Medicinal Chemistry Optimization of Antiplasmodial Imidazopyridazine Hits from High Throughput Screening of a SoftFocus Kinase Library: Part 1.

Claire Le Manach^{†,⊥}, Diego González Cabrera[†], Frederic Douelle[†], Aloysius T. Nchinda[†], Yassir Younis[†], Dale Taylor[∞], Lubbe Wiesner[∞], Karen L. White[§], Eileen Ryan[§], Corinne March[§], Sandra Duffy[⊙], Vicky M Avery[⊙], David Waterson[‡], Michael J. Witty[‡], Sergio Wittlin^{⊙,‡}, Susan A. Charman[§], Leslie J. Street[†] and Kelly Chibale^{,†,‡,⊥}*

Contents:

A. Additional spectroscopic data	S2-S10
B. HPLC method	S10
C. Metabolic stability - Microsome predicted hepatic extraction ratio (E_H)	S11
D. Physicochemical data	S13
E. SCID mouse studies	S17
F. Rat pharmacokinetic studies	S17
I. Additional references	S18

A. Additional Spectroscopic data

Pyridyl derivatives:

3-(pyridin-4-yl)-6-(3-(trifluoromethoxy)phenyl)imidazo[1,2-b]pyridazine (10):

Column chromatography: DCM/MeOH (98:2), Recrystallization in DCM/AcOEt, 68%.

^1H NMR (300 MHz, CDCl_3), δ **8.76** (d, 2H; $J = 6.0$); **8.29** (s, 1H); **8.16** (d, 1H; $J = 9.3$); **8.12** (d, 2H; $J = 6.0$); **7.96** (ddd, 1H); **7.92, 7.88** (m, 1H); **7.63** (d, 1H); **7.61** (d, 1H; $J = 9.3$); **7.42, 7.38** (m, 2H), ^{13}C NMR (75 MHz, CDCl_3), δ 150.9, 150.6, 150.2, 145.4, 140.8, 137.7, 136.0, 135.4, 130.9, 126.9, 125.5, 122.7, 120.2, 119.8, 116.3. MS (EI+): $m/z = 355.9$ (exact Mass = 356.0985)

6-(3-(tert-butyl)phenyl)-3-(pyridin-4-yl)imidazo[1,2-b]pyridazine (11):

Column chromatography: DCM/MeOH (99:1, 98:2, 95:5), Recrystallization in AcOEt,

63%. ^1H NMR (400 MHz, CDCl_3), δ **8.73** (d, 2H; $J = 5.6$); **8.26** (s, 1H); **8.18** (d, 2H, $J = 6.4$); **8.11** (m, 2H); **8.781** (dd, 2H; $J = 1.2$, $J = 7.2$); **7.63** (d, 1H; $J = 9.6$); **7.59** (dd, 1H, $J = 1.2$, $J = 8.0$); **7.51** (dd, 2H; $J = 8.0$); **1.43** (s, 9H), ^{13}C NMR (100 MHz, CDCl_3), δ 152.6, 152.3, 150.2, 140.7, 136.2, 135.1, 134.7, 129.0, 127.5, 126.2, 125.9, 124.3, 124.2, 120.0, 116.8, 53.3, 31.3. MS (EI+): $m/z = 327.8$ (exact Mass = 328.1688)

6-(4-fluorophenyl)-3-(pyridin-4-yl)imidazo[1,2-b]pyridazine (12):

Column chromatography: DCM/MeOH (99:1, 98:2, 95:5), Recrystallization in

DCM/MeOH, 44%. ^1H NMR (400 MHz, CDCl_3), δ **8.75** (d, 2H; $J = 6.6$); **8.26** (s, 1H); **8.13, 8.11** (m, 3H; $J = 6.6$, $J = 9.6$); **8.02** (dd, 2H; $J = 8.8$); **7.58** (d, 1H; $J = 9.6$); **7.29, 7.25** (dd, 2H; $J = 8.8$), ^{13}C NMR (100 MHz, CDCl_3), δ 165.5, 163.0, 161.6, 151.3, 150.3, 136.0, 134.9, 129.2, 129.0, 120.0, 127.1, 116.5, 116.3. MS (EI+): $m/z = 290.1$ (exact Mass = 290.0968)

6-(3-chlorophenyl)-3-(pyridin-4-yl)imidazo[1,2-b]pyridazine (13):

Column chromatography: DCM/MeOH (98:2), Recrystallization in DCM/MeOH, 51%.

