiperidin-1-yl)-6-(pyridin-3-yl)pyrimidin-2-yl)propyl)carbamate (167 mg, 0.35 mmol) was prepared in dichloromethane (10 mL) and trifluoroacetic acid (0.5 mL) added and the mixture stirred at rt for 24h. The mixture was then concentrated under reduced pressure, diluted with DCM (10 mL) filtered through an SCX-2 column and the column washed with DCM (20 mL), methanol (20 mL), and flushed with 7M ammonia in methanol. Ammonia filtrate then concentrated under reduced pressure to afford **69** (105 mg, 0.28 mmol, 79%) as a colourless oil. Purity by LCMS (UV chromatogram, 190-450 nm) > 95%. ¹H-NMR (500 MHz, CDCl₃) δ 9.11 (d, 1H, *J* = 2.2 Hz), 8.66 (dd, 1H, *J* = 1.7, 4.8 Hz), 8.28 (dt, 1H, *J* = 1.9, 8.3 Hz), 7.38 (dd, 1H, *J* = 4.8, 8.0 Hz), 6.73 (s, 1H), 4.55 (brd, 2H, *J* = 11.5 Hz), 3.28 (t, 4H, *J* = 4.6 Hz), 2.99 (t, 2H, *J* = 14.5 Hz), 2.84 (dt, 4H, *J* = 7.5, 19.5 Hz), 2.58 (t, 4H, *J* = 4.7 Hz), 2.53-2.47 (m, 1H), 2.04-1.96 (m, 4H), 1.65 (brs, 2H), 1.56-1.48 (m, 2H); LRMS (ES⁺) *m/z* 383 [M+H]⁺.

N,N-dimethyl-3-(4-(4-morpholinopiperidin-1-yl)-6-(pyridin-3-yl)pyrimidin-2-yl)propan-1-amine (70) A mixture of 3-[4-(4-morpholino-1-piperidyl)-6-(3-pyridyl)pyrimidin-2-yl]propan-1-amine (80 mg, 0.21 mmol) in chloroform (6mL) was prepared at rt and paraformaldehyde (31 mg, 1.05 mmol) added and the mixture heated to 65-68°C for 1h. The mixture was then cooled to rt and sodium triacetoxyborohydride (222 mg, 1.05 mmol) added in one portion and the mixture heated again to 65-68°C for 16h. The mixture was then cooled to rt and diluted with dichloromethane (10 mL) and purified through an SCX2 column. The filtrate was then repurified due to minor impurities by column chromatography (0-10% 7M ammonia methanol/dichloromethane) to afford **70** (57mg, 0.1388mmol, 66 %) as a colourless oil. Purity by LCMS (UV chromatogram, 190-450 nm) > 95%. ¹H-NMR (500 MHz, CDCl₃) δ 9.12 (d, 1H, *J* = 2.2 Hz), 8.66 (dd, 1H, *J* = 1.7, 4.8 Hz), 8.29 (dt, 1H, *J* = 1.8, 8.0 Hz), 7.38 (dd, 1H, *J* = 4.9, 7.9 Hz), 6.73 (s, 1H), 4.56 (brd, 2H, *J* = 12.1 Hz), 3.73 (t, 4H, *J* = 4.5 Hz), 2.96 (t, 2H, *J* = 14.5 Hz), 2.82 (t, 2H, *J* = 7.6 Hz), 2.58 (t, 4H, *J* = 4.6 Hz), 2.53-2.47 (m, 1H), 2.40 (t, 2H, *J* = 8.1 Hz), 2.27 (s, 6H), 1.96 (brd, 2H, *J* = 13.3 Hz), 1.60 (brs, 2H), 1.56-1.48 (m, 2H); LRMS (ES+) *m/z* 411 [M+H]⁺.

4-(1-(2-methyl-6-(pyridin-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (71) A mixture of 3-pyridylboronic acid (62.123mg, 0.5054mmol), Potassium phosphate (107.28mg, 0.5054mmol), palladium tetrakis(triphenylphosphine) (5.8373mg, 0.0051mmol), 4-[1-(6-chloro-2-methyl-pyrimidin-4-yl)-4-piperidyl]morpholine (50mg, 0.1685mmol) in 1,4-dioxane (1.6mL) and water (0.4000mL) was heated at 120°C in a Biotage Initiator microwave for 30mins. The reaction mixture was evaporated by blow-down and then dissolved in DCM/MeOH, loaded onto a primed SCX cartridge (5g) and eluted with methanol then 2M NH₃/MeOH. The appropriate fractions were evaporated by blow-down and the residue was dissolved in 10% water in DMF and purified by Mass Directed HPLC (5-95% MeCN, basic method). The appropriate fractions were combined and evaporated by blow-down and freeze-dried to give **71** (35 mg, 58% yield) as a colourless solid. Purity by LCMS (UV chromatogram, 190-450 nm) > 98%. ¹H NMR (500 MHz, CDCl₃) δ 9.10 (d, *J*=1.6 Hz, 1H), 8.67 (dd, *J*=1.7, 4.8 Hz, 1H), 8.28 (td, *J*=2.0, 7.9 Hz, 1H), 7.39 (dd, *J*=4.9, 7.9 Hz, 1H), 6.72 (s, 1H), 4.55 (t, *J*=5.5 Hz, 2H), 3.73 (s, 4H), 3.00 - 2.93 (m, 2H), 2.58 (s, 7H), 2.50 (s, 1H), 1.97 (d, *J*=12.6 Hz, 2H), 1.56 - 1.48 (m, 2H); LRMS (ES+) *m/z* 340 [M+H]⁺.

4-(1-(2-(4-fluorophenyl)-6-(pyridin-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (72) In a sealed 20 ml microwave vial, a solution of 4-[1-[2-methylsulfanyl-6-(3-pyridyl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.150 g, 0.40 mmol) in THF (8 ml) was degassed by bubbling argon through for 5 minutes. (4-fluorophenyl)boronic acid (0.056 g, 0.40 mmol), thiophene-2-carboxyloxycopper (0.115 g, 0.61 mmol) and Pd(PPh₃)₄ (0.047 g, 0.04 mmol) were added at room temperature. The reaction was heated under microwave irradiation at 120 °C for 1h. The reaction crude was applied to a SCX column (2 g) and the product was eluted with 2 M NH₃ in MeOH. Solvents were removed and the product was purified by mass directed autopreparative HPLC under basic conditions. The fractions containing product were pooled together and solvents were removed to obtain **72** as off-white solid (30 mg, 17% yield). Purity by LCMS (UV chromatogram, 190-450 nm) > 98%. ¹H-NMR (500 MHz, d₆ DMSO) δ 9.45 (d, 1H, *J* = 1.7 Hz), 8.70 (dd, 1H, *J* = 1.6, 4.7 Hz), 8.65-8.62 (m, 1H), 8.53-8.49 (m, 2H), 7.56

(ddd, 1H, $J = 0.7, 4.8, 8.0$ Hz), 7.41 (s, 1H), 7.35-7.30 (m, 2H), 4.79-4.65 (m, 2H), 3.57-3.55 (m, 4H), 3.04-3.00 (m, 2H), 1.92-1.90 (m, 2H), 1.45-1.37 (m, 2H); LRMS (ES⁺) m/z 419 [M+H]⁺.

