Supplementary Information

A Critical Evaluation of Pyrrolo[2,3-*d*]pyrimidine-4-amines as *Plasmodium falciparum* Apical Membrane Antigen 1 (AMA1) Inhibitors

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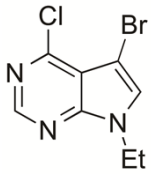
Contents

General Experimental

NMR spectra (^{1}H, ^{19}F, ^{13}C) were recorded on a Bruker Avance Nanobay III 400 MHz Ultrashield Plus spectrometer at 400.13, 376.50 and 100.62 MHz, respectively coupled to a BACS 60 automatic sample changer at 25 °C. Chemical shifts (δ) are recorded in parts per million (ppm) by correction with reference to the chemical shift of the solvent, according to the procedure described by Gottlieb.[45] Coupling constants (*J*) are recorded in Hz, and the significant multiplicities described by singlet (s), doublet (d), triplet (t), quadruplet (q), broad (br), multiplet (m), doublet of doublets (dd), and doublet of triplets (dt). LC-MS were run to verify reaction outcome and purity using an Agilent 6100 series Single Quad coupled to an Agilent 1200 series HPLC. The following buffers were used: buffer A, 0.1% formic acid in H_2O; buffer B, 0.1% formic acid in MeCN. The following gradient was used with a Phenomenex Luna 3 µM C8(2) 15 mm × 4.6 mm column, and a flow rate of 0.5 mL/min and total run time of 12 min; 0–4 min 95% buffer A and 5% buffer B, 4–7 min 0% buffer A and 100% buffer B, 7–12 min 95% buffer A and 5% buffer B. Mass spectra were acquired in positive and negative ion mode with a scan range of 0–1000 *m*/*z* at 5 V. UV detection was carried out at 254 nm. All compounds were of >95% purity. Thin layer chromatography was conducted on 0.2 mm plates using Merck silica gel 60 F_{254}. Column chromatography was achieved using Merck silica gel 60 (particle size 0.063–0.200 µm, 70–230 mesh). Calculated partition co-efficient values (cLogP) were calculated using ChemAxon's Instant JChem program. Instant JChem was used for structure database management, search and prediction (Instant JChem 5.9.4, 2013, ChemAxon; http://www.chemaxon.com). 4-Chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1**) was purchased from Astatech and used to synthesise 5-bromo-4-chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**3**)[26] and 5-bromo-4-chloro-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**7a**)[35] via literature procedures.

Synthetic procedures

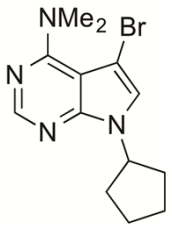
5-Bromo-4-chloro-7-ethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (7b)



To a solution of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1**) (1.00 g, 6.51 mmol) in DMF (8 mL) at 0 °C, was added NaH, as a 60% dispersion in mineral oil (389 mg, 9.77 mmol), portionwise in a teflon-capped sealed tube. The mixture was stirred at 0 °C for 30 min, whereby pressure builds up. The mixture was briefly degassed and EtI (1.04 mL, 13.02 mmol) was added and the whole heated at 60 °C for 5 h. The mixture was extracted with EtOAc (100 mL) and washed with H_2O (100 mL). The aqueous fraction was re-extracted with EtOAc (100 mL). The organic fractions were combined and washed with H_2O (5 × 50 mL), then sat. NaCl (10 mL). The organic phase was dried with $MgSO_4$, filtered and the filtrate evaporated under reduced pressure to give a brown gum (1.31 g). The residue was purified by column chromatography (petroleum spirits/EtOAc, 9:1) to give 4-chloro-7-ethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**6b**) (994 mg, 84%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.54 (s, 1H), 7.19 (d, *J* = 3.6 Hz, 1H), 6.49 (d, *J* = 3.6 Hz, 1H), 4.24 (q, *J* = 7.3 Hz, 2H), 1.40 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 152.0 (C), 150.6 (C), 150.4 (CH), 128.7 (CH), 117.5 (C), 99.3 (CH), 40.0 (CH_2), 15.5 (CH_3).