^1H NMR (400 MHz, CDCl_3), δ **8.75** (d, 2H; $J = 6.4$); **8.26** (s, 1H); **8.13** (d, 1H; $J = 9.6$);

8.10 (d, 2H; J = 6.4); **7.99, 7.97** (m, 1H); **7.93, 7.87** (m, 1H); **7.57** (d, 1H; J = 9.6); **7.53, 7.48** (m, 2H)

5-(3-(pyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl)isoquinoline (14):

Column chromatography: DCM/MeOH (98:2, 95:5), Recrystallization in DCM/MeOH, 20%. ¹H NMR (300 MHz, CDCl₃), δ **9.58** (d, 1H; J = 1.2); **8.74, 8.72** (m, 3H); **8.68** (d, 1H; J = 6.0); **8.54** (d, 1H; J = 9.6); **8.46** (d, 1H, J = 8.1); **8.31** (d, 2H, J = 6.3); **8.23** (dd, 1H, J = 1.2, J = 7.2); **8.21** (d, 1H; J = 6.0); **8.00** (dd, 1H; J = 7.2, 8.1); **7.82** (d, 1H, J = 9.3)

6-(3-fluorophenyl)-3-(pyridin-4-yl)imidazo[1,2-b]pyridazine (15):

Column chromatography: DCM/MeOH (98:2, 95:5), Recrystallization in DCM/MeOH, 40%. ¹H NMR (300 MHz, CDCl₃), δ **8.76** (d, 2H, J = 6.3); **8.28** (s, 1H); **8.16, 8.11** (m, 3H); **7.81** (ddd, 1H, J = 1.2, J = 1.5, J = 7.8); **7.74** (ddd, 1H, J = 1.2, J = 2.4, J = 9.9); **7.60** (d, 1H, J = 9.6); **7.59, 7.51** (m, 1H); **7.29, 7.20** (m, 1H)

4-(3-(3-(pyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl)phenyl)morpholine (16):

Column chromatography: DCM/MeOH (98:2, 95:5), Recrystallization in Hexane, 67%. ¹H NMR (300 MHz, CDCl₃), δ **8.73** (d, 2H; J = 6.2); **8.27** (s, 1H); **8.16** (d, 2H; J = 6.2); **8.11** (d, 1H; J = 9.6); **7.61** (d, 1H; J = 9.6); **7.58** (br, 1H); **7.49, 7.47** (m, 2H); **7.14, 7.07** (m, 1H); **3.93** (m, 2H); **3.29** (m, 2H), ¹³C NMR (75 MHz, CDCl₃), δ 152.5, 150.2, 136.2, 134.8, 130.0, 126.2, 120.0, 118.7, 117.6, 116.9, 114.0, 66.8, 49.2. MS (EI+): m/z = 356.96 (exact Mass = 357.1590)

6-(3,4-difluorophenyl)-3-(pyridin-4-yl)imidazo[1,2-b]pyridazine (17):

Column chromatography: DCM/MeOH (98:2), Recrystallization in DCM/MeOH, 53%. ¹H NMR (300 MHz, CDCl₃), δ **8.76** (d, 2H, J = 6.3); **8.27** (s, 1H); **8.15** (d, 1H; J = 9.6); **8.09** (d, 2H, J = 6.3); **7.87** (ddd, 1H, J = 2.4, 7.5, 11.4); **7.78, 7.72** (m, 1H); **7.54** (d, 1H; J = 9.6); **7.36** (ddd, 1H; J = 8.1, 9.9). MS (EI+): m/z = 308.0 (exact Mass = 308.0874)

6-(6-methoxypyridin-3-yl)-3-(pyridin-4-yl)imidazo[1,2-b]pyridazine (18):

Column chromatography: DCM/MeOH (97:3, 95:5, 90:10), Recrystallization in AcOEt/Hex, 49%. ¹H NMR (300 MHz, CD₃OD), δ **8.83** (d, 1H; J = 2.3); **8.74** (d, 2H; J = 1.5, J = 6.0); **8.25** (s, 1H); **8.23** (dd, 1H; J = 2.3, J = 8.9); **8.14**, **8.08** (m, 3H); **7.55** (d, 1H; J = 9.6); **6.94** (d, 1H; J = 9.0); **4.04** (s, 3H). ¹³C NMR (100 MHz, CDCl₃) 165.6, 162.5, 150.3, 150.0, 146.1, 140.6, 137.0, 136.0, 134.8, 126.6, 126.1, 124.7, 119.9, 115.8, 111.6, 53.9. MS (EI+): m/z = 302.8 (exact Mass = 303.1120)

(4-(3-(pyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl)phenyl)methanamine (19):

Column chromatography: DCM/MeOH (98:2, 95:5, 90:10), Recrystallization in DCM/Hex, 20%. ¹H NMR (400 MHz, CDCl₃), δ **8.66** (d, 2H, J = 6.4); **8.19** (s, 1H); **8.04** (d, 2H; J = 6.4); **7.90** (d, 2H, J = 9.6); **7.87** (d, 2H, J = 8.4); **7.53** (d, 2H, 8.4); **7.46** (d, 1H; J = 9.6); **3.81** (s, 2H)