4-(1-(2-(4-(methylsulfonyl)phenyl)-6-(pyridin-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (73) Prepared in an analogous 3-step procedure to that of compound **43**: In a 5ml microwave vial, a solution of 4-[1-[2-methylsulfonyl-6-(3-pyridyl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.08 g, 0.22 mmol) in 1,4-Dioxane (4 ml) was degassed by bubbling argon through for 5 minutes. (4-Methylsulfonylphenyl)boronic acid (0.09 g, 0.44 mmol), thiophene-2-carbonyloxycopper (0.08 g, 0.44 mmol) and Pd(PPh₃)₄ (0.02 g, 0.02 mmol) were added at room temperature. The reaction was heated at 130°C for 1 h under microwave irradiation. The reaction crude was applied to a SCX column (2 g) and the product was eluted with 2 M NH₃ in MeOH. Solvents were removed under reduced pressure and the product was purified by mass directed auto-preparative HPLC under basic conditions (5-95 prep basic). The fractions containing product were pooled together and solvents were removed to obtain **73** as white solid (24 mg, 22% yield). Purity by LCMS (UV chromatogram, 190-450 nm) >98%. ¹H-NMR (500 MHz, *d*6 DMSO) δ 9.28-9.27 (m, 1H), 8.73-8.70 (m, 3H), 8.46-8.43 (m, 1H), 8.05-8.04 (m, 2H), 7.47-7.44 (m, 1H), 6.93 (s, 1H), 4.71-4.66 (m, 2H), 3.78-3.76 (m, 4H), 3.13-3.96 (m, 5H), 3.68-3.57 (m, 5H), 2.17-2.00 (m, 2H), 1.65-1.61 (m, 2H); LRMS (ES⁺) m/z 479 [M+H]⁺.

1-(4-(4-morpholinopiperidin-1-yl)-6-(pyridine-3-yl)pyrimidin-2-yl)piperidine-4-carbonitrile (74) A solution of 4-[1-(6-chloro-2-iodo-pyrimidin-4-yl)-4-piperidyl]morpholine (0.15 g, 0.37 mmol), piperidine-4-carbonitrile (0.05 ml, 0.40 mmol) and DIPEA (0.13 ml, 0.40 mmol) in NMP (2 ml) was heated at 200°C for 15 min under microwaved irradiation. Reaction crude was diluted with MeOH (5 ml) and applied to a SCX cartridge (5g) and the product was diluted with a solution of 2N NH₃ in methanol. Solvents were removed under reduced pressure and the product was further purified by column chromatography (12g silica cartridge) using A: DCM, B: 10% MeOH in DCM as eluents and the following gradient: 1 min hold at 100%A, 18 min ramp to 50% B and 3 min hold at 50% B. The fractions containing product were pooled together and solvents were removed to obtain 1-(4-chloro-6-(4-morpholinopiperidin-1-yl)pyrimidin-2-yl)piperidine-4-carbonitrile as an off-white solid (148 mg, quantitative yield). Purity by LCMS (UV chromatogram, 190-450 nm) 91 %. ¹H-NMR (500 MHz, CDCl₃) δ 5.83 (s, 1H), 4.24 (broad peak, 2H), 4.00 (ddd, 2H, $J = 3.6, 6.9, 13.7$ Hz), 3.66-3.64 (m, 4H), 3.55 (ddd, 2H, $J = 3.4, 8.1, 13.7$ Hz), 2.83-2.77 (m, 3H), 2.50-2.48 (m, 4H), 2.42-2.36 (m, 1H), 1.93-1.85 (m, 2H), 1.80-1.73 (m, 2H), 1.42-1.35 (m, 2H); LRMS (ES⁺) m/z 391 [M+H]⁺.

To a stirred solution of 1-(4-chloro-6-(4-morpholinopiperidin-1-yl)pyrimidin-2-yl)piperidine-4-carbonitrile (0.15 g, 0.38 mmol) and 3-pyridylboronic acid (0.09 g, 0.76 mmol) in DMF (3 mL), Pd(PPh₃)₄ (0.015 g, 0.01 mmol) and an aqueous solution of potassium carbonate (2M, 0.5 ml) were added. The reaction was heated at 120°C for 20 min under microwave irradiation. The reaction crude was diluted with methanol (10 ml) and applied to a SCX column (2 g) and the product was eluted with 2M NH₃ in MeOH. The product was further purified by preparative HPLC under basic conditions. The fractions containing product were pooled together and

solvents were removed to obtain **74** as off-white solid (75 mg, 45% yield). Purity by LCMS (UV chromatogram, 190-450 nm) 96%. ¹H-NMR (500 MHz, CDCl₃) δ 9.15 (dd, 1H, *J*=0.7, 2.2 Hz), 8.65 (dd, 1H, *J*=1.7, 4.8 Hz), 8.28-8.26 (m, 1H), 7.36 (ddd, 1H, *J*=0.8, 4.8, 8.0 Hz), 6.38 (s, 1H), 4.50-4.47 (m, 4H), 4.13-4.11 (m, 2H), 3.73-3.71 (m, 4H), 3.49 (ddd, 2H, *J*=2.6, 5.4, 5.5 Hz), 2.96-2.91 (m, 2H), 2.58-2.56 (m, 4H), 2.51-2.45 (m, 1H), 1.95-1.93 (m, 2H), 1.54-1.46 (m, 2H); LRMS (ES⁺) *m/z* 434 [M+H]⁺.

4-(1-(2-(4,4-difluoropiperidin-1-yl)-6-(pyridin-3-yl)pyrimidin-4-yl)piperidin-4-

yl)morpholine (75) Prepared in an analogous 3-step procedure to that of compound **5**: To a stirred solution of 4-[1-[6-chloro-2-(4,4-difluoro-1-piperidyl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.16 g, 0.40 mmol) in DMF (6 mL), a solution of potassium phosphate (0.251 g, 1.19 mmol) in water (2 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min and then, Pd(PPh₃)₄ (0.013 g, 0.012 mmol) was added. The reaction was heated at 130°C under microwave irradiation for 20 min. Reaction crude was filtered through Celite[®], washed with DCM (10 mL) and partitioned between DCM (15 mL) and saturated aqueous solution of NaHCO₃ (5 mL). The organics phase was dried over MgSO₄ before concentration to dryness. The crude was then purified by mass directed autopreparative HPLC under basic conditions. The fractions containing product were pooled together and solvents were removed to obtain **75** as white solid (49 mg, 28% yield). Purity by LCMS (UV chromatogram, 190-450 nm) > 98%. ¹H-NMR (500 MHz, CDCl₃) δ 9.15 (dd, 1H, *J*=0.7, 2.2 Hz), 8.65 (dd, 1H, *J*=1.7, 4.8 Hz), 8.27-8.25 (m, 1H), 7.36 (ddd, 1H, *J*=0.7, 4.8, 7.9 Hz), 6.35 (s, 1H), 4.49-4.46 (m, 2H), 4.02-3.74 (m, 4H), 3.74-3.72 (m, 4H), 2.95-2.90 (m, 2H), 2.59-2.57 (m, 4H), 2.52-2.46 (m, 1H), 2.06-1.93 (m, 6H), 1.51 (ddd, 1H, *J*=4.3, 12.4, 16.1 Hz); LRMS (ES⁺) *m/z* 445 [M+H]⁺.

