# **Electronic supplementary information**

# Antiplasmodial imidazopyridazines: structure-activity relationship studies lead to the identification of analogues with improved solubility and hERG profiles

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## 1.0 Additional chemistry

## 1.1 Synthesis of the aniline precursor 5d for synthesis of analogue 22

Since the aniline starting material **5d**, used to synthesize compound **22**, could not be sourced commercially, it was synthesized in house according to synthetic scheme 1 below. Tosylation of the commercially available alcohol starting material **5a** in the presence of sodium hydroxide generated a good leaving group at the primary carbon (**5b**). Treatment of the tosylated intermediate **5b** with diethylamine afforded the amino-substituted intermediate **5c** in 87% yield. Reduction of the nitro group with acidified stannous chloride (SnCl<sub>2</sub>) gave the aniline precursor **5d** in quantitative yield.<sup>1</sup>



**Scheme 1** Synthetic approach for aniline precursor **5d**. Reagents and conditions: (i) aq NaOH, *p*-toluenesulfonyl chloride, THF, 0 - 11 °C, 2 h, 66%; (ii)  $Et_2NH$ ,  $Et_3N$ , 1,4-dioxane, 55 °C, 5 h, 87%; (iii) SnCl<sub>2</sub>, aq HCl, THF, reflux, 66 °C, 29 h, quantitative.

## 1.2 Synthesis of the hydroxy-substituted aniline precursor 5h for synthesis of analogue 23

The starting material **5h**, for the Buchwald-Hartwig amination towards compound **23** was realized via a three-step synthetic approach (scheme 2) from a commercially available 4aminophenol **5e**. Electrophilic aromatic substitutions of anilines often lead to undesirable polysubstituted products due to their high reactivity.<sup>2</sup> To circumvent such side reactions, the 4-aminophenol starting material was acetylated with acetic anhydride under reflux to get the acetylated intermediate **5f**. Installation of the Mannich base side chain to give **5g** was then achieved via an electrophilic aromatic substitution in presence of formaldehyde and diethylamine. Subsequent deacetylation of the amino group in aqueous HCl gave the Mannich base starting material **5h** in 43% yield.



**Scheme 2** Synthetic approach for the hydroxy-substituted aniline precursor **5h**. Reagents and conditions: (i) Acetic anhydride, THF, reflux, 60 °C, 1.75 h, 87%; (ii) aq HCHO,  $Et_2NH$ , ethanol, microwave irradiation, 80 °C, 1.5 h, quantitative; (iii) aq HCl, reflux, 80 °C, 2 h, 43%.

## 2.0 Additional synthetic procedures and characterization

2-Fluoro-5-nitrobenzyl 4-methylbenzenesulfonate (**5b**). To a solution of NaOH (0.534 g, 13 mmol) in 3.8 mL deionized water cooled to 0 °C was added a solution of 2-fluoro-5nitrobenzyl alcohol (**5a**) (1.00 g, 5.8 mmol) in tetrahydrofuran (THF) (17 mL). *p*-Toluenesulfonyl chloride (1.89 g, 9.9 mmol) dissolved in THF (5.0 mL) was then added dropwise. The reaction mixture was left to stir at 0 – 11 °C for 2 hours. After completion of reaction as signalled by TLC (ionisation not observed on LC-MS), the reaction mixture was diluted with deionized water (50 mL) and extracted with DCM (100 mL × 3). The organic layer was dried (MgSO<sub>4</sub>) and solvent removed *in vacuo* to give an oily residue which was further crystallized and purified by trituration in methanol to afford **5b** as a cream white solid (1.25 g, 66%); <sup>1</sup>H-NMR  $\delta_{\rm H}$  (300 MHz; CDCl<sub>3</sub>) 8.29 – 8.16 (m, 2H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.22 (t, *J* = 8.7 Hz, 1H), 5.20 (s, 2H), 2.48 (s, 3H). *N-Ethyl-N-(2-fluoro-5-nitrobenzyl)ethanamine* (*5c*). Diethylamine (0.600 mL, 5.9 mmol) and triethylamine (0.800 mL, 5.9 mmol) were added to a solution of 2-fluoro-5-nitrobenzyl 4-methylbenzenesulfonate (*5b*) (1.28 g, 3.9 mmol) in anhydrous 1,4-dioxane (7.8 mL). The reaction mixture was heated to 55 °C and stirred for 5 hours. The solvent was removed *in vacuo* and the resulting yellowish residue taken up in DCM (150 mL) before washing with deionized water (60 mL × 1), and saturated aqueous solution of NaCl (50 mL × 1). The organic layer was dried (MgSO<sub>4</sub>) before removing the solvent *in vacuo* to give **5c** as a yellow oil (0.771 g, 87%); <sup>1</sup>H-NMR  $\delta_{\rm H}$  (300 MHz; CDCl<sub>3</sub>) 8.48 (dd, *J* = 6.2, 2.9 Hz, 1H), 8.14 (ddd, *J* = 9.0, 4.4, 3.0 Hz, 1H), 7.17 (t, *J* = 8.9 Hz, 1H), 3.71 (s, 2H), 2.62 (q, *J* = 7.1 Hz, 4H), 1.10 (t, *J* = 7.1 Hz, 6H).

3-((Diethylamino)methyl)-4-fluoroaniline (5d). A solution of stannous chloride (SnCl<sub>2</sub>) (2.58 g, 13.6 mmol) in 1 M aqueous HCl (10.2 mL, 10.2 mmol) was added to a suspension of *N*-ethyl-*N*-(2-fluoro-5-nitrobenzyl)ethanamine (5c) (0.771 g, 3.4 mmol) in 15 mL THF. The reaction mixture was refluxed at 66 °C for 29 hours. THF was removed *in vacuo* where after the pH of the remaining aqueous mixture was adjusted to ~ 8 by gradual addition of saturated aqueous NaHCO<sub>3</sub>. The resulting turbidy emulsion was extracted with DCM (150 mL × 6). The combined organic layers were dried (MgSO<sub>4</sub>) after which the solvent was removed *in vacuo* to obtain the aniline compound 5d as a yellow oil (0.661 g, quantitative); <sup>1</sup>H-NMR  $\delta_{\rm H}$  (300 MHz; CDCl<sub>3</sub>) 6.86 – 6.78 (m, 2H), 6.53 (ddd, *J* = 8.6, 4.1, 3.0 Hz, 1H), 3.58 (s, 2H), 2.59 (q, *J* = 7.1 Hz, 4H), 1.09 (t, *J* = 7.1 Hz, 6H); LC-MS, ESI/APCI<sup>+</sup>: *m/z* [M + H]<sup>+</sup> = 197.1, calculated exact mass = 196.1376, t<sub>r</sub> = 0.3 min.

*N-(4-Hydroxyphenyl)acetamide* (*5f*). A solution of 4-aminophenol **5e** (1.50 g, 13.7 mmol) and acetic anhydride (1.30 mL, 13.7 mmol) in THF (20 mL) was refluxed at 60 °C for 1.75 hours. The solvent was then removed *in vacuo* and the resulting brown residue purified by trituration in diethyl ether for 30 minutes to give the acetylated compound, **5f**, as a cream white solid (1.81 g, 87%); <sup>1</sup>H-NMR  $\delta_{\rm H}$  (300 MHz; CD<sub>3</sub>OD) 7.31 (d, *J* = 9.0 Hz, 2H), 6.74 (d, *J* = 9.1 Hz, 2H), 2.10 (s, 3H); LC-MS, ESI/APCI<sup>+</sup>: *m/z* [M + H]<sup>+</sup> = 152.1, calculated exact mass = 151.0633, t<sub>r</sub> = 1.0 min.