(3-(3-(pyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl)phenyl)methanamine (20):

The Boc-protected amine (**10**) (117 mg, 0.291 mmol, 1 eq) was dissolved in DMF (0.4 mL) and a solution of 4M HCl in dioxane (875 µL, 3.5 mmol, 12 eq) was added. The solution became white and cloudy and was stirred at room temperature overnight. After concentration of the solvents the residue was dissolved in MeOH and water and treated with Amberlyst A-21. After 30 min stirring, the resin was filtered off. Recrystallization in DCM/Hex, 47%. ¹H NMR (300 MHz, DMSO), δ **8.72** (d, 2H; J = 6.0); **8.56** (s, 1H); **8.39** (d, 1H; J = 9.6); **8.32**, **8.28** (m, 3H; J = 9.6, J = 6.0); **8.14** (d, 1H; J = 7.6); **8.14** (d, 1H; J = 7.6); **7.98** (d, 1H; J = 9.6); **7.72**, **7.60** (m, 2H; J = 7.6); **4.15** (s, 1H), ¹³C NMR (100 MHz, DMSO), δ 151.9, 150.7, 141.0, 136.1, 135.9, 135.7, 131.3, 130.0, 128.3, 127.5, 127.3, 125.5, 120.8, 117.5

3-(pyridin-4-yl)-6-(6-(trifluoromethyl)pyridin-3-yl)imidazo[1,2-b]pyridazine (21):

Column chromatography: DCM/MeOH (97:3, 95:5, 90:10), Recrystallization in AcOEt/Hex, 49%. ¹H NMR (300 MHz, CD₃OD), δ **8.75** (dd, 2H; J = 1.8, J = 6.3); **8.27** (s, 1H); **8.17**, **8.03** (m, 5H, J = 1.8, J = 6.3, J = 9.3); **7.59** (d, 1H; J = 9.3); **7.42** (d, 2H; J = 8.1), ¹³C NMR (100 MHz, CDCl₃), δ 151.0, 150.4, 146.4, 140.6, 135.1, 133.9, 128.8, 126.6, 126.1, 121.5, 120.0, 116.2. MS (EI+): m/z = 355.9 (exact Mass = 356.0885)

3-(pyridin-4-yl)-6-(4-(trifluoromethoxy)phenyl)imidazo[1,2-b]pyridazine (22):

Column chromatography: DCM/MeOH (97:3, 95:5, 90:10), Recrystallization in AcOEt/Hex, 49%. ¹H NMR (300 MHz, CD₃OD), δ **8.75** (dd, 2H; J = 1.8, J = 6.3); **8.27** (s, 1H); **8.17, 8.03** (m, 5H); **7.59** (d, 1H; J = 9.3); **7.42** (d, 2H; J = 8.1), ¹³C NMR (100 MHz, CDCl₃), δ 151.0; 150.4 C-5; 146.4, 140.6; 135.1 C-3; 133.9; 128.8 C-7; 126.6 C-1; 126.1; 121.5 C-6; 120.0 C-4; 116.2 C-2. MS (EI+): m/z = 355.9 (exact Mass = 356.0885)

6-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)-3-(pyridin-4-yl)imidazo[1,2-b]pyridazine (23):

Column chromatography: DCM/MeOH (98:2, 95:5), Recrystallization in AcOEt, 49%. ¹H NMR (400 MHz, CDCl₃), δ **8.77** (d, 2H, J = 6.2); **8.31** (s, 1H); **8.16** (d, 1H; J = 8.8); **8.14** (d, 2H, J = 8.4); **8.11** (d, 2H, J = 6.2); **7.96** (m, 2H, J = 8.4); **7.62** (d, 1H; J = 8.8); **3.13** (s br, 4H); **2.52** (s br, 4H); **2.29** (s, 3H). MS (EI+): m/z = 434.2 (exact Mass = 434.1525)

6-(4-(tert-butyl)phenyl)-3-(pyridin-4-yl)imidazo[1,2-b]pyridazine (24):

Column chromatography: DCM/MeOH (98:2), Recrystallization in AcOEt, 47%. ¹H NMR (400 MHz, CDCl₃), δ **8.72** (d, 2H, J = 6.0); **8.23** (s, 1H); **8.14** (d, 2H; J = 6.0); **8.07** (d, 1H, J = 9.6); **7.95** (d, 2H, J = 8.2); **7.61, 7.57** (m, 3H); **1.39** (s, 9H). MS (EI+): m/z = 328.2 (exact Mass = 328.1688)