4-(1-(2-(2-methyl-1H-imidazol-1-yl)-6-pyridin-3-yl)pyrimidin-4-yl)piperidin-4-

yl)morpholine (76) Prepared in an analogous 3-step procedure to that of compound **40**: To a stirred solution of 4-[1-[6-chloro-2-(1-methylpyrazol-4-yl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.034 g, 0.09 mmol) and 3-pyridylboronic acid (0.034 g, 0.28 mmol) in DMF (3 mL), a solution of potassium phosphate (0.60 g, 0.28 mmol) in water (1 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min and then Pd(PPh₃)₄ (0.003 g, 0.003 mmol) was added. The reaction was heated at 130°C under microwave irradiation for 20 min. Reaction was filtered through Celite^R and partitioned between DCM (10 mL) and a saturated aqueous solution of NaHCO₃ (5 mL). The organics phase was dried over MgSO₄ before concentration to dryness. The crude was then purified by preparative mass directed autopreparative HPLC (method: 5-95 basic). The fractions containing product were pooled together and solvents were removed to obtain **76** as off-white solid (27 mg, 71% yield). Purity by LCMS (UV chromatogram, 190-450 nm) 99%. ¹H-NMR (500 MHz, CDCl₃) δ 9.22-9.21 (m, 1H), 8.72 (dd, 1H, *J*=1.6, 4.8 Hz), 8.35-8.33 (m, 1H), 7.91 (d, 1H, *J*=1.6 Hz), 7.45-7.42 (m, 1H), 6.96 (d, 1H, *J*=1.6 Hz), 6.81 (s, 1H), 4.55-4.49 (m, 2H), 3.73 (m, 4H), 3.09 (t, 2H, *J*=11.7 Hz), 2.89 (s, 3H), 2.61-2.52 (m, 5H), 2.02 (d, 2H, *J*=12.3 Hz), 1.67-1.53 (m, 2H); LRMS (ES⁺) *m/z* 406 [M+H]⁺.

4-(1-(2-(4-methylpiperazin-1-yl)-6-(pyridine-3-yl)pyrimidin-4-yl)piperidin-4-

yl)morpholine (77) Prepared in an analogous 3-step procedure to that of compound **5**: To a stirred solution of 4-[1-[6-chloro-2-(1-methylpyrazol-4-yl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.10 g, 0.26 mmol) and 3-pyridylboronic acid (0.97 g, 0.79 mmol) in DMF (3 mL), a solution of potassium phosphate (0.17 g, 0.79 mmol) in water (1 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min and then Pd(PPh₃)₄ (0.009 g, 0.008 mmol) was added. The reaction was heated at 130°C under microwave irradiation for 20 min. Reaction was filtered through Celite^R and partitioned between DCM (15 ml) and a saturated aqueous solution of NaHCO₃ (5 ml). The organics phase was dried over MgSO₄ before concentration to dryness. The crude was then purified by preparative mass directed autopreparative HPLC (method: 5-95 basic). The fractions containing product were pooled together and solvents were removed to obtain **77** as off-white solid (22 mg, 20% yield). Purity by LCMS (UV chromatogram, 190-450 nm) >98%. ¹H-NMR (500 MHz, CDCl₃) δ 9.15-9.14 (m, 1H), 8.64-8.63 (m, 1H), 8.28-8.26 (m, 1H), 7.36-7.34 (m, 1H), 6.33(s, 1H), 4.49-4.47 (m, 2H), 3.90-3.89 (m, 4H), 3.74-3.73 (m, 4H), 2.94-2.89 (m, 2H), 2.59-2.57 (m, 4H), 2.48-2.46 (m, 5H), 2.35 (s, 3H), 1.95-1.93 (m, 2H), 1.59-1.48 (m, 2H); LRMS (ES⁺) m/z 479 [M+H]⁺.

1,3-dimethyl-5-(4-(4-morpholinopiperidin-1-yl)-6-(pyridin-3-yl)pyrimidin-2-yl)-1H-

benzo[d]imidazole-2(3H)-one (78) Prepared in an analogous 3-step procedure to that of compound **43**: In a sealed 5 ml microwave vial, a solution of 4-[1-[2-methylsulfanyl-6-(3-pyridyl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.08 g, 0.21 mmol) in 1,4-Dioxane (4 ml) was degassed by bubbling argon through for 5 minutes. (1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)boronic acid (0.089 g, 0.43 mmol), thiophene-2-carbonyloxycopper (0.082 g, 0.43 mmol) and Pd(PPh₃)₄ (0.025 g, 0.02 mmol) were added at room temperature. The reaction was heated under microwave irradiation at 120 °C for 1h. The reaction crude was applied to a SCX column (2 g) and the product was eluted with 2 M NH₃ in MeOH. Solvents were removed and the product was purified by mass directed autopreparative HPLC under basic conditions (5-40% basic method). The fractions containing product were pooled together and solvents were removed to obtain **78** as white solid (24 mg, 23% yield). Purity by LCMS (UV chromatogram, 190-450 nm) > 98%. ¹H-NMR (500 MHz, CDCl₃) δ 9.23-9.29 (m, 1H), 8.70-8.69 (m, 1H), 8.44-8.42 (m, 1H), 8.37 (dd, 1H, *J* = 1.5, 8.2 Hz), 8.16 (d, 1H, *J* = 1.5 Hz), 7.44 (ddd, 1H, *J* = 0.5, 4.8, 8.0 Hz), 7.03 (d, 1H, *J* = 8.2 Hz), 6.83 (s, 1H), 4.68-4.66 (m, 2H), 3.75-3.73 (m, 4H), 3.53 (s, 3H), 3.47 (s, 3H), 3.10-3.04 (m, 2H), 2.61-2.53(m, 5H), 2.04-2.01 (m, 2H), 1.64-1.56 (m, 2H); LRMS (ES⁺) m/z 486 [M+H]⁺.