To a solution of 4-chloro-7-ethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**6b**) (510 mg, 2.82 mmol) in DCM (10 mL) was added *N*-bromosuccinimide (551 mg, 3.09 mmol) and the whole was stirred at 25 °C for 16 h. At this time the solution was evaporated under reduced pressure and H_2O (100 mL) added. The precipitate which formed was filtered, washed with H_2O and dried to give 5-bromo-4-chloro-7-ethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**7b**) (695 mg, 95%) as a light pink solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (s, 1H), 7.31 (s, 1H), 4.31 (q, *J* = 7.3 Hz, 2H), 1.47 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 152.2 (C), 151.0 (CH), 150.1 (C), 128.7 (CH), 115.0 (C), 87.5 (C), 40.4 (CH_2), 15.5 (CH_3).

5-Bromo-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-dimethylamine (4a)

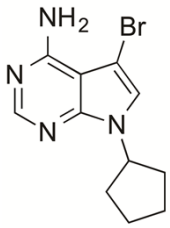


3 (400 mg, 1.33 mmol) was dissolved in *i*-PrOH (1.5 mL) and Me_2NH (as a 33% solution in EtOH) (300 µL) was added and the solution heated at 100 °C in a sealed tube for 16 h. H_2O (50 mL) was added to and the mixture extracted with EtOAc (2 × 20 mL), dried with $MgSO_4$, filtered and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography (petroleum spirits/EtOAc, 1:1) to give **4a** (327 mg, 79%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 7.11 (s, 1H), 5.21 – 5.14 (m, 1H), 3.27 (s, 6H), 2.23 – 2.19 (m, 2H), 1.88 – 1.74 (m,

6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.5 (C), 150.8 (C), 150.3 (CH), 122.4 (CH), 104.2 (C), 87.2 (C), 55.5 (CH), 42.6 (2 × CH_3), 32.9 (2 × CH_2), 24.2 (2 × CH_2).

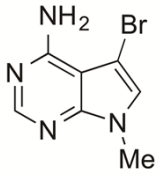
General procedure for formation of 5-bromo-7-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amines, (**4b**, **8a** and **8b**): Concentrated NH_4OH (5 mL) and *i*-PrOH (100 μL) were added to the required chloro intermediate (**3**, **7a** or **7b**) (500 mg) and the mixture was heated at 100 °C in a sealed tube for 40 h. At this time, H_2O (50 mL) was added and the reaction was either filtered and washed with H_2O to give **4b** or extracted with EtOAc (2 × 20 mL), dried with $MgSO_4$, filtered and the filtrate evaporated under reduced pressure to give **8a** and **8b**. These were then purified by column chromatography (petroleum spirits/EtOAc, 1:1).

5-Bromo-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (4b)



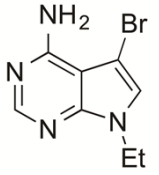
3 (500 mg, 1.66 mmol) gave **4b** (315 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.02 (s, 1H), 5.94 (br s, 2H), 5.17 – 5.10 (m, 1H), 2.24 – 2.19 (m, 2H), 1.88 – 1.75 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.6 (C), 151.7 (CH), 149.4 (C), 121.2 (CH), 102.0 (C), 86.4 (C), 55.6 (CH), 33.1 (2 × CH_2), 24.2 (2 × CH_2).

5-Bromo-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (8a)[35]



7a (900 mg, 3.65 mmol) gave **8a** (576 mg, 69%).
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, 1H), 6.91 (s, 1H), 5.92 (br s, 2H), 3.75 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): 157.0 (C), 152.8 (CH), 149.8 (C), 124.7 (CH), 102.0 (C), 86.1 (C), 31.5 (CH_3).