*N*-(*3*-((*Diethylamino*)*methyl*)-*4*-*hydroxyphenyl*)*acetamide* (*5g*). Diethylamine (0.800 mL, 7.94 mmol) and 37% aqueous formaldehyde (0.600 mL, 7.94 mmol) were added to a solution of *N*-(4-hydroxyphenyl)acetamide (*5f*) (0.600 g, 3.97 mmol) in 5.0 mL absolute ethanol. The reaction mixture was heated to 80 °C under microwave irradiation for 1.5 hours after which the solvent was removed *in vacuo*. The brown residue was taken up in 40 mL DCM and extracted with 1 M HCl (35 mL × 1). The pH of the acidic aqueous layer was then adjusted to ~ 12 by gradual addition of a saturated aqueous solution of NaOH. The resultant basic mixture was then extracted with DCM (50 mL × 3). The combined organic layers were dried (MgSO<sub>4</sub>) before removing the solvent *in vacuo* to afford **5g** as a brown oil, which, subsequently, crystallized upon standing (0.987 g, quantitative); <sup>1</sup>H-NMR  $\delta_{\rm H}$  (300 MHz; CDCl<sub>3</sub>) 7.35 (d, *J* = 2.4 Hz, 1H), 7.15 (br s, 1H), 7.07 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.76 (d, *J* = 8.6 Hz, 1H), 3.77 (s, 2H), 2.64 (q, *J* = 7.2 Hz, 4H), 2.15 (s, 3H), 1.12 (t, *J* = 7.2 Hz, 6H); LC-MS, ESI/APCI<sup>+</sup>: *m/z* [M + H]<sup>+</sup> = 237.2, calculated exact mass = 236.1525, t<sub>r</sub> = 0.58 min.

4-Amino-2-((diethylamino)methyl)phenol (5h). N-(3-((Diethylamino)methyl)-4hydroxyphenyl)acetamide (5g) (0.987 g, 4.18 mmol) was refluxed at 80 °C in 6 M aqueous HCl (4.20 mL, 25.1 mmol) for 2 hours. The reaction mixture was left to cool to room temperature before adjusting the pH to ~ 8 by gradual addition of a saturated aqueous solution of NaHCO<sub>3</sub>. The black solution obtained was extracted with DCM (100 mL × 5). The combined organic layers were dried (MgSO<sub>4</sub>), where after the solvent was removed *in vacuo*. The obtained black residue was subjected to chromatography on silica (4% CH<sub>3</sub>OH/DCM) to give the phenolic compound **5h** as a brown oil (0.352 g, 43%); <sup>1</sup>H-NMR  $\delta_{\rm H}$  (300 MHz; CDCl<sub>3</sub>) 6.67 (d, *J* = 8.4 Hz, 1H), 6.56 (dd, *J* = 8.4, 2.8 Hz, 1H), 6.41 (d, *J* = 2.7 Hz, 1H), 3.70 (s, 2H), 2.64 (q, *J* = 7.2 Hz, 4H), 1.12 (t, *J* = 7.2 Hz, 6H); LC-MS, ESI/APCI<sup>+</sup>: *m/z* [M + H]<sup>+</sup> = 195.2, calculated exact mass = 194.1419, t<sub>r</sub> = 0.57 min.

6-Chloro-3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (**4b**). Purified by flash chromatography (0 – 2% CH<sub>3</sub>OH/DCM). Yellow solid (0.577 g, 65%); <sup>1</sup>H-NMR δ<sub>H</sub> (300 MHz; CDCl<sub>3</sub>) 8.30 (d, J = 8.8 Hz, 2H), 8.23 (s, 1H), 8.18 - 8.07 (m, 4H), 3.13 (s, 3H); LC-MS, ESI/APCI<sup>+</sup>: m/z [M + H]<sup>+</sup> = 308.0, calculated exact mass = 307.0182, t<sub>r</sub> = 3.1 min.

6-Chloro-3-(3-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazine (**4c**). Purified by flash chromatography (0 – 3% CH<sub>3</sub>OH/DCM). Yellow solid (0.1713 g, 66%); <sup>1</sup>H-NMR δ<sub>H</sub> (300 MHz; CDCl<sub>3</sub>) 8.32 (s, 1H), 8.28 – 8.20 (m, 1H), 8.19 (s, 1H), 8.05 (d, J = 9.4 Hz, 1H), 7.74 – 7.69 (m, 2H), 7.18 (d, J = 9.4 Hz, 1H), 2.84 (s, 3H).

6-Chloro-3-(3-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (**4d**). Purified by flash chromatography (0 – 4% CH<sub>3</sub>OH/DCM). Yellow solid (0.1797 g, 66%); <sup>1</sup>H-NMR δ<sub>H</sub> (300 MHz; CDCl<sub>3</sub>) 8.63 (s, 1H), 8.41 (d, *J* = 8.1 Hz, 1H), 8.20 (s, 1H), 8.04 (d, *J* = 9.5 Hz, 1H), 7.99 (d, *J* = 8.9 Hz, 1H), 7.76 (t, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 9.4 Hz, 1H), 3.16 (s, 3H); LC-MS, APCI<sup>+</sup>: m/z [M + H]<sup>+</sup> = 308.0, calculated exact mass = 307.0182, t<sub>r</sub> = 3.4 min.

*N*-(4-(6-Chloroimidazo[1,2-b]pyridazin-3-yl)-3-fluorobenzyl)-*N*-ethylethanamine (4e). Purified by flash chromatography (0 – 5% CH<sub>3</sub>OH/DCM), then prep-TLC (developed in 6% CH<sub>3</sub>OH/DCM). Yellow oil (0.097 g, 26%); <sup>1</sup>H-NMR  $\delta_{\rm H}$  (300 MHz; CDCl<sub>3</sub>) 8.23 – 8.10 (m, 2H), 8.00 (d, *J* = 9.4 Hz, 1H), 7.40 – 7.30 (m, 2H), 7.12 (d, *J* = 9.4 Hz, 1H), 3.70 (s, 2H), 2.65 (q, *J* = 7.1 Hz, 4H), 1.13 (t, *J* = 7.1 Hz, 6H); LC-MS, ESI/APCI<sup>+</sup>: *m*/*z* [M + H]<sup>+</sup> = 333.1, calculated exact mass = 332.1204, t<sub>r</sub> = 2.5 min.

*N*-(4-(6-Chloroimidazo[1,2-b]pyridazin-3-yl)-2-fluorobenzyl)-*N*-ethylethanamine (**4f**). Purified by flash chromatography (0 – 3% CH<sub>3</sub>OH/DCM). Yellow crystalline solid (0.135 g, 37%); <sup>1</sup>H-NMR δ<sub>H</sub> (300 MHz; CDCl<sub>3</sub>) 8.10 (s, 1H), 7.98 (d, *J* = 9.4 Hz, 1H), 7.89 – 7.77 (m, 2H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.12 (d, *J* = 9.4 Hz, 1H), 3.76 (s, 2H), 2.65 (q, *J* = 7.1 Hz, 4H), 1.14 (t, *J* = 7.1 Hz, 6H).

N-(5-(6-Chloroimidazo[1,2-b]pyridazin-3-yl)-2-fluorobenzyl)-N-ethylethanamine (4g). Purified by flash chromatography (0 – 4% CH<sub>3</sub>OH/DCM). Yellow oil (0.182 g, 50%); <sup>1</sup>H-NMR  $\delta_{\rm H}$  (300 MHz; CDCl<sub>3</sub>) 8.24 (s, 1H), 8.09 (s, 1H), 7.98 (d, *J* = 9.4 Hz, 1H), 7.95 - 7.91 (m, 1H), 7.26 – 7.16 (m, 1H), 7.10 (d, *J* = 9.4 Hz, 1H), 3.86 (s, 2H), 2.73 (d, *J* = 6.5 Hz, 4H), 1.21 (t, *J* = 6.5 Hz, 6H); LC-MS, ESI/APCl<sup>+</sup>: m/z [M + H]<sup>+</sup> = 333.1, calculated exact mass = 332.1204, t<sub>r</sub> = 2.8 min. 3-(4-(Methylsulfinyl)phenyl)-6-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (5). Purified by prep-TLC (developed in 4% CH<sub>3</sub>OH/DCM). Yellow solid (0.067 g, 60%), mp 260.0 – 262.5 °C (from 7% MeOH in DCM);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 4 : 96) 0.33; <sup>1</sup>H-NMR δ<sub>H</sub> (400 MHz; CDCl<sub>3</sub>) 8.34 (m, 3H), 8.23 (m, 3H), 8.16 (d, J = 8.0 Hz, 2H), 7.85 (d, J = 7.7 Hz, 2H), 7.65 (s, 1H), 3.15 (s, 3H), 2.84 (s, 3H); <sup>13</sup>C-NMR δ<sub>C</sub> (101 MHz; CDCl<sub>3</sub>) 150.25, 145.50, 142.06, 140.63, 131.18, 128.31 (4C), 128.09 (3C), 127.49 (2C), 126.74, 124.15 (2C), 115.95, 44.50, 44.04; LC-MS, APCI<sup>+</sup>: m/z [M + H]<sup>+</sup> = 412.0, calculated exact mass = 411.0711, purity: 97.5%, t<sub>r</sub> = 3.4 min.