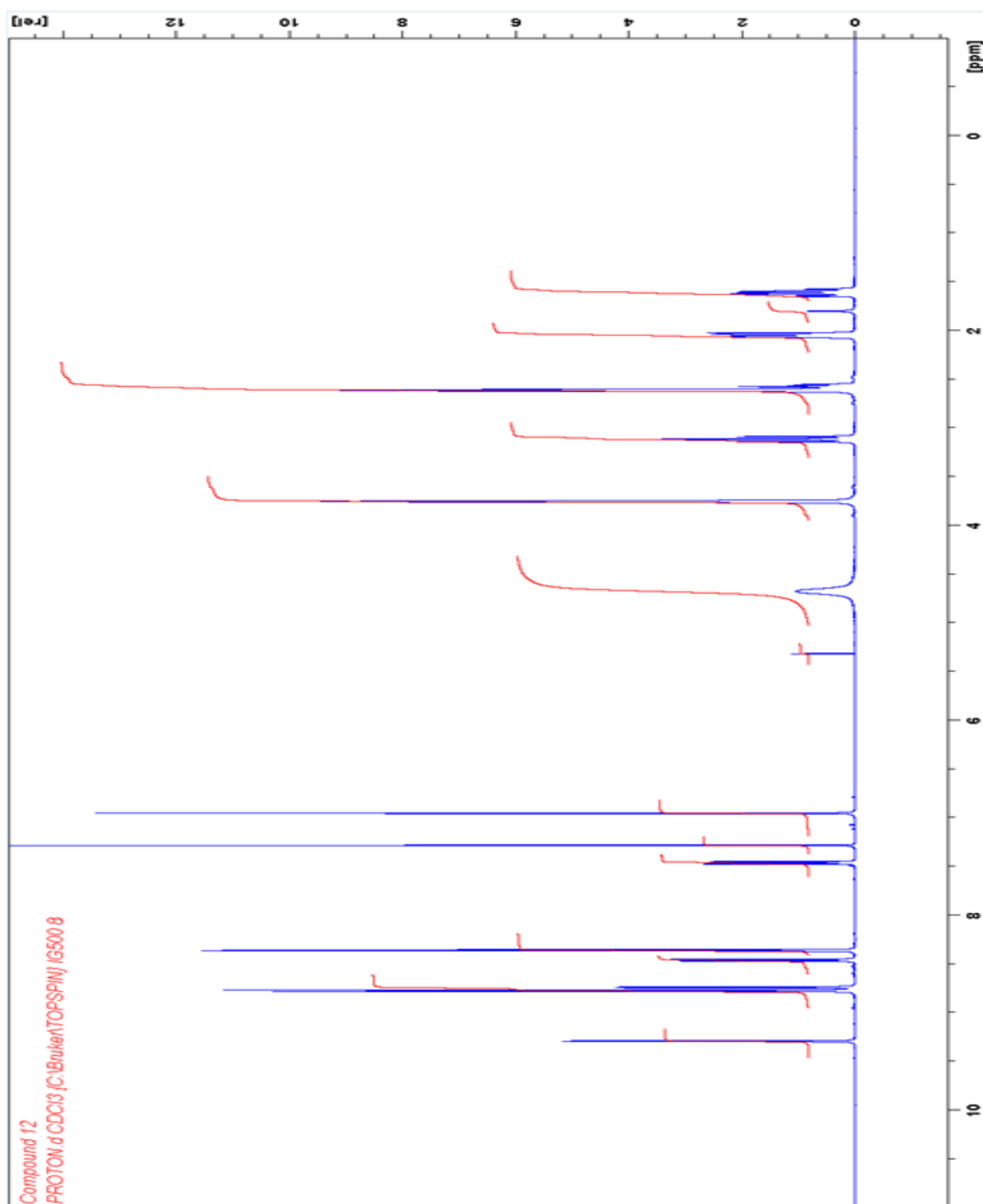
4-(1-(2-(3,5-dimethylisoxazol-4-yl)-6-(pyridine-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (79) To a solution of 4-[1-(6-chloro-2-iodo-pyrimidin-4-yl)-4-piperidyl]morpholine (0.15 g, 0.37 mmol) and (3,5-dimethylisoxazol-4yl)boronic acid (0.05 mg, 0.37) in DME (3 ml) in a 5 ml sealed microwave tube, Pd(PPh₃)₂Cl₂ (0.01 g, 0.02 mmol) and an aqueous 2M Na₂CO₃ solution (0.55 ml) were added. The reaction was heated at 120°C for 20 min under microwave irradiation. The reaction mixture was diluted with methanol (10 ml) and applied to a SCX column (5 g) and the product was eluted with 2M NH₃ in MeOH. Solvents were removed under reduced pressure and the product was further purified by column

chromatography (12g silica cartridge) using A: DCM, B: 10% MeOH in DCM as eluents and the following gradient: 1 min hold at 100%A, 18 min ramp to 50% B and 3 min hold at 50% B. The fractions containing product were pooled together and solvents were removed to obtain 4-(1-(6-chloro-2-(3,5-dimethylisoxazol-4-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine as an off-white solid (100 mg, 72% yield, 71% purity by LCMS). The product was used for the next step without further purification. To a stirred solution of 4-(1-(6-chloro-2-(3,5-dimethylisoxazol-4-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (0.10 g, 0.26 mmol) and 3-pyridylboronic acid (0.065 g, 0.52 mmol) in DME (3 ml), Pd(PPh₃)₄ (0.015 g, 0.01 mmol) and an aqueous solution of potassium phosphate (2M, 0.5 ml) were added. The reaction was heated at 120°C for 20 min under microwave irradiation. The reaction crude was diluted with methanol (5 ml) and applied to a SCX column (1 g) and the product was eluted with 2M NH₃ in MeOH. The product was further purified by preparative HPLC under basic conditions. The fractions containing product were pooled together and solvents were removed to obtain xx as off-white solid (26 mg, 23% yield). Purity by LCMS (UV chromatogram, 190-450 nm) 98%. ¹H-NMR (500 MHz, CDCl₃) δ 9.23 (broad peak, 1H), 8.71-8.70 (m, 1H), 8.34-8.32 (m, 1H), 7.42 (dd, 1H, *J*=4.8, 7.8 Hz), 6.81 (s, 1H), 4.55-4.531 (m, 2H), 3.73 (broad peak, 4H), 3.09-3.03 (m, 2H), 2.83 (s, 3H), 2.65 (s, 3H), 2.58-2.52 (m, 5H), 2.02-2.00 (m, 2H), 1.60-1.53 (m, 2H); LRMS (ES⁺) *m/z* 421 [M+H]⁺.