4-((4-(3-(pyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl)phenyl)sulfonyl)morpholine (25):

Column chromatography: DCM/MeOH (98:2), (95:5), (9:1), (85:15), Recrystallization in AcOEt, 54%. ¹H NMR (300 MHz, CDCl₃), δ **8.77** (d, 2H; J = 6.0); **8.31** (s, 1H); **8.22, 8.19** (m, 3H); **8.11** (d, 2H; J = 6.0); **7.96** (d, 2H; J = 8.1); **7.65** (d, 1H; J = 9.5); **3.78** (t, 2H; J = 4.8); **3.10** (t, 2H; J = 4.8). ¹³C NMR (75 MHz, CDCl₃), δ 150.4, 145.2, 139.9, 137.1, 135.8, 135.5, 128.7, 127.8, 126.9, 126.3, 120.0, 116.2, 66.1, 46.0. MS (EI+): m/z = 420.6 (exact Mass = 421.1209)

tert-butyl 4-(3-(pyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl)benzylcarbamate (26):

Column chromatography: DCM/MeOH (98:2), Recrystallization in AcOEt, 40%. ¹H NMR (400 MHz, CDCl₃), δ **8.74** (d, 2H, J = 6.0); **8.25** (s, 1H); **8.14, 8.10** (m, 3H); **7.99** (d, 2H, J = 8.0); **7.60** (d, 1H, J = 9.6); **7.49** (d, 2H, J = 8.0); **4.98** (s br, 1H); **4.47** (s, 2H); **1.49** (s, 9H). MS (EI+): m/z = 400.9 (exact Mass = 401.1852)

8-(3-(pyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl)quinoline (27):

Column chromatography: DCM/MeOH (98:2), (95:5), Recrystallization in AcOEt, 67%. ¹H NMR (300 MHz, CDCl₃), δ **8.97** (dd, 1H; J = 2.0, J = 4.0); **8.66** (d, 2H; J = 6.4); **8.30** (dd, 1H; J = 1.6, J = 8.0); **8.27** (s, 1H); **8.191, 8.127** (m, 3H); **8.09** (d, 1H; J = 9.4); **8.03** (dd, 1H; J = 1.6, J = 8.4); **7.61** (d, 1H; J = 9.4); **7.75** (dd, 1H; J = 6.8, J = 8.0); **7.51** (dd, 1H; J = 4.0), ¹³C NMR (75 MHz, CDCl₃), δ 153.2, 150.7, 150.2, 145.9, 140.9, 136.9, 136.3, 135.0, 134.4, 131.2, 130.3, 128.7, 126.5, 125.7, 124.2, 122.7, 121.6, 119.9. MS (EI+): m/z = 323.11 (exact Mass = 323.1171)

4-(4-(3-(pyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl)phenyl)morpholine (28):

Column chromatography: DCM/MeOH (98:2, 95:5), Recrystallization in AcOEt, 39%. ¹H NMR (300 MHz, CDCl₃), δ **8.73** (d, 2H, J = 6.0); **8.21** (s, 1H); **8.15** (d, 2H; J = 6.0); **8.05** (d, 1H, J = 9.6); **7.95** (d, 2H, J = 9.0); **7.57** (d, 1H, J = 9.6); **7.05** (d, 2H; J = 9.0); **3.90** (t, 4H; J = 4.8); **3.31** (t, 4H; J = 4.8)

6-(4-(methylsulfonyl)phenyl)-3-(pyridin-4-yl)imidazo[1,2-b]pyridazine (29):

Column chromatography: DCM/MeOH (95:5, 90:10), Recrystallization in AcOEt/Hex, 38%. ¹H NMR (300 MHz, CD₃OD), δ **8.78** (dd, 2H; J = 1.8, J = 6.3); **8.33** (s, 1H); **8.14, 8.05** (m, 7H); **7.65** (d, 1H; J = 9.3); **3.19** (s, 3H), ¹³C NMR (100 MHz, CDCl₃), δ 150.4, 142.3, 135.8, 128.4, 128.1, 126.9, 120.0, 116.2, 44.5. MS (EI+): m/z = 349.8 (exact Mass = 350.0837). m.p. = 286°C.

5-(3-(pyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl)benzo[c][1,2,5]oxadiazole (31):

Recrystallization in DCM, 33%. ¹H NMR (400 MHz, CDCl₃), δ **8.80** (d, 2H; J = 6.4); **8.44** (dd, 1H; J = 1.2); **8.33** (s, 1H); **8.26** (dd, 1H; J = 2.4, J = 9.6); **8.24** (d, 1H; J = 9.2); **8.12, 8.07** (m, 3H); **7.73** (d, 1H; J = 9.2). MS (EI+): m/z = 314.0 (exact Mass = 314.0916)

Methylsulfonylphenyl derivatives:

3-(4-(methylsulfonyl)phenyl)-6-(3-(trifluoromethyl)phenyl)imidazo[1,2-b]pyridazine (41):

Crystallization of the product during the concentration, Recrystallization in AcOEt, 36%.