*N*-*Ethyl*-*N*-(2-fluoro-4-(3-(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazin-6yl)benzyl)ethanamine (**7**). Purified by flash chromatography (0 – 4% CH<sub>3</sub>OH/DCM), trituration in diethyl ether. Yellow solid (0.112 g, 57%), mp 162.0 – 165.0 °C (from diethyl ether);  $R_f$ (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 6 : 94) 0.23; <sup>1</sup>H-NMR  $\delta_H$  (300 MHz; CDCl<sub>3</sub>) 8.35 (d, *J* = 8.4 Hz, 2H), 8.20 (s, 1H), 8.14 (d, *J* = 9.4 Hz, 1H), 7.98 – 7.78 (m, 4H), 7.74 (d, *J* = 11.4 Hz, 1H), 7.58 (d, *J* = 9.4 Hz, 1H), 3.89 (s, 2H), 3.50 (q, *J* = 7.0 Hz, 4H), 2.83 (s, 3H), 1.23 (t, *J* = 7.0 Hz, 6H); <sup>13</sup>C-NMR  $\delta_C$  (CDCl<sub>3</sub>) 162.9, 160.5, 145.0, 140.0, 134.4 (2C), 132.9, 131.4, 127.9, 127.4 (2C), 126.5, 124.1 (2C), 122.9, 115.7 (2C), 114.0 (d, *J* = 24.4 Hz), 65.8 (2C), 46.8, 44.0, 15.2 (2C); LC-MS, ESI/APCI<sup>+</sup>: *m/z* [M + H]<sup>+</sup> = 437.2, calculated exact mass = 436.1733, purity: 99.6%, t<sub>r</sub> = 2.7 min.

*N*-*Ethyl*-*N*-(2-fluoro-5-(3-(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazin-6yl)benzyl)ethanamine (**8**). Purified by flash chromatography (0 – 4% CH<sub>3</sub>OH/DCM), trituration in diethyl ether. Yellow solid (0.243 g, 80%), mp 122.4 – 124.5 °C (from diethyl ether);  $R_f$ (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 6 : 94) 0.33; <sup>1</sup>H-NMR  $\delta_{\rm H}$  (300 MHz; CDCl<sub>3</sub>) 8.37 (d, *J* = 8.6 Hz, 2H), 8.23 (d, *J* = 6.6 Hz, 1H), 8.17 (s, 1H), 8.11 (d, *J* = 9.5 Hz, 1H), 7.98 – 7.87 (m, 1H), 7.80 (d, *J* = 8.6 Hz, 2H), 7.63 (d, *J* = 9.4 Hz, 1H), 7.25 – 7.15 (m, 1H), 3.78 (s, 2H), 2.81 (s, 3H), 2.67 (q, *J* = 7.1 Hz, 4H), 1.14 (t, *J* = 7.1 Hz, 6H); <sup>13</sup>C-NMR  $\delta_{\rm C}$  (100.6 MHz; CDCl<sub>3</sub>) 163.8, 162.0, 152.0, 144.8, 134.0, 131.7, 127.3 (3C), 126.4, 124.0 (6C), 116.3, 116.1, 49.6, 46.9 (2C), 44.0, 15.2 (2C); LC-MS, ESI/APCI<sup>+</sup>: m/z [M + H]<sup>+</sup> = 437.1, calculated exact mass = 436.1733, purity: 99.9%, t<sub>r</sub> = 2.6 min.

*N*-*Ethyl*-*N*-(2-fluoro-4-(3-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6yl)benzyl)ethanamine (**9**). Purified by flash chromatography (0 – 1% CH<sub>3</sub>OH/DCM), trituration in methanol. Yellow solid (0.0627 g, 13%), mp 183.0 – 186.0 °C (from MeOH);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 6 : 94) 0.28; <sup>1</sup>H-NMR  $\delta_H$  (400 MHz; CDCl<sub>3</sub>) 8.41 (d, *J* = 8.8 Hz, 2H), 8.23 (s, 1H), 8.15 (d, *J* = 9.5 Hz, 1H), 8.12 (d, *J* = 8.7 Hz, 2H), 7.79 (br s, 2H), 7.71 (d, *J* = 10.9 Hz, 1H), 7.61 (d, *J* = 9.5 Hz, 1H), 3.82 (s, 2H), 3.15 (s, 3H), 2.70 (br d, *J* = 6.6 Hz, 4H), 1.17 (t, *J* = 7.1 Hz, 6H); <sup>13</sup>C-NMR  $\delta_C$  (100.6 MHz; CDCl<sub>3</sub>) 162.9, 160.4, 150.9, 140.4, 139.2, 134.9, 134.0, 132.4, 127.9 (4C), 126.9 (2C), 126.6, 122.7, 116.3, 113.8 (d, *J* = 24.7 Hz), 49.6, 47.0 (2C), 44.6, 11.4 (2C); LC-MS, ESI/APCI<sup>+</sup>: *m*/*z* [M + H]<sup>+</sup> = 453.1, calculated exact mass = 452.1682, purity: 99.2%, t<sub>r</sub> = 2.5 min. *N*-*Ethyl-N*-(3-fluoro-4-(6-(3-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazin-3-

yl)benzyl)ethanamine (**10**). Purified by prep-TLC (developed in 6% CH<sub>3</sub>OH/DCM). Yellow oil (0.0483 g, 38%);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 6 : 94) 0.26; <sup>1</sup>H-NMR  $\delta_H$  (300 MHz; CDCl<sub>3</sub>) 8.51 (t, J = 7.7 Hz, 1H), 8.30 (s, 1H), 8.26 (s, 1H), 8.21 – 8.13 (m, 2H), 7.82 – 7.71 (m, 3H), 7.67 (d, J = 9.5 Hz, 1H), 7.61 (d, J = 12.2 Hz, 1H), 4.15 (s, 2H), 3.08 (br s, 4H), 2.83 (s, 3H), 1.44 (t, J = 6.6 Hz, 6H); <sup>13</sup>C-NMR  $\delta_C$  (100.6; CDCl<sub>3</sub>) 160.8, 158.3, 150.6, 147.2, 139.5, 137.1, 136.8, 130.2, 129.8, 129.6

(2C), 126.5 (3C), 125.0, 122.1, 118.2, 116.0, 55.5, 46.2 (2C), 44.2, 9.0 (2C); LC-MS, ESI/APCI<sup>+</sup>: *m*/*z* [M + H]<sup>+</sup> = 437.2, calculated exact mass = 436.1733, purity: 98.5%, t<sub>r</sub> = 2.9 min.