5-Bromo-7-ethyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (8b)

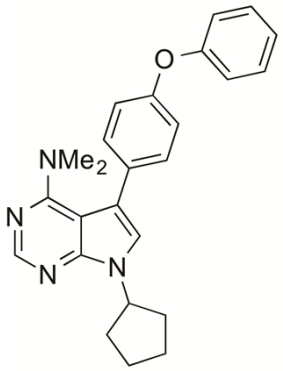


7b (500 mg, 1.91 mmol) gave **8b** (365 mg, 79%).
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, 1H), 6.97 (s, 1H), 5.75 (br s, 2H), 4.20 (q, *J* = 7.3 Hz, 2H), 1.43 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.0 (C), 152.6 (CH), 149.3 (C), 123.1 (CH), 102.1 (C), 86.1 (C), 39.8 (CH_2), 15.8 (CH_3).

General procedure for formation of 5-substituted-7-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amines, (**5a**–**c** and **9a**–**k**): To a degassed biphasic solution of THF (1.5 mL) and 1M Na_2CO_3 (0.5 mL), was added the required bromo intermediate (**4a**–**b** or **8a**–**b**) (for **4a**, 50 mg, 0.16 mmol, for **4b**, 50 mg, 0.18 mmol, for **8a**, 50 mg, 0.22 mmol and for **8b**, 50 mg, 0.21 mmol), R^1–$B(OH)_2$ (3.0 eq.) and $PdCl_2(PPh_3)_2$ (0.1 eq.) and the mixture heated at 100 °C for 2 h. The reaction mixture was

diluted with 3 mL EtOAc and the organic layer filtered through cotton wool. The filtrate was directly applied to a silica column and eluted with EtOAc/Et_3N (99:1). The purified fractions were evaporated at reduced pressure to give compounds **5a**–**c** or **9a**–**k** typically as off-white amorphous solids.

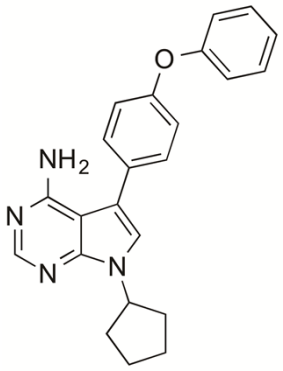
7-cyclopentyl-5-(4-phenoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-dimethylamine (5a)[26]



4a (50 mg, 0.16 mmol) gave **5a** (42 mg, 65%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (s, 1H), 7.44 – 7.41 (m, 2H), 7.37 – 7.33 (m, 2H), 7.13 – 7.08 (m, 1H), 7.06 – 7.02 (m, 5H), 5.28 – 5.20 (m, 1H), 2.85 (s, 6H), 2.28 – 2.21 (m, 2H), 1.91 – 1.76 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 160.5 (C), 157.4 (C), 156.0 (C), 151.9 (C), 150.2 (CH), 131.7 (C), 129.9 (4 × CH), 123.4 (CH), 120.2 (CH), 119.1 (2 × CH), 119.0 (2 × CH), 116.7 (C), 102.8 (C), 55.1 (CH), 41.3 (2 × CH_3), 33.0 (2 × CH_2), 24.2 (2 × CH_2).

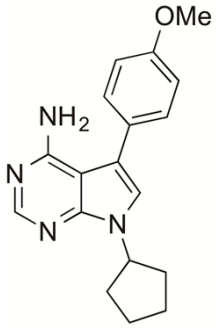
7-cyclopentyl-5-(4-phenoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (5b)[26]



4b (50 mg, 0.18 mmol) gave **5b** (36 mg, 54%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (s, 1H), 7.46 – 7.42 (m, 2H), 7.40 – 7.35 (m, 2H), 7.17 – 7.12 (m, 1H), 7.11 – 7.05 (m, 4H), 7.02 (s, 1H), 5.27 – 5.17 (m, 1H), 5.07 (br s, 2H), 2.31 – 2.21 (m, 2H), 1.92 – 1.76 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.1 (C), 157.0 (C), 156.9 (C), 151.9 (CH), 150.8 (C), 130.3 (2 × CH), 130.1 (C), 130.0 (2 × CH), 123.8 (CH), 119.9 (CH), 119.3 (2 × CH), 119.2 (2 × CH), 115.8 (C), 101.3 (C), 55.1 (CH), 33.1 (2 × CH_2), 24.3 (2 × CH_2).