¹H NMR (300 MHz, CDCl₃), δ **8.42** (d, 2H; J = 8.7); **8.25** (s, 1H); **8.22** (d, 1H; J = 7.8); **8.18** (d, 1H; J = 9.3); **8.10** (d, 2H; J = 8.7); **7.81** (d, 1H; J = 7.8); **7.72** (t, 1H; J = 7.8); **7.65** (d, 1H; J = 9.3); **3.75** (t, 2H; J = 6.0); **3.11** (s, 3H). MS (EI+): m/z = 416.5 (exact Mass = 417.0759)

N-(2-hydroxyethyl)-3-(3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)benzamide (42):

Crystallization of the product during the concentration, Recrystallization in AcOEt, 46%.

¹H NMR (300 MHz, CD₃OD/CDCl₃), δ **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; 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** (dd, 1H; J = 7.8); **3.75** (t, 2H; J = 6.0); **3.56** (t, 2H; J = 6.0); **3.12** (s, 3H). MS (EI+): m/z = 436.0 (exact Mass = 436.1205)

3-(3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)benzamide (43):

Column chromatography: DCM/MeOH (98:2, 95:5, 9:1, 85:15), Recrystallization in

AcOEt, 38%. ¹H NMR (300 MHz, dMSO-d₆), δ **8.59**, **8.55** (m, 3H); **8.50** (s, 3H); **8.39** (d, 1H; J = 9.6); **8.33** (dd, 1H; J = 1.8, J = 7.8); **8.10** (d, 2H; J = 8.7); **8.09**, **8.05** (m, 1H); **8.03** (d, 1H; J = 9.6); **7.70** (dd, 1H; J = 7.8); **3.28** (s, 3H). m.p. = 260°C.

3-(3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)benzonitrile (44):

Column chromatography: DCM/MeOH (98:2, 95:5), Recrystallization in AcOEt, 66%.

¹H NMR (300 MHz, dMSO-d₆), δ **8.61** (t, 1H; J = 2.0); **8.55** (d, 2H; J = 8.8); **8.53** (s, 1H); **8.53**, **8.50** (m, 1H); **8.43** (d, 1H; J = 9.6); **8.11** (d, 2H; J = 8.8); **8.06** (d, 1H; J = 9.7); **8.07**, **8.04** (m, 1H); **7.83** (dd, 1H; J = 7.6, J = 8.4); **3.29** (s, 3H). MS (EI+): m/z = 374.0 (exact Mass = 374.0837). m.p. = 185°C.

**N,N-dimethyl-3-(3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)aniline
(45):**

Column chromatography: DCM/MeOH (98:2, 95:5), Recrystallization in AcOEt, 78%. ¹H NMR (300 MHz, dMSO-d₆), δ **8.57** (d, 2H; J = 8.4); **8.47** (s, 3H); **8.30** (d, 1H; J = 9.3); **8.07** (d, 2H; J = 8.4); **7.93** (d, 1H; J = 9.3); **7.48, 7.36** (m, 3H); **7.01, 6.90** (m, 1H); **3.26** (s, 3H); **3.03** (s, 6H). MS (EI+): m/z = 392.0 (exact Mass = 392.4810)

**3-(4-(methylsulfonyl)phenyl)-6-(4-(trifluoromethyl)phenyl)imidazo[1,2-b]pyridazine
(46):**

Column chromatography: AcOEt/MeOH (1:0, 9:1), Recrystallization in MeOH/AcOEt, 53%. ¹H NMR (400 MHz, CD₃OD), δ **8.38** (d, 2H; J = 8.6); **8.24** (s, 1H); **8.17** (d, 1H; J = 9.2); **8.12** (d, 2H; J = 8.4); **8.09** (d, 2H; J = 8.6); **7.83** (d, 2H; J = 8.4); **7.63** (d, 1H; J = 9.2). MS (EI+): m/z = 417.0 (exact Mass = 417.0759)

(47): Column chromatography: DCM/MeOH (98:2), Recrystallization in DCM/MeOH, 25%. ¹H NMR (300 MHz, CD₃OD), δ **8.25** (d, 2H; J = 8.7); **8.06, 8.00** (m, 2H); **7.98** (d, 1H; J = 9.3); **7.91** (d, 2H; J = 8.7); **7.76** (dd, 1H; J = 7.8); **7.65** (dd, 1H; J = 9.3); **7.45** (dd, 1H; J = 7.8); **4.29, 4.14** (d, 1H); **2.97** (s, 3H); **2.02, 1.85** (m, 2H) **1.66, 1.33** (m, 6H). ¹³C NMR (100 MHz, CD₃OD/CDCl₃), 167.3, 151.6, 140.0, 138.7, 135.5, 135.2, 133.7, 139.4, 129.0, 128.5, 127.5, 136.8, 125.9, 125.8, 117.0, 51.7, 43.9, 32.3, 23.6. MS (EI+): m/z = 460.0 (exact Mass = 460.1569)