N-Ethyl-N-(2-fluoro-4-(6-(3-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazin-3-

yl)benzyl)ethanamine (**11**). Purified by flash chromatography (0 – 4% CH<sub>3</sub>OH/DCM), prep-TLC (silica, developed in 10% CH<sub>3</sub>OH/DCM). Yellow oil (0.0484 g, 27%);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 6 : 94) 0.15; <sup>1</sup>H-NMR  $\delta_H$  (300 MHz; CDCl<sub>3</sub>) 8.30 (s, 1H), 8.25 – 8.09 (m, 3H), 8.01 (d, *J* = 11.6 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.85 – 7.60 (m, 4H), 3.81 (s, 2H), 2.84 (s, 3H), 2.70 (br d, *J* = 7.1 Hz, 4H), 1.17 (t, *J* = 7.0 Hz, 6H); <sup>13</sup>C-NMR  $\delta_C$  (100.6 MHz; CDCl<sub>3</sub>) 162.7, 160.2, 150.6, 147.3, 139.9, 137.1, 134.3, 132.6, 130.3, 129.6, 127.3, 126.6, 125.0 (2C), 122.5, 122.1, 115.6, 113.1 (d, *J* = 25.4 Hz), 49.3, 46.6 (2C), 44.1, 15.2 (2C); LC-MS, ESI/APCI<sup>+</sup>: *m/z* [M + H]<sup>+</sup> = 437.2, calculated exact mass = 436.1733, purity: 99.9%, t<sub>r</sub> = 2.8 min.

*N*-*Ethyl*-*N*-(2-fluoro-5-(6-(3-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazin-3yl)benzyl)ethanamine (**12**). Purified by prep-TLC (developed in 6% CH<sub>3</sub>OH/DCM). Yellow oil (0.101 g, 42%); *R*<sub>f</sub> (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 6 : 94) 0.21; <sup>1</sup>H-NMR  $\delta_{\rm H}$  (300 MHz; CDCl<sub>3</sub>) 8.39 – 8.29 (m, 2H), 8.25 (dt, *J* = 7.4, 1.6 Hz, 1H), 8.16 – 8.07 (m, 2H), 8.00 (ddd, *J* = 8.4, 4.9, 2.4 Hz, 1H), 7.80 – 7.66 (m, 2H), 7.61 (d, *J* = 9.5 Hz, 1H), 7.25 – 7.15 (m, 1H), 3.80 (s, 2H), 2.82 (s, 3H), 2.68 (q, *J* = 7.1 Hz, 4H), 1.12 (t, *J* = 7.1 Hz, 6H); <sup>13</sup>C-NMR  $\delta_{\rm C}$  (100.6 MHz; CDCl<sub>3</sub>) 162.2, 159.8, 150.3, 147.1, 139.3, 137.3, 133.6, 130.1 (2C), 129.6, 128.3, 127.2, 126.4, 124.8, 124.6, 122.1, 115.6 (d, *J* = 22.9 Hz), 115.0, 49.9, 47.0 (2C), 44.1, 11.6 (2C); LC-MS, ESI/APCI<sup>+</sup>: *m/z* [M + H]<sup>+</sup> = 437.2, calculated exact mass = 436.1733, purity: 98.2%, t<sub>r</sub> = 3.0 min.

3,6-Bis(3-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (**28**). Purified by prep-TLC (developed in 4% CH<sub>3</sub>OH/DCM). Crystallized in diethyl ether. Yellow solid (0.114 g, 62%); mp 228.7 – 230.8 °C (from diethyl ether);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 4 : 96) 0.20; <sup>1</sup>H-NMR  $\delta_H$  (400 MHz; CDCl<sub>3</sub>) 9.17 (s, 1H), 8.71 (s, 1H), 8.55 – 8.21 (m, 4H), 8.17 (d, *J* = 7.6 Hz, 1H), 8.04 (d, *J* = 7.4 Hz, 1H), 7.94 – 7.74 (m, 3H), 3.24 (s, 3H), 3.22 (s, 3H); <sup>13</sup>C-NMR  $\delta_C$  (101 MHz; CDCl<sub>3</sub>) 151.08, 142.30, 141.61, 136.18, 132.12 (3C), 131.56, 130.55, 130.11, 129.33 (2C), 127.04 (2C), 126.47, 126.20, 125.47, 117.29, 44.76, 44.53; LC-MS, APCI<sup>+</sup>: m/z [M + H]<sup>+</sup> = 428.1, calculated exact mass = 427.0660, purity: 95.9%, t<sub>r</sub> = 3.9 min.

6-(4-(Methylsulfinyl)phenyl)-3-(3-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (**30**). Purified by prep-TLC (developed in 4% CH<sub>3</sub>OH/DCM). Crystallized in diethyl ether. Yellow solid (0.0441 g, 45%), mp 218.6 – 221.0 °C (from diethyl ether);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 4 : 96) 0.32; <sup>1</sup>H-NMR δ<sub>H</sub> (400 MHz; CDCl<sub>3</sub>) 9.09 (d, J = 1.9 Hz, 1H), 8.35 (d, J = 7.9 Hz, 1H), 8.33 – 8.19 (m, 4H), 8.03 – 7.97 (m, 1H), 7.88 (d, J = 8.1 Hz, 2H), 7.81 – 7.68 (m, 2H), 3.16 (s, 3H), 2.83 (s, 3H); <sup>13</sup>C-NMR δ<sub>C</sub> (101 MHz; CDCl<sub>3</sub>) 150.81, 148.32, 141.42, 137.76, 131.20, 130.03, 129.88, 128.01 (4C), 126.63, 126.37, 125.34, 124.48 (3C), 116.07, 44.58, 43.94; LC-MS, APCl<sup>+</sup>: m/z [M + H]<sup>+</sup> = 412.0, calculated exact mass = 411.0711, purity: 97.9%, t<sub>r</sub> = 3.5 min.

3-(3-(Methylsulfinyl)phenyl)-6-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazine (**31**). Purified by prep-TLC (developed in 4% CH<sub>3</sub>OH/DCM). Crystallized in diethyl ether. Yellow solid (0.0436 g, 37%), mp 218.0 – 221.5 °C (from diethyl ether);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 4 : 96) 0.29; <sup>1</sup>H-NMR δ<sub>H</sub> (400 MHz; CDCl<sub>3</sub>) 8.72 (d, J = 1.7 Hz, 1H), 8.34 – 8.25 (m, 4H), 8.23 (d, J = 7.9 Hz, 1H), 8.17 (d, J = 8.4 Hz, 2H), 7.75 – 7.66 (m, 2H), 7.63 (dt, J = 8.0, 1.2 Hz, 1H), 3.15 (s, 3H), 2.84 (s, 3H); <sup>13</sup>C-NMR  $\delta_{C}$  (101 MHz; CDCl<sub>3</sub>) 150.22, 149.15, 146.89, 142.09, 140.40, 135.72, 129.69 (2C), 128.91, 128.33 (3C), 128.09 (2C), 126.64, 123.00, 121.56, 115.90, 44.48, 44.18; LC-MS, APCl<sup>+</sup>: m/z [M + H]<sup>+</sup> = 412.0, calculated exact mass = 411.0711, purity: 99.6%, t<sub>r</sub> = 3.5 min.

3-(3-(Methylsulfinyl)phenyl)-6-(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazine (**32**). Purified by prep-TLC (developed in 4% CH<sub>3</sub>OH/DCM). Crystallized in diethyl ether. Yellow solid (0.0666 g, 58%), mp 174.0 – 176.2 °C (from diethyl ether);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 4 : 96) 0.14; <sup>1</sup>H-NMR δ<sub>H</sub> (400 MHz; CDCl<sub>3</sub>) 8.74 – 8.68 (m, 1H), 8.30 – 8.18 (m, 5H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.74 – 7.66 (m, 2H), 7.66 – 7.62 (m, 1H), 2.84 (s, 3H), 2.83 (s, 3H); <sup>13</sup>C-NMR δ<sub>C</sub> (101 MHz; CDCl<sub>3</sub>) 150.82, 148.20, 146.80, 137.97, 133.59, 129.90, 129.68 (2C), 128.91, 128.06 (3C), 126.47, 124.43 (2C), 122.86, 121.53, 115.96, 44.15, 43.95; LC-MS, APCI<sup>+</sup>: *m/z* [M + H]<sup>+</sup> = 396.1, calculated exact mass = 395.0762, purity: 99.9%, t<sub>r</sub> = 3.5 min.