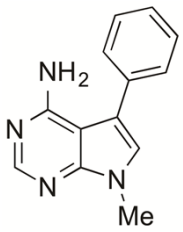
7-cyclopentyl-5-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (5c)[26]



4b (50 mg, 0.18 mmol) gave **5c** (29 mg, 53%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1H), 7.39 (d, J = 8.7 Hz, 2H), 7.00 – 6.96 (m, 3H), 5.37 (br s, 2H), 5.22 – 5.17 (m, 1H), 3.85 (s, 3H), 2.25 – 2.20 (m, 2H), 1.90 – 1.75 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.1 (C), 156.8 (C), 151.0 (CH), 150.5 (C), 130.1 (2 × CH), 127.2 (C), 119.8 (CH), 116.2 (C), 114.6 (2 × CH), 101.3 (C), 55.5 (CH_3), 55.1 (CH), 33.1 (2 × CH_2), 24.3 (2 × CH_2).

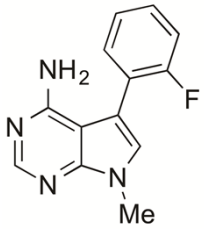
7-Methyl-5-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9a)



8a (50 mg, 0.22 mmol) gave **9a** (22 mg, 45%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 7.50−7.43 (m, 4H), 7.38−7.34 (m, 1H), 6.94 (s, 1H), 5.24 (br s, 2H), 3.84 (s, 3H). HR-ESMS calcd. for $C_{13}H_{13}N_4^+$ [M + H] 225.1135, found 225.1135.

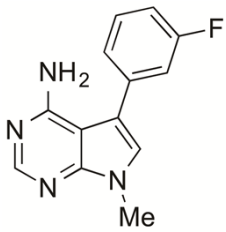
5-(2-Fluorophenyl)-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9b)



8a (50 mg, 0.22 mmol) gave **9b** (27 mg, 51%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (s, 1H), 7.43 (td, *J* = 7.6, 1.8 Hz, 1H), 7.35 (tdd, *J* = 7.1, 5.1, 1.9 Hz, 1H), 7.27−7.18 (m, 2H), 7.04 (d, *J* = 1.1 Hz, 1H), 5.04 (br s, 2H), 3.86 (s, 3H). HR-ESMS calcd. for $C_{13}H_{12}FN_4^+$ [M + H] 243.1041, found 243.1040.

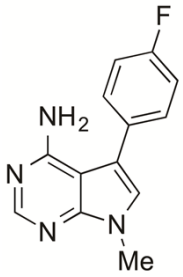
5-(3-Fluorophenyl)-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9c)



8a (50 mg, 0.22 mmol) gave **9b** (16 mg, 30%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (s, 1H), 7.42 (td, *J* = 7.9, 6.1 Hz, 1H), 7.28−7.24 (m, 1H), 7.18 (ddd, *J* = 9.7, 2.4, 1.7 Hz, 1H), 7.05 (tdd, *J* = 8.5, 2.6, 1.0 Hz, 1H), 6.96 (s, 1H), 5.16 (br s, 2H), 3.85 (s, 3H). HR-ESMS calcd. for $C_{13}H_{12}FN_4^+$ [M + H] 243.1041, found 243.1040.

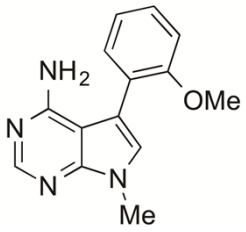
5-(4-Fluorophenyl)-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9d)



8a (50 mg, 0.22 mmol) gave **9d** (25 mg, 47%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 7.45−7.41 (m, 2H), 7.17−7.12 (m, 2H), 6.90 (s, 1H), 5.14 (br s, 2H), 3.83 (s, 3H). HR-ESMS calcd. for $C_{13}H_{12}FN_4^+$ [M + H] 243.1041, found 243.1041.