**6-(3-chloro-4-fluorophenyl)-3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine
(48):**

Column chromatography: DCM/MeOH (98:2, 95:5, 9:1, 85:15), Recrystallization in AcOEt, 58%. ¹H NMR (300 MHz, CDCl₃), δ **8.37** (d, 2H; J = 8.4); **8.22** (s, 1H); **8.15** (d, 1H; J = 9.3); **8.11** (d, 2H; J = 8.4); **8.05** (dd, 1H; J = 2.4, J = 8.4); **7.94, 7.86** (m, 1H); **7.55** (d, 1H; J = 9.3); **7.34** (dd, 1H; J = 8.4); **3.13** (s, 3H), ¹³C NMR (100 MHz, CDCl₃), δ 161.0, 158.0, 149.9, 140.1, 139.0, 135.1, 129.5, 127.9, 127.0, 126.9, 126.8, 117.4, 115.9, 44.6. MS (EI+): m/z = 400.5 (exact Mass = 401.0401)

(51): Column chromatography: DCM/MeOH (98:2), Recrystallization in AcOEt, 44%. (300 MHz, CDCl₃), 8.38 (d, 2H; J = 8.7); 8.25 (s, 1H); 8.32 (dd, 1H; J = 2.4, 7.2); 8.14 (d, 1H; J = 9.3); 8.07 (d, 2H; J = 8.7); 7.85, 7.80 (m, 1H); 7.59 (dd, 1H; J = 1.8, 9.3); 7.30 (dd, 1H; J = 8.7, 10.5); 5.93 (s, 1H); 3.10 (s, 3H); 1.50 (s, 9H). MS (EI+): m/z 466.1 (exact Mass = 466.1475)

N-(2-(dimethylamino)ethyl)-3-(3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)benzamide (50):

No work up. The reaction mixture was concentrated and the residue purified on sílica gel. Column chromatography: DCM/MeOH (95:5, 90:10, 80:20, 70:30), Recrystallization in AcOEt, 62%. ¹H NMR (300 MHz, d₆-DMSO), δ **8.59, 5.56** (m, 3H); **8.50** (s, 1H); **8.39** (d, 1H; J = 9.6); **8.32** (d, 1H; J = 8.1); **8.11, 8.07** (m, 3H); **8.03** (d, 2H; J = 9.6); **7.70** (dd, 1H; J = 7.8); **3.53, 3.27** (m, 2H); **3.27** (s, 3H); **2.59, 2.49** (m, 2H); **2.27** (s, 6H). MS (EI+): m/z = 463.2 (exact Mass = 463.1678)

6-(3-fluoro-4-(trifluoromethyl)phenyl)-3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (51):

Column chromatography: DCM/MeOH (98:2, 95:5, 9:1, 85:15), Recrystallization in AcOEt, 30%. ¹H NMR (300 MHz, CDCl₃), δ **8.37** (d, 2H; J = 8.7); **8.26** (s, 1H); **8.20** (d, 1H; J = 9.3); **8.12** (d, 2H; J = 8.7); **7.93, 7.77** (m, 3H); **7.61** (d, 1H; J = 9.3); **3.13** (s, 3H). MS (EI+): m/z = 434.99 (exact Mass = 435.0665)

3-(4-(methylsulfonyl)phenyl)-6-(6-(trifluoromethyl)pyridin-3-yl)imidazo[1,2-b]pyridazine (52):

Column chromatography: AcOEt/Hex (5:1, 1:0), AcOEt/MeOH (95:5, 9:1), Recrystallization in MeOH/AcOEt, 37%. ¹H NMR (400 MHz, CD₃OD), δ **9.38** (d, 1H; J = 1.6); **8.49** (ddd, 1H; J = 0.8, J = 1.8, J = 8.4); **8.35** (dd, 2H; J = 2.0, J = 8.8); **8.28** (s, 1H); **8.25** (d, 1H; J = 9.6); **8.11** (dd, 2H; J = 2.0, J = 8.); **7.90** (dd, 1H; J = 0.8, J = 8.3); **7.65** (d, 1H; J = 9.5); **3.13** (s, 3H). ¹³C NMR (100 MHz, CDCl₃), δ 148.4, 146.2, 135.8,