3-(4-(Methylsulfinyl)phenyl)-N-(3-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6amine (14). A mixture of 6-chloro-3-(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazine (4a) (0.100 g, 0.34 mmol), 3-(methylsulfonyl)aniline (0.0640 g, 0.37 mmol), Pd<sub>2</sub>(dba)<sub>3</sub> (0.0249 g, 0.027 mmol), BrettPhos (0.0219 g, 0.041 mmol) and Cs<sub>2</sub>CO<sub>3</sub> (0.222 g, 0.68 mmol) in 1.5 mL anhydrous 1,4-dioxane in a sealed tube was flashed with nitrogen for 25 minutes. The reaction mixture was then heated to 120 °C and stirred at this temperature for 24 hours. The solvent was removed in vacuo and the resulting black residue taken up in DCM (50 mL), washed with deionized water (30 mL × 1), saturated aqueous solutions of NaHCO<sub>3</sub> (30 mL × 2) and NaCl (30 mL  $\times$  1). The organic layer was dried (MgSO<sub>4</sub>), after which the solvent was removed in vacuo. The yellowish residue was then subjected to prep-TLC (developed in 5% CH<sub>3</sub>OH/DCM) to afford **14** as a brown solid (0.0228 g, 16%); R<sub>f</sub> (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 6 : 94) 0.24; <sup>1</sup>H-NMR  $\delta_{H}$  (400 MHz; DMSO-d<sub>6</sub>) 9.89 (s, 1H), 8.32 – 8.24 (m, 3H), 8.10 – 8.02 (m, 2H), 7.99 (dd, J = 8.2, 1.2 Hz, 1H), 7.85 (d, J = 8.5 Hz, 2H), 7.66 (t, J = 7.9 Hz, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.03 (d, J = 9.7 Hz, 1H), 3.19 (s, 3H), 2.81 (s, 3H);  ${}^{13}$ C-NMR  $\delta_{c}$  (101 MHz; DMSO-d<sub>6</sub>) 151.01, 145.41, 142.07, 141.40, 138.32, 132.42, 131.55, 130.59, 127.51, 127.36, 127.25 (2C), 124.77 (2C), 123.68, 120.34, 116.42, 113.82, 44.10, 43.72; LC-MS, APCI<sup>+</sup>: *m*/*z* [M + H]<sup>+</sup> = 427.1, calculated exact mass = 426.0820, purity: 99.9%,  $t_r$  = 3.8 min.

*N*,3-Bis(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazin-6-amine (**16**). Purified by prep-TLC (developed once in 6% CH<sub>3</sub>OH/DCM, then once in 7% CH<sub>3</sub>OH/DCM), trituration in ethyl acetate for 20 h. Grey solid (0.0111 g, 8%);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 4 : 96) 0.045; <sup>1</sup>H-NMR  $\delta_H$  (400 MHz; DMSO-d<sub>6</sub>) 9.84 (s, 1H), 8.31 (d, *J* = 8.5 Hz, 2H), 8.07 (s, 1H), 8.03 (d, *J* = 9.7 Hz, 1H), 7.91 (d, *J* = 8.7 Hz, 2H), 7.86 (d, *J* = 8.5 Hz, 2H), 7.70 (d, *J* = 8.7 Hz, 2H), 7.04 (d, *J* = 9.7 Hz, 1H), 2.83 (s, 3H), 2.75 (s, 3H); <sup>13</sup>C-NMR  $\delta_C$  (101 MHz; DMSO-d<sub>6</sub>) 150.98, 145.52, 142.95, 138.55, 138.18, 132.22, 131.59, 127.29 (2C), 127.18, 126.80, 125.30 (2C), 124.53 (2C), 119.27 (2C), 113.89, 43.70 (2C); LC-MS, APCI<sup>+</sup>: *m*/*z* [M + H]<sup>+</sup> = 411.1, calculated exact mass = 410.0871, purity: 96.8%, t<sub>r</sub> = 3.6 min.

## 3-(4-(Methylsulfinyl)phenyl)-N-(1-(methylsulfonyl)piperidin-4-yl)imidazo[1,2-

*b]pyridazin-6-amine* (**21**). Purified by prep-TLC (developed once in 4% CH<sub>3</sub>OH/DCM, then twice in 5% CH<sub>3</sub>OH/DCM). Brown solid (0.022 g, 30%);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 6 : 94) 0.36 <sup>1</sup>H-NMR  $\delta_{\rm H}$  (400 MHz; DMSO-d<sub>6</sub>) 8.41 (d, J = 8.6 Hz, 2H), 8.04 (s, 1H), 7.87 – 7.74 (m, 3H), 7.16 (d, J =

6.5 Hz, 1H), 6.76 (d, J = 9.7 Hz, 1H), 3.90 – 3.75 (m, 1H), 3.68 – 3.55 (m, 2H), 3.07 – 2.97 (m, 2H), 2.94 (s, 3H), 2.80 (s, 3H), 2.26 – 2.13 (m, 2H), 1.69 – 1.52 (m, 2H); <sup>13</sup>C-NMR  $\delta_{c}$  (101 MHz; DMSO-d<sub>6</sub>) 153.21, 144.72, 138.10, 132.18, 131.40, 126.38, 126.27 (2C), 126.12, 124.54 (2C), 113.10, 48.00, 44.76 (2C), 43.62, 35.15, 30.82 (2C); LC-MS, APCI<sup>+</sup>: m/z [M + H]<sup>+</sup> = 434.1, calculated exact mass = 433.1242, purity: 98.6%, t<sub>r</sub> = 3.5 min.

N-(1-Methylpiperidin-4-yl)-3-(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazin-6amine (20). A suspension of 6-chloro-3-(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazine (4a) (0.0700 g, 0.24 mmol), Pd<sub>2</sub>(dba)<sub>3</sub> (0.0176 g, 0.019 mmol), XPhos (0.0137 g, 0.029 mmol), and sodium tert-butoxide (0.0461 g, 0.48 mmol) in 1.5 mL of anhydrous 1,4-dioxane in a sealed tube was flashed with nitrogen for 20 minutes. 4-Amino-1-methylpiperidine (0.06 mL, 0.48 mmol) was then added and the reaction mixture heated to 100 °C and stirred at this temperature for 22 hours. The solvent was removed in vacuo and the resulting black residue taken up in DCM (50 mL), washed with saturated aqueous solutions of NaHCO<sub>3</sub> (30 mL  $\times$  3) and NaCl (30 mL  $\times$  3). The organic layer was dried (MgSO<sub>4</sub>) after which the solvent was removed in vacuo. The resulting residue was subjected to flash column chromatography (0 -14% CH<sub>3</sub>OH/DCM). Further purification by prep-TLC (developed once in 20% CH<sub>3</sub>OH/DCM, then once in 25% CH<sub>3</sub>OH/DCM) furnished the target compound **20** as a brown solid (0.0121 g, 14%); *R*<sub>f</sub> (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 10 : 90) 0.076; <sup>1</sup>H-NMR δ<sub>H</sub> (400 MHz; DMSO-d<sub>6</sub>) 8.43 (d, *J* = 8.6 Hz, 2H), 8.02 (s, 1H), 7.87 – 7.66 (m, 3H), 7.07 (d, J = 6.7 Hz, 1H), 6.75 (d, J = 9.7 Hz, 1H), 3.75 – 3.56 (m, 1H), 2.92 – 2.72 (m, 5H), 2.24 (s, 3H), 2.19 – 2.02 (m, 4H), 1.65 – 1.43 (m, 2H); <sup>13</sup>C-NMR δ<sub>c</sub> (101 MHz; DMSO-d<sub>6</sub>) 153.28, 144.68, 138.05, 132.23, 131.26, 126.19 (3C), 125.99, 124.30 (2C), 113.18, 54.54 (2C), 48.56, 46.36, 43.66, 31.37 (2C); LC-MS, APCI<sup>+</sup>: *m*/*z* [M + H]<sup>+</sup> = 370.1, calculated exact mass = 369.1623, purity: 99.9%, t<sub>r</sub> = 1.9 min.