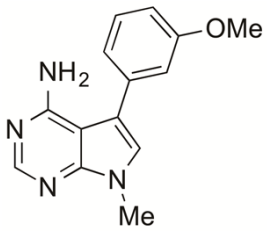
5-(2-Methoxyphenyl)-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9e)



8a (50 mg, 0.22 mmol) gave **9e** (28 mg, 50%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1H), 7.36−7.29 (m, 2H), 7.07−7.00 (m, 2H), 6.96 (s, 1H), 5.09 (br s, 2H), 3.83 (s, 6H). HR-ESMS calcd. for $C_{14}H_{15}N_4O^+$ [M + H] 255.1240, found 255.1240.

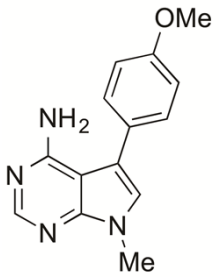
5-(3-Methoxyphenyl)-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9f)



8a (50 mg, 0.22 mmol) gave **9f** (5 mg, 9%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (s, 1H), 7.39−7.35 (m, 1H), 7.07−7.04 (m, 1H), 7.02−6.99 (m, 1H), 6.95 (s, 1H), 6.91 (ddd, *J* = 8.3, 2.6, 0.9 Hz, 1H), 5.16 (br s, 2H), 3.86 (s, 3H), 3.85 (s, 3H). HR-ESMS calcd. for $C_{14}H_{15}N_4O^+$ [M + H] 255.1240, found 255.1241.

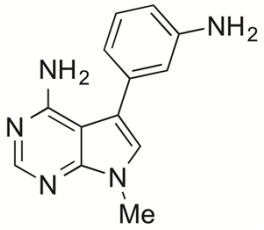
5-(4-Methoxyphenyl)-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9g)



8a (50 mg, 0.22 mmol) gave **9g** (27 mg, 48%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 7.39 (d, *J* = 8.8 Hz, 2H), 6.99 (d, *J* = 8.8 Hz, 2H), 6.88 (s, 1H), 5.05 (br s, 2H), 3.86 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.1 (C), 157.2 (C), 152.1 (CH), 150.3 (C), 130.2 (2 × CH), 127.2 (C), 128.5 (CH), 115.9 (C), 114.6 (2 × CH), 101.4 (C), 55.5 (CH_3), 31.3 (CH_3). HR-ESMS calcd. for $C_{14}H_{15}N_4O^+$ [M + H] 255.1240, found 255.1240.

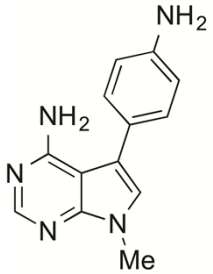
5-(3-Aminophenyl)-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9h)



8a (50 mg, 0.22 mmol) gave **9h** (24 mg, 46%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 7.23 (t, *J* = 7.7 Hz, 1H), 6.92 (s, 1H), 6.85 (ddd, *J* = 7.5, 1.5, 1.0 Hz, 1H), 6.83−6.76 (m, 1H), 6.67 (ddd, *J* = 8.0, 2.4, 1.0 Hz, 1H), 5.20 (s, 2H), 3.83 (s, 3H). HR-ESMS calcd. for $C_{13}H_{14}N_5^+$ [M + H] 240.1244, found 240.1236.

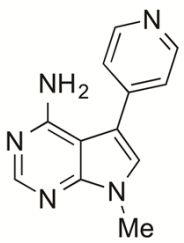
5-(4-Aminophenyl)-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9i)



8a (50 mg, 0.22 mmol) gave **9i** (16 mg, 30%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (s, 1H), 7.28 (d, *J* = 8.5 Hz, 2H), 6.87 (s, 1H), 6.79 (d, *J* = 8.5 Hz, 1H), 5.14 (br s, 2H), 3.84 (s, 3H), 3.78 (br s, 2H). HR-ESMS calcd. for $C_{13}H_{14}N_5^+$ [M + H] 240.1244, found 240.1238.