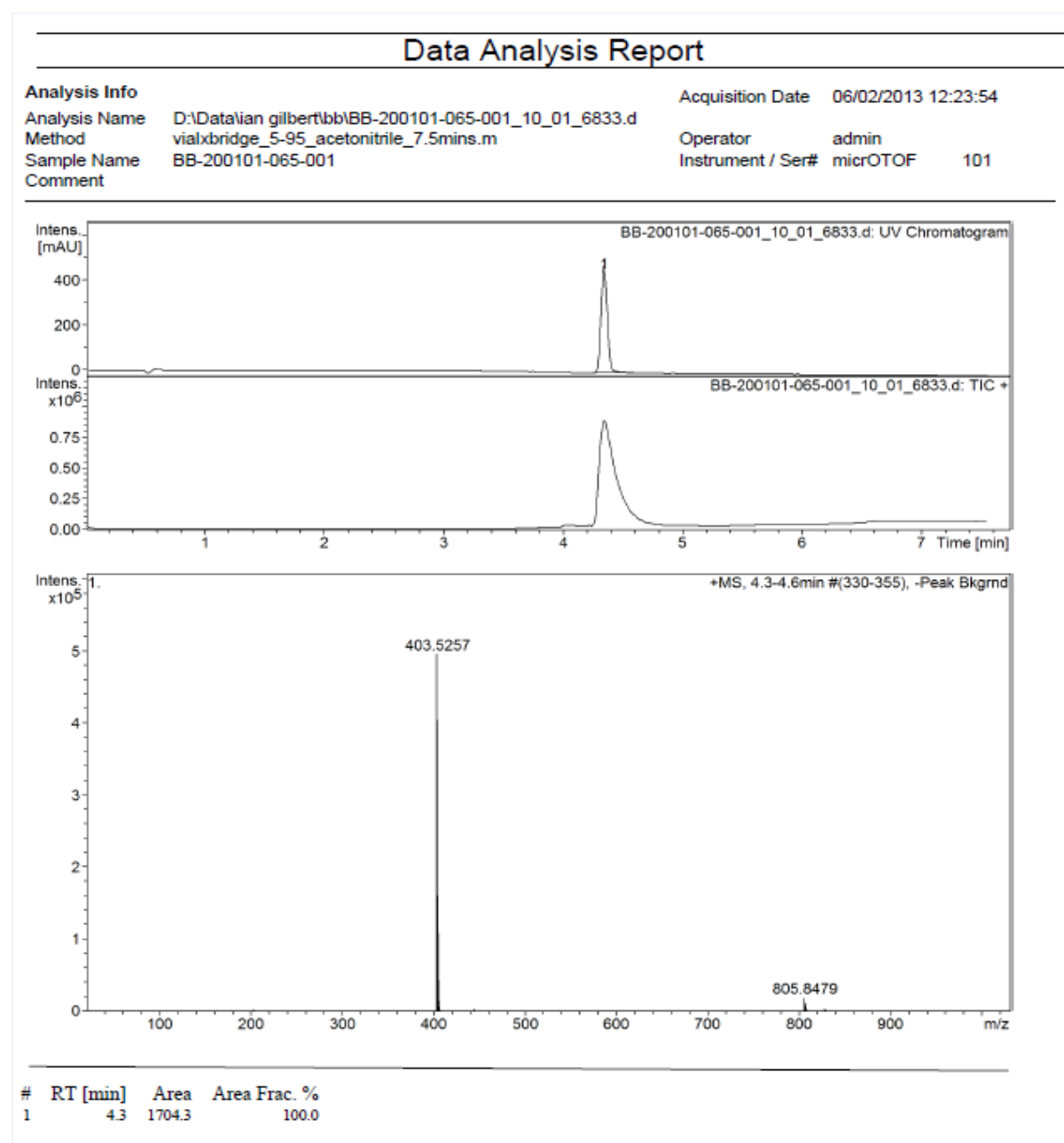
4-(1-(2-(2-fluoropyridin-4-yl)-6-(pyridin-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (80) Prepared in an analogous 4-step procedure to that of compound **51**: A mixture of 4-(1-(6-chloro-2-(2-fluoropyridin-4-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (131 mg, 0.35 mmol) was dissolved in DMF (4 mL) and to it added 3-pyridyl boronic acid (85 mg, 0.69 mmol), potassium phosphate (147 mg, 0.69 mmol) in water (0.5 mL) and Pd tetrakis (20 mg, 0.02 mmol). Mixture then heated in mw at 110°C for 1h. The mixture was then diluted with ethyl acetate (20 mL) and filtered through a celite column, the filtrate then partitioned and organics washed with 5% LiCl aq. (3 x 10 mL), brine (10 mL), dried over MgSO₄ and concentrated under reduced pressure. Mixture then purified by column (0-10% 7M ammonia in methanol/dichloromethane) to afford 4-(1-(2-(2-fluoropyridin-4-yl)-6-(pyridin-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (109 mg, 0.26 mmol, 75 %) as a colourless solid. Purity by LCMS (UV chromatogram, 190-450 nm) > 95%. ¹H-NMR (500 MHz, CDCl₃) 9.26 (d, 1H, *J* = 1.5 Hz), 8.73 (dd, 1H, *J* = 1.8, 4.7 Hz), 8.43 (dt, 1H, *J* = 1.8, 8.1 Hz), 8.34 (d, 1H, *J* = 5.0 Hz), 8.24 (d, 1H, *J* = 4.7 Hz), 7.98 (s, 1H), 7.47-7.44 (m, 1H), 6.96 (s, 1H), 4.65 (brs, 2H), 3.74 (t, 4H, *J* = 4.3 Hz), 3.11 (t, 2H, *J* = 11.7 Hz), 2.61-2.54 (m, 5H), 2.04 (brd, 2H, *J* = 15.1 Hz), 1.63-1.56 (m, 2H); LRMS (ES⁺) *m/z* 421 [M+H]⁺.

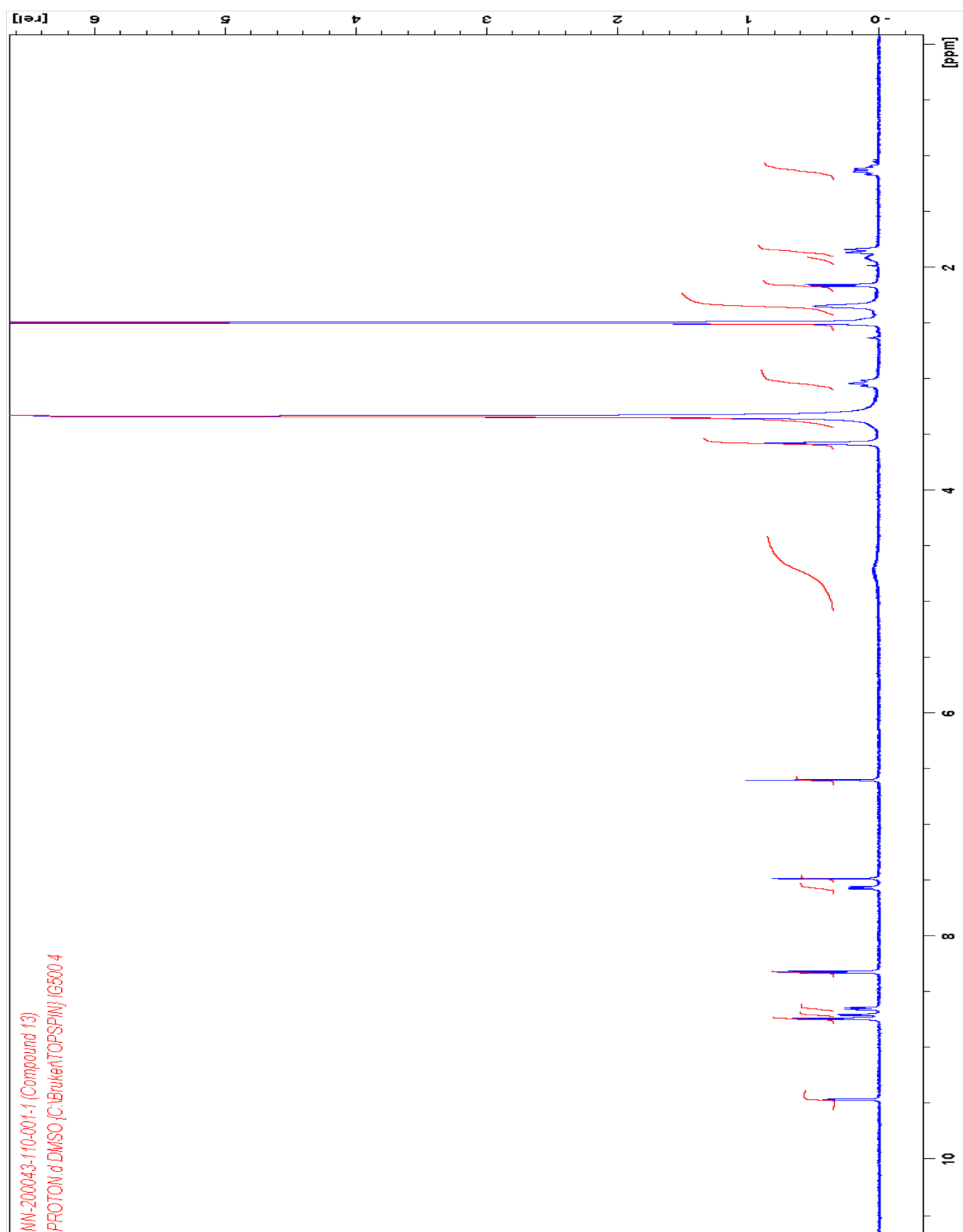
4-(1-(2-(2-methoxypyridin-4-yl)-6-(pyridin-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (81) In a sealed 5 ml microwave vial, a solution of 4-[1-[2-methylsulfanyl-6-(3-pyridyl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.10 g, 0.27 mmol) in 1,4-Dioxane (4 ml) was degassed by bubbling argon through for 5 minutes. (2-methyl-4-pyridyl)boronic acid (0.08 g, 0.54 mmol), thiophene-2-carboxylcopper (0.10 g, 0.54 mmol) and Pd(PPh₃)₄ (0.03 g, 0.03 mmol) were added at room temperature. The reaction was heated in a sealed tube at 130°C for 1h. The reaction crude was applied to a SCX column (2 g) and the product was eluted with 2 M NH₃ in MeOH. Solvents were removed under reduced pressured and the product was