N-(3-((Diethylamino)methyl)-4-fluorophenyl)-3-(4-(methylsulfinyl)phenyl)imidazo[1,2b]pyridazin-6-amine (22). A mixture of 6-chloro-3-(4-(methylsulfinyl)phenyl)imidazo[1,2b]pyridazine (4a) (0.070 g, 0.24 mmol), 3-((diethylamino)methyl)-4-fluoroaniline (0.0518 g, 0.26 mmol), Pd<sub>2</sub>(dba)<sub>3</sub> (0.0088 g, 0.0096 mmol), BrettPhos (0.0077 g, 0.014 mmol), and Cs<sub>2</sub>CO<sub>3</sub> in anhydrous 1,4-dioxane (1.7 mL) in a sealed tube was flashed with nitrogen for 25 minutes. The reaction mixture was then heated to 120 °C and stirred for 12 hours. Significant amounts of starting material, as monitored by LC-MS, were detected. At this point, an extra 0.04 eq of Pd<sub>2</sub>(dba)<sub>3</sub> were added and the reaction mixture left to stir for another 4 hours under nitrogen atmosphere. The solvent was removed in vacuo. The resulting dark-brown residue was taken up in 100 mL DCM, washed with deionized water (50 mL × 2), saturated aqueous solutions of NaHCO<sub>3</sub> (50 mL  $\times$  2) and NaCl (50 mL  $\times$  2). After drying the organic layer (MgSO<sub>4</sub>), the solvent was removed in vacuo. The obtained crude mixture was subjected to prep-TLC (developed in 5 : 94.5 : 0.5% CH<sub>3</sub>OH/DCM/25% aq NH<sub>4</sub>OH). The obtained target compound, **22**, was further purified by automated flash column chromatography (0 – 4%  $CH_3OH/DCM$ ) followed by trituration in diethyl ether to give the title compound as a yellow solid (0.0158 g, 10%); mp 118.0 – 120.0 °C (from diethyl ether);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 6 : 94) 0.1; <sup>1</sup>H-NMR  $\delta_H$  (300 MHz; CDCl<sub>3</sub>) 8.24 (d, J = 8.5 Hz, 2H), 7.92 (s, 1H), 7.84 - 7.63 (m, 4H), 7.58 - 7.51 (m, 1H), 7.33 (s, 1H), 7.04 (t, J = 9.1 Hz, 1H), 6.79 (d, J = 9.5 Hz, 1H), 3.72 (s, 2H), 2.81 (s, 3H), 2.65 (q, J = 7.0 Hz, 4H), 1.10 (t, J = 7.1 Hz, 6H); LC-MS, ESI/APCI<sup>+</sup>: m/z [M + H]<sup>+</sup> = 452.2, calculated exact mass = 451.1842, purity: 98.0%, t<sub>r</sub> = 2.8 min.

2-((Diethylamino)methyl)-4-((3-(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazin-6yl)amino)phenol (23). A suspension of 6-chloro-3-(4-(methylsulfinyl)phenyl)imidazo[1,2b]pyridazine (4a) (0.100 g, 0.34 mmol), 4-amino-2-((diethylamino)methyl)phenol (0.0733 g, 0.38 mmol), Pd<sub>2</sub>(dba)<sub>3</sub> (0.0250 g, 0.028 mmol), BrettPhos (0.0220 g, 0.04 mmol) and Cs<sub>2</sub>CO<sub>3</sub> (0.222 g, 0.68 mmol) in anhydrous 1,4-dioxane (1.3 mL) in a sealed tube was purged with nitrogen for 30 minutes. The reaction mixture was then heated to 120 °C and stirred under nitrogen for 18 hours after which the solvent was removed *in vacuo*. The resulting black residue was taken up in DCM (50 mL), washed with deionized water (30 mL × 2), saturated aqueous solutions of NaHCO<sub>3</sub> (30 mL × 2) and NaCl (30 mL × 1). After drying (MgSO<sub>4</sub>) the organic layer, the solvent was removed *in vacuo* where after the crude mixture obtained was subjected to prep-TLC (developed in 8% CH<sub>3</sub>OH/DCM) to deliver the aminated compound, **23**, as a brown solid (0.0275 g, 18%);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 8 : 92) 0.12; <sup>1</sup>H-NMR  $\delta_H$  (400 MHz; CD<sub>3</sub>OD) 8.32 (d, *J* = 8.5 Hz, 2H), 7.89 (s, 1H), 7.80 (d, *J* = 8.5 Hz, 2H), 7.74 (d, *J* = 9.7 Hz, 1H), 7.54 (d, *J* = 2.5 Hz, 1H), 7.33 (dd, *J* = 8.6, 2.7 Hz, 1H), 6.91 (d, *J* = 9.7 Hz, 1H), 6.75 (d, *J* = 8.7 Hz, 1H), 3.82 (s, 2H), 2.87 (s, 3H), 2.71 (q, *J* = 7.1 Hz, 4H), 1.15 (t, *J* = 7.2 Hz, 6H); <sup>13</sup>C-NMR  $\delta_C$  (100.6; CD<sub>3</sub>OD) 153.3, 151.7, 143.3, 137.8, 132.3, 131.9, 129.9, 127.2 (2C), 124.9, 123.8 (2C), 121.9, 120.5 (2C), 116.7, 115.5, 114.0, 56.3, 46.4 (2C), 42.3, 10.2 (2C); LC-MS, ESI/APCI<sup>+</sup>: *m/z* [M + H]<sup>+</sup> = 450.2, calculated exact mass = 449.1885, purity: 95.0%, t<sub>r</sub> = 3.2 min.

3-(4-(methylsulfinyl)phenyl)-N-(4-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-6amine (17). A mixture of 6-chloro-3-(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazine (4a) (0.130 g, 0.45 mmol), 4-(methylsulfonyl)aniline (0.0925 g, 0.54 mmol), Pd<sub>2</sub>(dba)<sub>3</sub> (0.020 g, 0.022 mmol), (R)-BINAP (0.0168 g, 0.027 mmol) and K<sub>2</sub>CO<sub>3</sub> (1.24 g, 9.00 mmol) in 1.8 mL anhydrous toluene was heated to 120 °C in a sealed tube and stirred at this temperature for 17 hours. However, no significant amount of product was detected on LC-MS. At this point, anhydrous 1,4-dioxane (3.5 mL) was added and the reaction mixture stirred for another 17 hours where after a significant formation of the product was observed on LC-MS. The reaction mixture was then diluted with deionised water (40 mL) followed by extraction with DCM (70 mL). The solvents were removed in vacuo and the resulting residue taken up in DCM (70 mL), washed with a saturated aqueous solution of NaCl (40 mL × 3) and dried (MgSO<sub>4</sub>). The solvent was then removed in vacuo. The obtained crude mixture was subjected to prep-TLC (developed in 5.5% CH<sub>3</sub>OH/DCM) to afford the aminated target compound **17** as a brown solid (0.0251 g, 13%), mp 296.7 – 299.2 °C (from 6% MeOH in DCM); R<sub>f</sub> (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 6 : 94) 0.25; <sup>1</sup>H-NMR  $\delta_{H}$  (300 MHz; DMSO-d<sub>6</sub>) 10.05 (s, 1H), 8.30 (d, J = 8.5 Hz, 2H), 8.13 – 8.04 (m, 2H), 7.98 – 7.86 (m, 6H), 7.07 (d, J = 9.7 Hz, 1H), 3.19 (s, 3H), 2.84 (s, 3H);  $^{13}$ C-NMR  $\delta_{c}$  (100.6 MHz; DMSO-d<sub>6</sub>) 150.7, 145.8, 145.2, 138.3, 135.9, 133.3, 132.5, 131.5, 128.8 (2C), 127.6, 127.4 (2C), 124.6 (2C), 118.4 (2C), 113.9, 44.5, 43.7; LC-MS, ESI/APCI<sup>+</sup>: *m/z* [M + H]<sup>+</sup> = 427.1, calculated exact mass = 426.0820, purity: 98.0%, t<sub>r</sub> = 3.2 min.