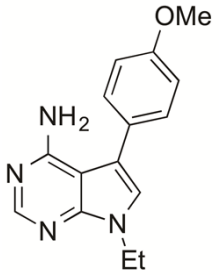
7-Methyl-5-(pyridin-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9j)



8a (50 mg, 0.22 mmol) gave **9j** (10 mg, 20%).

^{1}H NMR (400 MHz, DMSO): δ 8.58 (dd, *J* = 4.4, 1.6 Hz, 2H), 8.19 (s, 1H), 7.57 (s, 1H), 7.45 (dd, *J* = 4.4, 1.6 Hz, 2H), 6.30 (br s, 2H), 3.76 (s, 3H). HR-ESMS calcd. for $C_{12}H_{12}N_5^+$ [M + H] 226.1087, found 226.1081.

7-Ethyl-5-(4-methoxyphenyl)- 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9k)



8b (50 mg, 0.21 mmol) gave **9k** (38 mg, 68%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (s, 1H), 7.40 (d, *J* = 8.7 Hz, 2H), 6.99 (d, *J* = 8.7 Hz, 2H), 6.93 (s, 1H), 5.07 (br s, 2H), 4.27 (q, *J* = 7.3 Hz, 2H), 3.86 (s, 3H), 1.49 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.0 (C), 157.3 (C), 152.0 (CH), 150.3 (C), 130.1 (2 × CH), 127.3 (C), 121.8 (CH), 115.9 (C), 114.6 (2 × CH), 101.4 (C), 55.5 (CH_3), 39.4 (CH_2), 15.8 (CH_3). HR-ESMS calcd. for $C_{15}H_{17}N_4O^+$ [M + H] 269.1397, found 269.1397.

3D7 *Pf*AMA1 DI+II Expression

3D7 *Pf*AMA1$_{[104-442]}$ was produced according to the procedures described by Lim *et al*.[29] In brief, the protein was first expressed using the high-cell-density methodology and then purified using Ni^{2+}-affinity chromatography. The denatured protein was refolded in a redox environment containing reduced and oxidised glutathione at 4:1 ratio, and the refolded protein was subsequently purified using anion-exchange chromatography. Pure fractions were combined and buffer-exchanged into 20 mM phosphate buffer, pH 7.4, using an Amicon Ultra-4 centrifugal unit (Millipore) for NMR experiments.

NMR Aggregation Studies of 5a−c

All three compounds (**5a**, **5b** and **5c**) were dissolved at concentrations of 5, 10, 20, 40 and 80 μM in 20 mM phosphate buffer at pH 7.4 containing 10 % ^2H_2O, 1 % $^2H_6$-DMSO and 1 μM 4,4-dimethyl-4-silapentane-1-sulfonic acid. Tween 20 at a final concentration of 0.05 % v/v was added into samples containing 80 μM of the compounds. ^{1}H NMR spectra for all the samples were acquired using Bruker Avance III 600 MHz spectrometer at 25°C with 128 scans. Excitation sculpting was employed to suppress the water proton resonance. The relaxation delay was 5 s. Bruker TopSpin 3.2 software was used to integrate the proton resonances of the compounds.

SPR Binding Experiments

The interaction of **5a**−**5c** and **9a**−**9k** with AMA1 was determined using a Biacore T200 biosensor. The methodology was similar to the protocol described by Lim *et al*.,[29] except that running buffer containing phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4{\cdot}2H_2O$, 2 mM KH_2PO_4), 3 mM EDTA (ethylenediaminetetraacetic acid), 0.05 % v/v Tween 20 and 5 % DMSO, pH 7.4 was used instead. Compounds were tested at 6.25, 12.5, 25, 50 and 100 μM.