purified by mass directed auto-preparative HPLC under basic conditions (5-95 prep basic). The fractions containing product were pooled together and solvents were removed to obtain xx as white solid (10 mg, 20% yield). Purity by LCMS (UV chromatogram, 190-450 nm) >98%. ¹H-NMR (500 MHz, CDCl₃) δ 9.32 (dd, 1H, J=0.6, 2.3 Hz), 9.26 (dd, 1H, J=0.7, 2.2 Hz), 8.70 (dd, 1H, J=1.7, 4.8 Hz), 8.65 (dd, 1H, J=2.4, 8.7 Hz), 8.44-8.42 (m, 1H), 7.44-7.41 (m, 1H), 6.85 (s, 1H), 6.82 (d, 1H, J=8.7 Hz), 4.68-4.65 (m, 2H), 4.02 (s, 3H), 3.77-3.76 (m, 4H), 3.08-3.02 (m, 2H), 2.64-2.61 (m, 5H), 2.05-2.00 (m, 2H), 1.67-1.50 (m, 2H); LRMS (ES+) m/z 433 [M+H]⁺.

Compound 12 ^1H NMR

Compound 12 LCMS



Compound 13 ^1H NMR

Compound 13 LCMS

Chromatogram List Report

Analysis Info

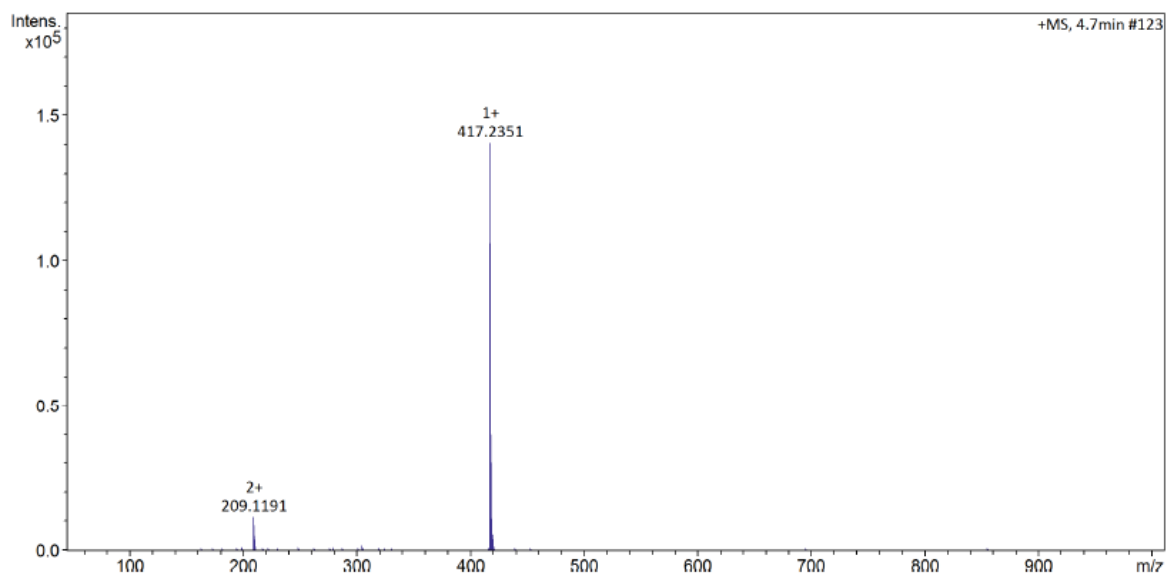
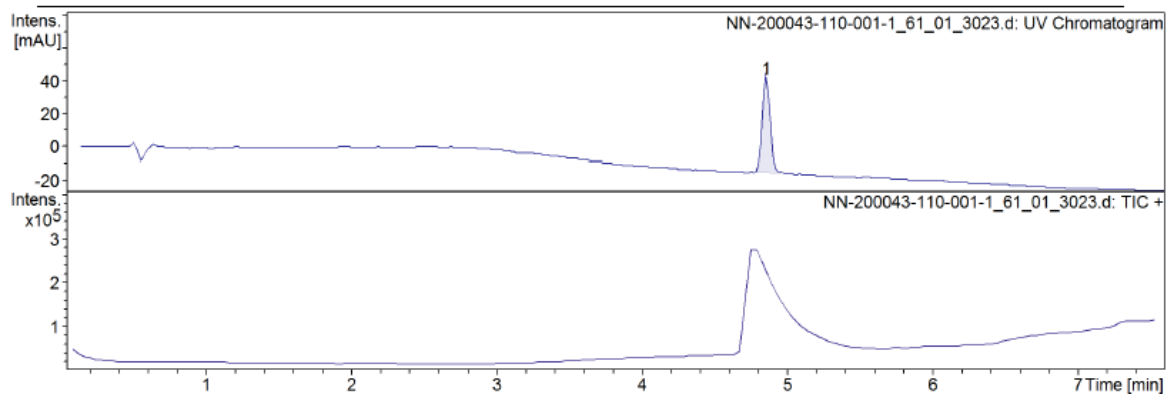
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Sample Name NN-200043-110-001-1
Comment

Acquisition Date 22/08/2012 14:02:49

Operator admin

Instrument microTOF 213750.00

101



#	RT [min]	Area	Area Frac. %
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Biology Methodology

Plasmodium falciparum screening

Assays against *P. falciparum* were conducted as previously described.¹⁻⁴ Cultures of the widely-used malaria reference strain of chloroquine-sensitive *Plasmodium falciparum* strain 3D7 were maintained in a 5% suspension of human red blood cells (obtained from East of Scotland Blood Transfusion Service, Ninewells Hospital, Dundee) cultured in RPMI 1640 medium (pH 7.3) supplemented with 0.5% Albumax II (Gibco Life Technologies, San Diego, CA), 12 mM sodium bicarbonate, 0.2 mM hypoxanthine and 20 mg/L gentamicin at 37°C, in a humidified atmosphere of 1% O₂, 3% CO₂ with a balance of nitrogen. Growth inhibition was quantified using a fluorescence assay utilising the binding of SYBR green to double stranded DNA, which emits a fluorescent signal at 528nm after excitation at 485nm.⁵ Mefloquine (potency range 30-60 nM) was used as a drug control to monitor the quality of the assay ($Z' = 0.6$ to 0.8 , Signal to background ≥ 3 , where Z' is a measure of the discrimination between the positive and negative controls on a screen plate). A 96-well [³H]-Hypoxanthine incorporation assay was also developed as a secondary assay in order to validate key compounds from each hit series in an orthogonal platform.⁶ Compound bioactivity from both assays was expressed as EC₅₀, the effective concentration of compound causing 50% inhibition of parasite growth.