4-((3-(4-(Methylsulfinyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)amino)tetrahydro-2Hthiopyran 1,1-dioxide (**19**). A suspension of 6-chloro-3-(4-(methylsulfinyl)phenyl)imidazo[1,2b]pyridazine (**4a**) (0.0700 g, 0.24 mmol), 4-aminotetrahydro-2H-thiopyran 1,1-dioxide hydrochloride (0.0483 g, 0.26 mmol), Pd<sub>2</sub>(dba)<sub>3</sub> (0.0176 g, 0.019 mmol), XPhos (0.0137 g, 0.029 mmol), sodium *tert*-butoxide (0.0461 g, 0.48 mmol) and triethylamine (TEA) (0.2 mL, 0.96 mmol) in 1.5 mL anhydrous 1,4-dioxane in a sealed tube was flashed with nitrogen for 30 minutes. The reaction mixture was then heated to 120 °C and stirred at this temperature for 17 hours. Only small quantities of the desired product were detected on LC-MS. At this point, the reaction mixture was charged with additional amounts of the amine hydrochloride (1.1 eq), Pd<sub>2</sub>(dba)<sub>3</sub> (0.08 eq), TEA (8.0 eq), sodium tert-butoxide (2.0 eq) and 0.5 mL anhydrous 1,4-dioxane. The reaction mixture was again purged with nitrogen for 2 minutes after which it was heated to 120 °C and stirred for another 9 hours. The solvent was removed in vacuo and the resulting black residue taken up in DCM (50 mL), washed with saturated aqueous solutions of NaHCO<sub>3</sub> (30 mL  $\times$  3) and NaCl (30 mL  $\times$  3). The organic layer was dried (MgSO<sub>4</sub>) where after the solvent was removed in vacuo. The crude mixture was then subjected to prep-TLC (developed once in 5% CH<sub>3</sub>OH/DCM, then twice in 6% CH<sub>3</sub>OH/DCM) to afford the aminated target compound 19 as a brown solid (0.0142 g, 15%); mp 273.1 - 274.5 °C (from 7% MeOH in DCM); R<sub>f</sub> (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 6 : 94) 0.38; <sup>1</sup>H-NMR δ<sub>H</sub> (400 MHz; DMSO-d<sub>6</sub>) 8.42 (d, J = 8.5 Hz, 2H), 8.05 (s, 1H), 7.87 – 7.76 (m, 3H), 7.25 (d, J = 6.7 Hz, 1H), 6.74 (d, J = 9.7 Hz, 1H), 4.15 - 3.98 (m, 1H), 3.46 - 3.27 (m, 2H), 3.25 - 3.14 (m, 2H), 2.80 (s, 3H), 2.48 - 2.35 (m, 2H), 2.16 – 1.98 (m, 2H);  ${}^{13}$ C-NMR  $\delta_{c}$  (101 MHz; DMSO-d<sub>6</sub>) 153.01, 144.71, 138.08, 132.05, 131.42, 126.51, 126.36 (2C), 126.18, 124.60 (2C), 113.00, 49.06 (2C), 46.84, 43.68, 29.08 (2C); LC-MS, APCI<sup>+</sup>: m/z [M + H]<sup>+</sup> = 405.1, calculated exact mass = 404.0977, purity: 97.2%, t<sub>r</sub> = 3.0 min.

6-(3-(Methylsulfinyl)phenyl)-N-(3-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-3amine (**25**). Purified by flash column chromatography (0 - 4% CH<sub>3</sub>OH/DCM), trituration in diethyl ether. Brown solid (0.0127 g, 10%); mp 150.4 – 152.0 °C (from diethyl ether);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 4 : 96) 0.19; <sup>1</sup>H-NMR δ<sub>H</sub> (400 MHz; DMSO-d<sub>6</sub>) 9.09 (s, 1H), 8.38 (d, J = 9.5 Hz, 1H), 8.29 (s, 1H), 8.22 (d, J = 7.9 Hz, 1H), 8.05 (d, J = 9.6 Hz, 1H), 8.02 (s, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.77 (t, J = 7.8 Hz, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.45 (s, 1H), 7.36 (d, J = 7.6 Hz, 1H), 7.24 (dd, J= 8.1, 2.3 Hz, 1H), 3.16 (s, 3H), 2.81 (s, 3H); <sup>13</sup>C-NMR δ<sub>C</sub> (101 MHz; DMSO-d<sub>6</sub>) 151.43, 148.10, 145.50, 142.26, 136.13, 134.94, 130.83, 130.55, 129.79, 128.30, 125.97, 125.83, 122.75 (2C), 119.50, 117.75 (2C), 112.76, 44.03, 43.62; LC-MS, APCI<sup>+</sup>: m/z [M + H]<sup>+</sup> = 427.1, calculated exact mass = 426.0820, purity: 95.1%, t<sub>r</sub> = 3.5 min.

*N*,6-*Bis*(3-(*methylsulfinyl*)*phenyl*)*imidazo*[1,2-*b*]*pyridazin-3-amine* (**26**). Purified by prep-TLC (developed in 4% CH<sub>3</sub>OH/DCM). Crystallized in diethyl ether. Brick-red solid (0.0186 g, 15%); mp 110.0 – 113.5 °C (from diethyl ether);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 4 : 96) 0.15; <sup>1</sup>H-NMR  $\delta_{\rm H}$  (400 MHz; DMSO-d<sub>6</sub>) 8.90 (s, 1H), 8.35 – 8.27 (m, 2H), 8.23 (d, *J* = 7.9 Hz, 1H), 8.00 – 7.90 (m, 2H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.76 (t, *J* = 7.7 Hz, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.24 (s, 1H), 7.07 (m, 2H), 2.81 (s, 3H), 2.69 (s, 3H); <sup>13</sup>C-NMR  $\delta_{\rm C}$  (101 MHz; DMSO-d<sub>6</sub>) 150.92, 148.05 (2C), 145.68, 136.37, 135.03, 130.50 (2C), 129.73, 128.59, 126.18, 125.77, 123.92, 122.72, 116.94, 116.61, 114.38, 109.29, 43.81, 43.62; LC-MS, APCI<sup>+</sup>: *m*/*z* [M + H]<sup>+</sup> = 411.1, calculated exact mass = 410.0871, purity: 96.6%, t<sub>r</sub> = 3.5 min.

6-(3-(Methylsulfinyl)phenyl)-N-(4-(methylsulfinyl)phenyl)imidazo[1,2-b]pyridazin-3amine (**27**). Purified by prep-TLC (developed twice in 6% CH<sub>3</sub>OH/DCM), trituration in diethyl ether. Brown solid (0.0204 g, 17%); mp 161.6 – 163.7 °C (from diethyl ether);  $R_f$  (CH<sub>3</sub>OH : CH<sub>2</sub>Cl<sub>2</sub> 4 : 96) 0.10; <sup>1</sup>H-NMR δ<sub>H</sub> (400 MHz; DMSO-d<sub>6</sub>) 8.83 (s, 1H), 8.29 (d, J = 1.8 Hz, 1H), 8.25 (d, J = 9.6 Hz, 1H), 8.20 (d, J = 7.8 Hz, 1H), 7.87 – 7.81 (m, 3H), 7.75 (t, J = 7.8 Hz, 1H), 7.53 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H), 2.81 (s, 3H), 2.69 (s, 3H); <sup>13</sup>C-NMR δ<sub>C</sub> (101 MHz; DMSOd<sub>6</sub>) 150.41, 147.99, 147.68, 136.69, 135.60, 135.32, 130.45, 129.64, 127.95, 126.83, 126.71, 125.84 (2C), 125.56, 122.63, 115.43, 114.92 (2C), 43.75, 43.63; LC-MS, APCI<sup>+</sup>: m/z [M + H]<sup>+</sup> = 411.1, calculated exact mass = 410.0871, purity: 99.9%, t<sub>r</sub> = 3.4 min.