135.6, 128.0, 127.3, 127.1, 120.8, 115.6, 44.5. MS (EI+): m/z = 417.9 (exact Mass = 418.0711)

3,6-bis(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (53):

Column chromatography: AcOEt/Hex (5:1, 1:0), AcOEt/MeOH (95:5, 9:1), Recrystallization in MeOH/AcOEt, 37%. ¹H NMR (400 MHz, CDCl₃), δ **8.38** (d, 2H; J = 8.4); **8.27** (s, 1H); **8.23, 8.10** (m, 7H); **7.65** (d, 1H; J = 9.6); **3.15 – 3.14** (2s, 2x3H), ¹³C NMR (100 MHz, CDCl₃), δ 148.4, 146.2, 135.8, 135.6, 128.0, 127.3, 127.1, 120.8, 115.6, 44.5. MS (EI+): m/z = 427.0 (exact Mass = 427.0660)

(54): Column chromatography: DCM/MeOH (98:2), Recrystallization in DCM/MeOH, 43%. ¹H NMR (300 MHz, CDCl₃), δ **8.39** (d, 2H; J = 8.7); **8.32** (dd, 1H; J = 2.4, 7.2); **8.26** (s, 1H); **8.14** (d, 1H; J = 9.3); **8.07** (d, 2H; J = 8.7); **8.03** (m, 1H); **7.60** (dd, 1H; J = 1.8, 9.3); **7.32** (dd, 1H; J = 8.7, 10.5); **5.98** (d, 1H; J = 7.5); **4.07, 3.96** (m, 1H); **3.12** (s, 3H); **2.12, 2.05** (m, 2H); **1.84, 1.65** (m, 4H); **1.52, 1.38** (m, 2H). ¹³C NMR (100 MHz, CD₃OD/CDCl₃), 151.5, 150.0, 147.6, 140.0, 134.9, 130.2, 130.1, 129.9, 127.8, 126.8, 126.0, 118.9, 118.8, 117.0, 116.8, 49.0, 44.5, 33.2, 25.4, 24.8. MS (EI+): m/z = 491.9 (exact Mass = 492.1631)

6-(3-(tert-butyl)phenyl)-3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (55):

Column chromatography: DCM/MeOH (98:2), Recrystallization in AcOEt, 58%. ¹H NMR (400 MHz, dms_o-d₆), δ **8.59** (d, 2H; J = 8.6); **8.49** (s, 1H); **8.33** (d, 1H; J = 9.0); **8.17** (t, 1H; J = 1.5); **8.06** (d, 2H; J = 8.6); **8.01, 7.92** (m, 2H); **7.63** (dd, 1H; J = 1.5, J = 8.4); **7.54** (dd, 1H; J = 7.8); **3.27** (s, 3H); **1.40** (s, 9H). MS (EI+): m/z = 404.9 (exact Mass = 405.1511)

B. HPLC Method

Using a XBridge C18 column, 1 µL 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).

C. Metabolic stability – Microsome predicted hepatic extraction ratio (E_H)

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 MgCl₂ (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 ST1. Microsome predicted hepatic extraction ratio (E_H)

4-pyridyl series		4-Methylsulfonylphenyl series		
Compd	E_H	Compd	E_H values	
	values			
	Human		Human	Rat

1	0.86	31	0.92	0.76
2	0.82	32	0.98	0.96
8	0.76	33^a	< 0.28	< 0.19
9	0.79	34	0.73	0.64
10	0.81	35^b	< 0.42	< 0.30
11	0.62	36	0.96	0.89
12	0.87	37	0.49	0.46
13	0.86	38^c	< 0.28	< 0.28
14	0.82	39	0.55	-
15	0.80	40	0.67	-
16	0.81	41	< 0.28	0.25
21	0.47	42	< 0.28	-
22	0.40	43	< 0.28	< 0.19
29	< 0.28	44	< 0.28	< 0.19
		46	< 0.28	-
		48	< 0.28	-

^a E_H (mouse) = 0.78

^b E_H (mouse) = < 0.33

^c E_H (mouse) = 0.83

D. Physicochemical data.

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%. Samples were then analysed via Nephelometry to determine a solubility range as described by Bevan and Lloyd.²

Partition coefficient values (logD) of the test compounds were estimated by correlation of their chromatographic retention properties against the characteristics of a series of standard compounds with known partition coefficient values. The method employed is a gradient HPLC based derivation of the method developed by Lombardo et al.³