Mammalian Cell Growth Inhibition assay

A counter-screen against normal diploid human fibroblasts (MRC-5 cell line) was carried out to exclude non-selective, and toxic compounds. The assay was essentially carried out as described previously.⁷ Cells were plated and incubated overnight to allow them to adhere as monolayers. A working stock of each test compound was transferred to an intermediate 384-well plate and pre-diluted with minimum essential media (MEM). The pre-diluted stocks were then transferred onto the cell monolayers, and the plates were incubated for 68 h. Resazurin, to a final concentration of 50 µM, was added to each well, after which plates were incubated for a further 3 h and measured for fluorescence ($\lambda_{\text{ex}}=528$ nm, $\lambda_{\text{em}}=590$ nm)

In vitro Cell Assay Data Analysis.

All data was processed using IDBS ActivityBase[®] raw data was converted into per cent inhibition through linear regression by setting the high inhibition control as 100% and the no inhibition control as 0%. Quality control criteria for passing plates were as follows: $Z' > 0.5$, $S:B > 3$, $\%CV_{(\text{no inhibition control})} < 15$. The formula used to calculate Z' is

$$1 - \frac{3 \times (StDev_high + StDev_low)}{ABS(Mean_high - Mean_low)}$$

All EC₅₀ Curve fitting was undertaken using XLFit version 4.2 using Model 205 with the following 4 parametric equation: $y = A + \frac{B - A}{1 + (C/x)^D}$ $A + \frac{(B-A)}{(1+(C/x)^D)}$, where A =% inhibition at bottom, B =% inhibition at top, C = EC₅₀, D = slope, x = inhibitor concentration and y = %

inhibition. If curve did not reach 100% of inhibition, B was fixed to 100 only when at least 50% of inhibition was reached.

Aqueous solubility

The aqueous solubility of the test compounds was measured using laser nephelometry, as described previously.⁸ Compounds were subject to serial dilution from 10 mg/mL to 0.5 mg/mL in DMSO. An aliquot was then mixed with MilliQ water to obtain an aqueous dilution plate with a final concentration range of 100 – 5 µg/mL, with a final DMSO concentration of 1.0%. Triplicate aliquots were transferred to a flat bottomed polystyrene plate which was immediately read on the NEPHELOstar (BMG Lab Technologies). The amount of laser scatter caused by insoluble particulates (relative nephelometry units, RNU) was plotted against compound concentration using a segmental regression fit, with the point of inflection being quoted as the compounds aqueous solubility (µg/mL). Assays were run in triplicate.

Intrinsic Clearance (CLi) experiments

The procedure was carried out as reported previously.⁸ Test compound (0.5 µM) was incubated with female CD1 mouse liver microsomes (Xenotech LLC TM ; 0.5 mg/mL 50 mM potassium phosphate buffer, pH 7.4) and the reaction started with addition of excess NADPH (8 mg/mL 50 mM potassium phosphate buffer, pH 7.4). Immediately, at time zero, then at 3, 6, 9, 15 and 30 min an aliquot (50µL) of the incubation mixture was removed and mixed with acetonitrile (100 µL) to stop the reaction. Internal standard was added to all samples, the samples centrifuged to sediment precipitated protein and the plates then sealed prior to UPLCMSMS analysis using a Quattro Premier XE (Waters Corporation, USA).

XLfit (IDBS, UK) was used to calculate the exponential decay and consequently the rate constant (k) from the ratio of peak area of test compound to internal standard at each timepoint. The rate of intrinsic clearance (CLi) of each test compound was then calculated using the following calculation:

$$CLi \text{ (mL/min/g liver)} = k \times V \times \text{Microsomal protein yield}$$

Where V (mL/mg protein) is the incubation volume/mg protein added and microsomal protein yield is taken as 52.5mg protein per g liver. Verapamil (0.5µM) was used as a positive control to confirm acceptable assay performance. Experiments were performed using a single time-course experiment.

Plasma Protein Binding (PPB) experiments

This was based on a previously described method, except using NMRI mice.⁹ In brief, a 96 well equilibrium dialysis apparatus was used to determine the free fraction in plasma for each compound (HT Dialysis LLC, Gales Ferry, CT). Membranes (12-14 kDA cut-off) were conditioned in deionised water for 60 min, followed by conditioning in 80:20 deionised water:ethanol for 20 min, and then rinsed in isotonic buffer before use. Female CD1 mouse plasma was removed from the freezer and allowed to thaw on the day of experiment. Thawed plasma was then centrifuged (Allegra X12-R, Beckman Coulter, USA), spiked with test

compound (final concentration 10 µg/mL), and 150 µL aliquots (n=6 replicate determinations) loaded into the 96-well equilibrium dialysis plate. Dialysis against isotonic buffer (150 µL) was carried out for 5 h in a temperature controlled incubator at ~37°C (Barworld scientific Ltd, UK) using an orbital microplate shaker at 100 revolutions/minute (Barworld Scientific Ltd, UK). At the end of the incubation period, 50 µL aliquots of plasma or buffer were transferred to micronic tubes (Micronic B.V., the Netherlands) and the composition in each tube balanced with control fluid (50 µL), such that the volume of buffer to plasma is the same. Sample extraction was performed by the addition of 200 µL of acetonitrile containing an appropriate internal standard. Samples were allowed to mix for 1 min and then centrifuged at 3000 rpm in 96-well blocks for 15 min (Allegra X12-R, Beckman Coulter, USA) after which 150 µL of supernatant was removed to 50 µL of water. All samples were analysed by UPLC-MS/MS on a Quattro Premier XE Mass Spectrometer (Waters Corporation, USA). The unbound fraction was determined as the ratio of the peak area in buffer to that in plasma. Experiments were run in triplicate.

Fluorescence-based CYP Inhibition using recombinantly expressed CYP batosomes

Fluorogenic CYP inhibition studies were conducted at 37 °C in 96-well, flat-bottom, clear polystyrene plates. Incubation mixtures containing EasyCYP batosomes (1000 pmoles/mL, 10 mg/mL CypexTM), fluorogenic substrate (CypexTM) and 50 mM potassium phosphate buffer (pH 7.4) were prepared at the following final concentrations: CYP1A2, 5 pmol/mL + 0.5 µM Ethoxyresorufin (ER); CYP2C9, 10 pmol/mL + 30 µM 7-methoxy-4-(trifluoromethyl)-coumarin (MFC); CYP2C19, 5 pmol/mL + 25 µM 3-Cyano-7-Ethoxycoumarin (CEC); CYP2D6, 10 pmol/mL + 6 µM 7-methoxy-4-(aminomethyl)-coumarin (MAMC); CYP3A4, 10 pmol/mL + 1 µM Diethoxyfluorescein (DEF) & 10 pmol/mL + 15 µM 7-Benzylxyquinoline (BQ). Batosome control protein was included in reactions to give a final concentration of 0.025 mg/mL. Reaction times were verified to be within the limits of kinetics linearity. Test compounds were prepared as 5 mM solutions in DMSO and serially diluted 1 in 3.03, 1 in 3.3 alternatively in a v-bottomed 96 well plate to give a concentration range of 5, 1.65, 0.5, 0.165, 0.05, 0.0165, 0.05 mM. Positive control inhibitor, miconazole, was prepared as a 0.5 mM solution in DMSO and similarly diluted.