# 3.0 Biological and solubility evaluation

In vitro antiplasmodial testing.<sup>3</sup> Compounds were screened against multidrug resistant (K1) and sensitive (NF54) strains of *P. falciparum in vitro* using the modified [<sup>3</sup>H]-hypoxanthine incorporation assay.<sup>3</sup> P. falciparum was cultivated in a variation of the medium previously described,<sup>4,5</sup> consisting of RPMI 1640 supplemented with 0.5% ALBUMAX<sup>®</sup> II, 25 mM Hepes, 25 mM NaHCO<sub>3</sub> (pH 7.3), 0.36 mM hypoxanthine and 100 μg/mL neomycin. Human erythrocytes served as host cells. Cultures were maintained at 37 °C in an atmosphere of 3% O<sub>2</sub>, 4% CO<sub>2</sub> and 93% N<sub>2</sub> in humidified modular chambers. Compounds were dissolved by sonication in DMSO (10 mg/mL) and diluted in hypoxanthine-free culture medium. Infected erythrocytes (100 µL per well with 2.5% hematocrit and 0.3% parasitemia) were added to each drug titrated in 100 µL duplicates over a 64-fold range. After 48 hours incubation, 0.5  $\mu$ Ci of [<sup>3</sup>H]hypoxanthine in 50  $\mu$ L medium was added and plates were incubated for an additional 24 hours. Parasites were harvested onto glass-fiber filters and radioactivity was counted using a Betaplate liquid scintillation counter (Wallac, Zurich). The results were recorded as counts per minute (cpm) per well at each drug concentration and expressed as a percentage of the untreated controls. Fifty percent inhibitory concentrations ( $IC_{50}$ ) were estimated by linear interpolation.<sup>6</sup>

*In vitro* cytotoxicity assay.<sup>7</sup> Cytotoxicity was measured using the MTT-assay which measures cellular growth and survival calorimetrically. All growth and chemosensitivity was measured by the formation of tetrazolium salt. The test samples were assayed in triplicate on one occasion. Stock solutions of 2 mg/mL of test samples in DMSO were prepared with poorly soluble samples being tested as suspensions. The test samples were kept at -20 °C until required. In all experiments, emetine was used as a reference drug. 10-fold serial dilutions in complete medium to give 6 concentrations were made from an initial concentration of 100  $\mu$ g/mL with the lowest concentration being 0.001  $\mu$ g/mL. The cell viability was not affected by the highest concentration of the solvent to which the cells were exposed. The full dose-response curves plotted using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4 software enabled the determination of the IC<sub>50</sub> values.

In vitro hERG testing. A QPatch hERG assay employing a four-point concentrationresponse format was used to carry out hERG inhibition studies by the UK-based Metrion Biosciences Ltd. The hERG gene was stably expressed in a CHO cell line (ATCC) which was grown and passaged under standard culture conditions. The external (e) and internal (i) recording solutions were of the following compositions (mM): NaCl – 140 (e) : 0 (i); KCl – 2 (e) : 70 (i); KF - 0 (e) : 60 (i); HEPES - 10 (e) : 10 (i); MgCl<sub>2</sub> - 1 (e) : 0 (i); CaCl<sub>2</sub> - 2 (e) : 0 (i); Glucose - 5 (e) : 0 (i); EGTA - 0 (e) : 5 (i); Mg<sub>2</sub>ATP - 0 (e) : 5 (i) and pH – 7.4 (NaOH) (e) : 7.2 (KOH) (i). The external recording solution was regularly prepared and kept at 4 °C until required and was maintained at room temperature during recording. The internal recording solution was prepared and kept at – 20 °C until required. The QPatch is a chip-based planar patch clamp which is automated. Using suction, cells added to each well are drawn across a small aperture creating a Giga-ohm seal between the membrane surface and a treated silicon surface. A small volume of bathing solution containing the test compound or control bathing solution is added to a reservoir on the chip which perfuses across the cell through quartz-lined microfluidic channels. The solution is removed by capillary action before the next sample is added. Using the industry +40/-40 voltage protocol, currents were triggered from a holding potential of -90 mV at a stimulus frequency of 0.1 Hz.

By cumulatively adding four escalating concentrations of the test compounds to an individual cell, the concentration response curves were established. This was done by firstly allowing the whole-cell configuration to be achieved followed by the addition of the vehicle (0.1% DMSO v/v in external recording solution) to each well in two bolus additions allowing a two-minute recording time between each addition. This was followed by the addition of four concentrations  $(0.3 - 10 \mu M)$  of test compounds in two bolus additions at 2-minute intervals. The effect on the hERG tail current amplitude was measured during the 4-minute recording time. The concentrations (0.3, 1, 3 and 10  $\mu$ M) of the test samples were prepared in such a way to have a final concentration of 0.1% of DMSO v/v in the external recording solution. For each compound, the experiments at each concentration were done in triplicate and using a bioinformatics suite developed and running in Pipeline Pilot (Biovia, USA), the percent inhibition, as a reduction in mean peak current relative to the value measured at the end of the vehicle control period, was calculated. Such percent inhibition data were used to construct the concentration-response curves which enabled calculation of the IC<sub>50</sub> values. For compounds which could not achieve 50% inhibition even at the highest tested concentration of 10  $\mu$ M, extrapolated IC<sub>50</sub> values for such are reported. In this regard, all IC<sub>50</sub> values above 10  $\mu$ M reported in this article were extrapolated and should be treated with caution.

**Solubility determination.**<sup>8</sup> A miniaturised shake flask method was used to perform the solubility assay. From 10 mM stock solutions of the test compounds in DMSO, calibration standards (10 - 220  $\mu$ M in DMSO) were prepared. The 10 mM stock solutions were also used to spike (1:50) duplicate aqueous samples in phosphate buffered saline (pH 6.5). The DMSO was dried off in a GeneVac (MiVac, 90 min, 37 °C) after which the samples were incubated while shaking for 20 hours at 25 °C. Thereafter, the solutions were filtered, and their absorbance measured using HPLC-DAD (Agilent 1200 Rapid Resolution HPLC with a diode array detector). The calibration standards were used to plot the calibration curves, which were used to determine the solubility of the aqueous samples.

## 4.0 Additional references

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# 5.0 Copies of NMR spectra





















































nmrdata.2061.fid PCM 061 in D6-DMSO 1H Spectrum Peter 6000 5500 1 ,SO₂Me 5000 н'n 11 SOMe 4500 4000 3500 3000 H (t) 7.77 J(7.84) L (dd) 7.24 J(8.09, 2.30) 2500 G (d) 7.86 J(7.76) K (d) 7.36 J(7.65) D (d) 8.22 J(7.86) 2000 I (t) 7.50 J(7.91) B (d) 8.38 J(9.49) E (d) 8.05 M (s) 3.16 A (s) 9.09 (s) .81 1500 Μ J(9.63) J (s) 7.45 F (s) 8.02 C (s) 8.29 ...38 8.8 -<mark>1.32-</mark> 1.32-204 30-19 1000 8.1 8.0 7.9 7.8 f1 (ppm) 8.4 8.2 7.7 7.6 7.2 7.5 7.4 7.3 500 M h ı - 0 1.00<u>4</u> 1.194 1.324 1.324 1.20<sup>4</sup> 1.304 1.12<sup>4</sup> 1.264 1.264 1.384 1.28 1.394 1.294 1.294 3.59<u>≖</u> 3.46-≖ -500 8.5 7.5 8.0 7.0 4.5 3.5 . 3.0 9.0 6.5 5.5 5.0 4.0 6.0 f1 (ppm)















151 150 149 148 147 146 145 144 143 142 141 140 139 138 137 136 135 134 133 132 131 130 129 128 127 126 125 124 123 122 121 120 119 118 117 fl (ppm)









52 151 150 149 148 147 146 145 144 143 142 141 140 139 138 137 136 135 134 133 132 131 130 129 128 127 126 125 124 123 122 121 120 119 118 117 116 46 45 44 f1 (ppm)