Table ST2. Physicochemical data

Compd	pKa ^a	PSA ^b b(Å ²)	Log D pH 7.4	Solubility (µg/mL)	
				pH 2.0	pH 6.5
Pyridyl derivatives					
1	3.8 - pyridine	-	2.9	> 100	6.3-12.5
	1.7 - imidazole				
2	3.8 - pyridine	-	2.2	> 100	12.5- 25.0
	1.6 - imidazole				
9	3.8 - pyridine	81.4	3.6	25-50	3.1-6.3
	1.8 - imidazole				
11	3.9 - pyridine	43.1	> 5.3	> 100	< 1.6
	1.9 - imidazole				

4.3 - isoquinoline					
14	3.7 - pyridine	56.0		50-100	1.6-3.1
	0.9 - imidazole		2.9		
15	3.8 - pyridine	43.1		> 100	1.6-3.1
	1.5 - imidazole		3.7		
	3.8 - pyridine				
16	3.7 - morpholine	55.6		25-50	1.6-3.1
	1.2 - imidazole		3.4		
18	3.7 - pyridine	-		> 50	0.8-1.6
	0.9 - imidazole		3.0		
21	3.7 - pyridine	-		> 96	6.3-12.5
	0.8 - imidazole		3.1		
22	3.8 - pyridine	-		25-50	< 1.6
	1.4 - imidazole		4.5		
29	3.7 - pyridine	-		>50	6.3-12.5
	1.1 - imidazole		2.1		
Methylsulfonylphenyl derivatives					
31	2.8 - imidazole	127.3		12.5-25.0	< 1.6
			2.9		
32	2.8 - imidazole	127.3		1.6-3.1	< 1.6

			3.5		
33	2.9 - imidazole	141.3	2.2	6.3-1.25	1.6-3.1
34	6.9 - piperazine	96.3		> 100	> 100
	3.4 - imidazole		2.6		
35	2.7 - imidazole	115.2	2.5	12.5-25.0	3.1-6.3
36	3.1 - imidazole	93.0	3.1	25-50	3.1-6.3
37	3.1 - imidazole	127.3	3.0	12.5-25	1.6-3.1
38	7.5 - piperazine	105.1		> 100	25-50
	3.1 - imidazole		2.3		
40	3.1 - imidazole	72.7	4.3	3.1-6.3	< 1.6
41	2.9 - imidazole	72.7	3.9	6.3-12.5	< 1.6
42	3.0 - imidazole	122.0	1.9	> 100	50-100
43	3.0 - imidazole	115.8		25-50	3.1-6.3

			2.0		
44	2.7 - imidazole	96.5	3.1	12.5-25	1.6-3.1
45	3.4 - aniline 3.1 - imidazole	76.0	3.8	25-50	< 1.6
47	3.0 - imidazole	101.8	3.3	6.3-12.5	3.1-6.3
48	2.5 - imidazole	72.7	3.9	3.1-6.3	1.6-3.1
49	2.5 - imidazole	101.8	3.3	25-50	1.6-3.1
50	8.8 - dimethylamine 3.0 - imidazole	105.1	2.5	> 100	> 100
55	3.4 - imidazole	72.7	4.6	3.1-6.3	< 1.6

^a pKa: determined using a ACD LogD suite of software programs.

^b Polar Surface Area: calculated using a simplified 2-dimensional modeling approach, which has been validated against a more sophisticated 3-dimensional modelling strategy. It is usually accepted that PSA values of less than approximately 120 Å² will provide acceptable oral drug absorption and membrane permeability.

E. SCID mouse studies

Table ST3. Comparison with standard antimalarial drugs tested in the *P.falciparum* Human Mouse model

Compound	ED ₉₀ (mg·kg ⁻¹)	AUC _{ED90} (µg·h·ml ⁻¹ ·day ⁻¹)
Atovaquone	0.05	0.35
Pyrimethamine	0.9	1.3
Piperaquine	3.7	0.2
Amodiaquine	4.2	0.063
Chloroquine	5	1
Mefloquine	7.7	8.2
Artesunate	10	ND
Lumefantrine	12	3.5
MMV652103	1.54	0.15

F. Pharmacodynamics Studies

Methods

a) Solubility estimates and logD measurements.

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%. Samples were then analysed via Nephelometry to determine a solubility range as described by Bevan and Lloyd.²

Partition coefficient values (logD) of the test compounds were estimated by correlation of their chromatographic retention properties against the characteristics of a series of standard compounds with known partition coefficient values. The method employed is a gradient HPLC based derivation of the method developed by Lombardo et al.³

b) In vitro microsomal stability assay methods.

Compounds were incubated at 37°C at 1 µM 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 MgCl₂ (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

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.
- (3) Lombardo, F.; Shalaeva, M. Y.; Tupper, K. A. and Gao, F. ElogD_{oct}: A Tool for Lipophilicity Determination in Drug Discovery. 2. Basic and Neutral Compounds, *J. Med. Chem.* 2001, 44, 2490-2497.
- (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.