For each isoform, 220 µL of incubation mix was added to each well of a 96-well, flat-bottom, clear polystyrene plate. 5 µL of each concentration was then mixed with 220 µL of the incubation mix and pre-incubated at 37°C for 5 mins (final test compound concentration range: 100, 33, 10, 3.3, 1.0, 0.33, 0.1 or 10, 3.3, 1.0, 0.33, 0.1, 0.033, 0.01 and 0 µM; miconazole 10, 3.3, 1.0, 0.33, 0.1, 0.033, 0.01 and 0 µM). Reactions were initiated by the addition of 25 µL regenerating cofactor solution (23 mM Glucose-6-Phosphate, 2.2 mM NADP, 6 Units per mL Glucose-6-Phosphate Dehydrogenase (from Baker's yeast *S. cerevisiae*) in 2% w/v NaHCO₃, Sigma) and subsequent production of fluorescence metabolite measured at 1 minute intervals over a 10 minute period using a BMG Optima fluorescence detector (ER: Exc 530nm, Em 590nm, MFC: Exc 410 nm, Em 530nm, CEC: Exc 410 nm, Em 530 nm, MAMC: Exc 405 nm, Em 460 nm, DEF: Exc 405 nm, Em 560 nm, 7BQ: Exc 485 nm, Em 530 nm).

Fluorescence responses were calculated as a percentage of uninhibited control and plotted against compound concentration to provide IC₅₀ values using the following equation.

$$y = \frac{Range}{1 + \left(\frac{x}{IC_{50}} \right)^s}$$

***In vivo* pharmacokinetics**

Compound was dosed as a bolus solution intravenously (**12**) at 3mg free base/kg (dose volume: 5 mL/kg; dose vehicle: 10% DMSO, 90% saline) to female NMRI mice (n=3) or dosed orally (**12** and **13**) by gavage as a solution at 10 mg free base/kg (dose volume: 10mL/kg; Dose vehicle: 5 or 10% DMSO; 40% PEG400; 50 or 55% distilled water) to female NMRI mice (n=3/dose level). Female NMRI mice were chosen as these represent the sex and strain used for the *P. berghei* mouse model of malaria. Blood samples (10 µl) were taken from each mouse at 5, 15 and 30 minutes, 1, 2, 4, 6, and 8 hours post-dose, mixed with two volumes of distilled water and stored frozen until UPLC/MS/MS analysis. The level of each compound in mouse blood was determined by UPLC-MS/MS as previously reported.¹⁰ Pharmacokinetic parameters were derived from the blood concentration time curve using PKsolutions software v 2.0 (Summit Research Services, USA).

***In vivo* antimalarial efficacy studies in *P. berghei*. (Swiss TPH)**

In vivo efficacy was conducted as previously described¹¹ with the modification that female NMRI mice (n = 3) were infected with a GFP-transfected *P. berghei* ANKA strain (donated by A. P. Waters and C. J. Janse, Leiden University, The Netherlands), and parasitaemia determined using standard flow cytometry techniques. Compounds **12** and **13** were prepared in 10% DMSO, 40% PEG 400 and 50% water prior to administration orally, once daily for 4 days. Blood samples were collected on day 4 (96 h after infection). Animals were observed for signs of overt toxicity/poor tolerability every 15 min for the first hour post dosing and then hourly up to 4 h after dosing each day. The animals were selected randomly for each group but were not blinded.

***In vivo* antimalarial efficacy studies in *P. falciparum*. (GlaxoSmithKline)**

Assays against *P. falciparum* were conducted as previously described.¹² Age-matched female immunodeficient NOD-scid IL-2R γ c-null mice (The Jackson Laboratory, Bar Harbor, ME) were engrafted with human erythrocytes (Red Cross Transfusion Blood Bank in Madrid, Spain) by daily injection with 1 mL of a 50% hematocrit erythrocyte suspension (RPMI 1640 medium, 25% (vol/vol) decomplexed human serum, 3.1 mM hypoxanthine) by intraperitoneal route throughout the experiment. When mice reached approximately 40% human erythrocytes in peripheral blood, they were intravenously infected with 2×10⁷ *P. falciparum* Pf3D^{70087/N9}-infected erythrocytes (day 0). Drug treatments were administered on day 3 after infection to mice randomly allocated to treatments once a day for 4 consecutive days by oral gavage at 10

mL/kg. Compound **13** was prepared in 10% DMSO; 40% PEG 400, 50% water before administration. Parasitaemia was measured by flow cytometry in samples of peripheral blood stained with the fluorescent nucleic acid dye SYTO-16 and anti-murine erythrocyte TER119 monoclonal antibody (Pharmingen, San Diego, CA, USA) in serial 2 μ L blood samples taken every 24 h until assay completion.

The blood levels of **13** in the mice of the efficacy experiment were measured in serial samples of peripheral blood (25 μ L) taken by tail puncture at 0.25, 0.5, 1, 3, 6, 8, 12 and 23 hours after the first administration. The blood samples were immediately lysed by mixing with 25 μ L of distilled water, frozen on dry ice and stored at -80°C until analysis. The compounds were extracted from 10 μ L of each lysate by liquid-liquid extraction in the MultiScreen Solvinert 0.45 μ m Hydrophobic PTFE 96- well plate system (Millipore) and stored frozen at -80°C until analysis by LC/MS/MS in AB Sciex API4000 (AB Sciex, Framingham, MA). The compound concentration versus time were analysed by non-compartmental analysis (NCA) using Phoenix® Version 6.3 (Pharsight Corporation, Mountain View, CA, USA). Additional statistical analysis was performed with GraphPad Prism® Version 6.02 (GraphPad Software Inc, San Diego CA, USA).

Efficacy was expressed as the daily exposure (AUC, μ g·h/mL/day) of **13** in whole blood necessary to reduce parasitaemia at day 7 by 90 % with respect to vehicle-treated mice (AUC_{ED90}). The AUC_{ED90} was estimated by fitting a four parameter logistic equation for the log₁₀ [parasitaemia at day 7 for individual *i*] versus the AUC_{0-23h} of **13** in blood for individual *i* using GraphPad Prism 6.0. (*i* = inhibitor concentration).

β -Haematin formation assay (GlaxoSmithKline)

The *in vitro* β -haematin formation assay was conducted as previously described.¹³ Briefly, the compounds were dissolved in DMSO and 1/3 serial dilutions were tested in a final reaction volume of 0.4 mL using oleoyl glycerol as catalytic agent. Each assay was run at least in triplicate. Non-linear regression analysis was used to fit the normalized results of the dose response curves and IC₅₀ values determined using the GraFit5 software package (GraphFit program; Erithacus Software, Horley, Surrey, UK).

***In vitro* parasite reduction ratio (PRR) assay (GlaxoSmithKline)**

The *in vitro* Parasite Rate Reduction assay (PRR) was conducted as previously described.¹⁴ Briefly, parasites were exposed to **13** for 120 h at a concentration corresponding to 10 x EC₅₀. Drug was renewed daily over the entire treatment period. Samples of parasites were taken from the treated culture at intervals (24, 48, 72, 96 and 120 h time points), drug was washed out and drug-free parasites were cultured in 96-well plates by adding fresh erythrocytes and new culture media. The number of viable parasites was determined by the serial dilution technique. Four independent serial dilutions were done with each sample to correct for experimental variation.

Human ether-à-go-go related gene (hERG) K⁺ assay (Outsourced)

Compounds were tested for inhibition of the human ether-à-go-go-related gene (hERG) K⁺ channel using IonWorks patch clamp electrophysiology. Eight-point concentration-response

SUPPORTING INFORMATION

curves were generated on 2 occasions using 3-fold serial dilutions from the maximum final assay concentration.

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