CPMG Binding Assays

CPMG experiments were conducted using 20 mM phosphate buffer at pH 7.4 containing 10 % ^2H_2O and 1 % $^2H_6$-DMSO. CPMG spin-locks of 0 and 200 ms with 1 ms delay between the hard 180° pulses were employed. Spectra for samples with 80 µM of **5c** in the presence and absence of 10 µM 3D7 *Pf*AMA1$_{[104-442]}$ were acquired first before adding R1 (VFAEFLPLFSKFGSRMHILK)[12] or RON2 (DITQQAKDIGAGPVASCFTTRMSPPQQICLNSVVNTALS) peptides to a final concentration of 15 µM.

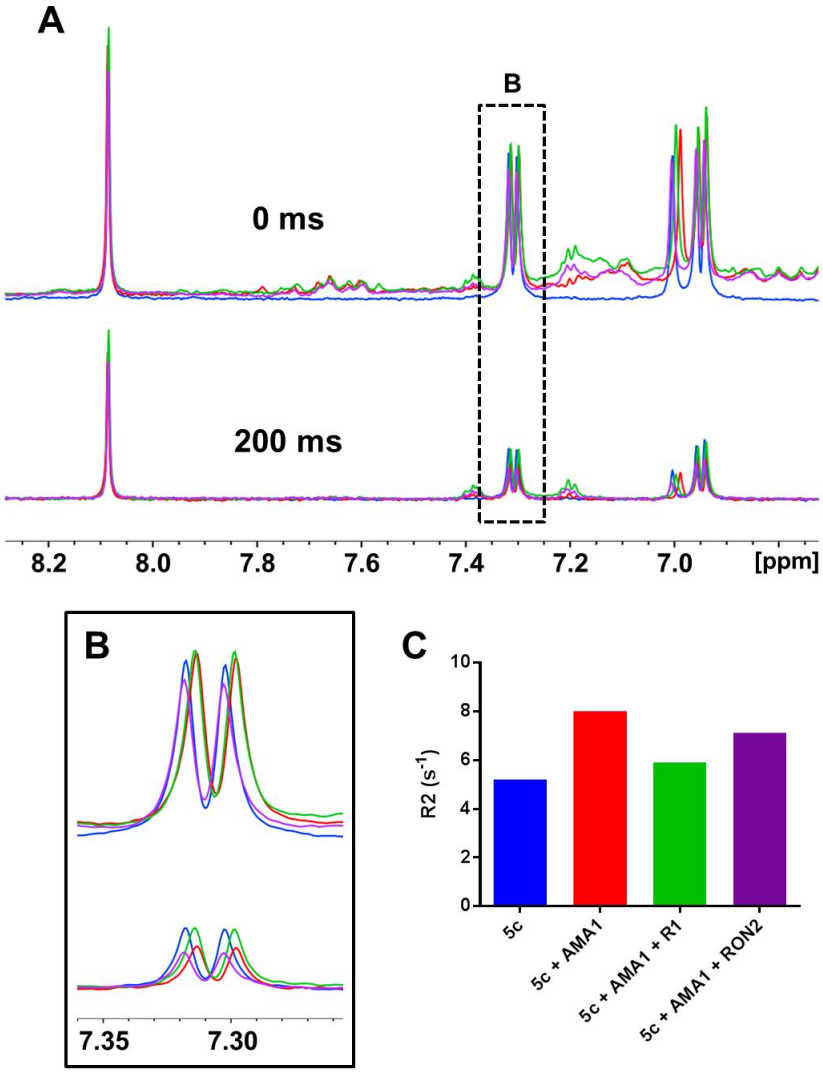
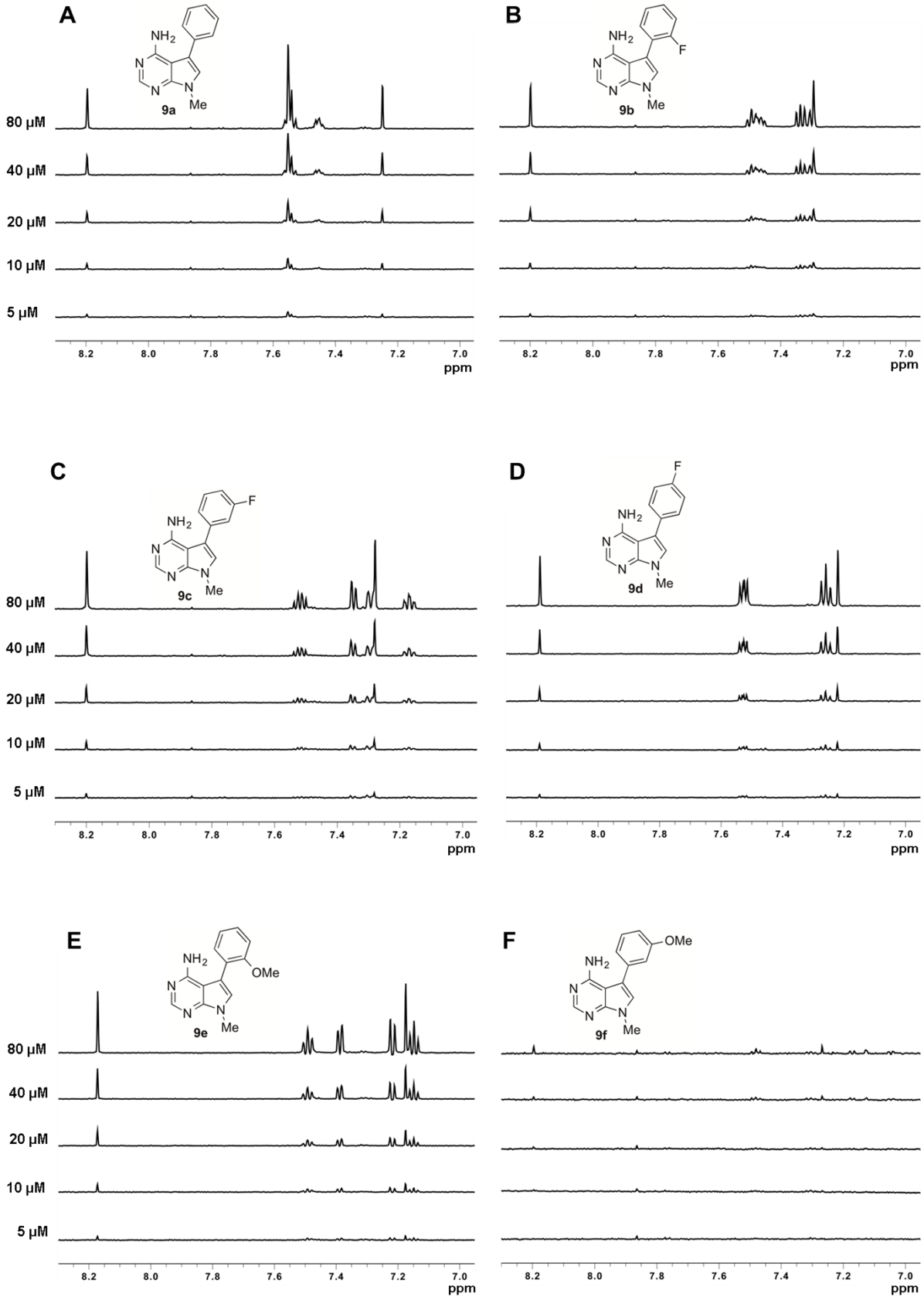


Figure S1. Binding monitored by CPMG. (A and B) All CPMG spectra were acquired with both 0 (top spectra) and 200 ms (bottom spectra) spin-relaxation filters in the presence of 0.05 % v/v Tween 20. Blue and red spectra were from samples containing **5c** in presence or absence of 3D7 *Pf*AMA1, respectively. Green and purple spectra correspond to samples containing **5c** and 3D7 *Pf*AMA1 with the additions of R1 and RON2 peptides, respectively. (C) Transverse relaxation rate (R2) for different samples used in the binding studies.

NMR Aggregation Studies of 9a−k

In a similar fashion to **5a−c**, the aggregation of **9a−k** was studied by 1D ^{1}H NMR.



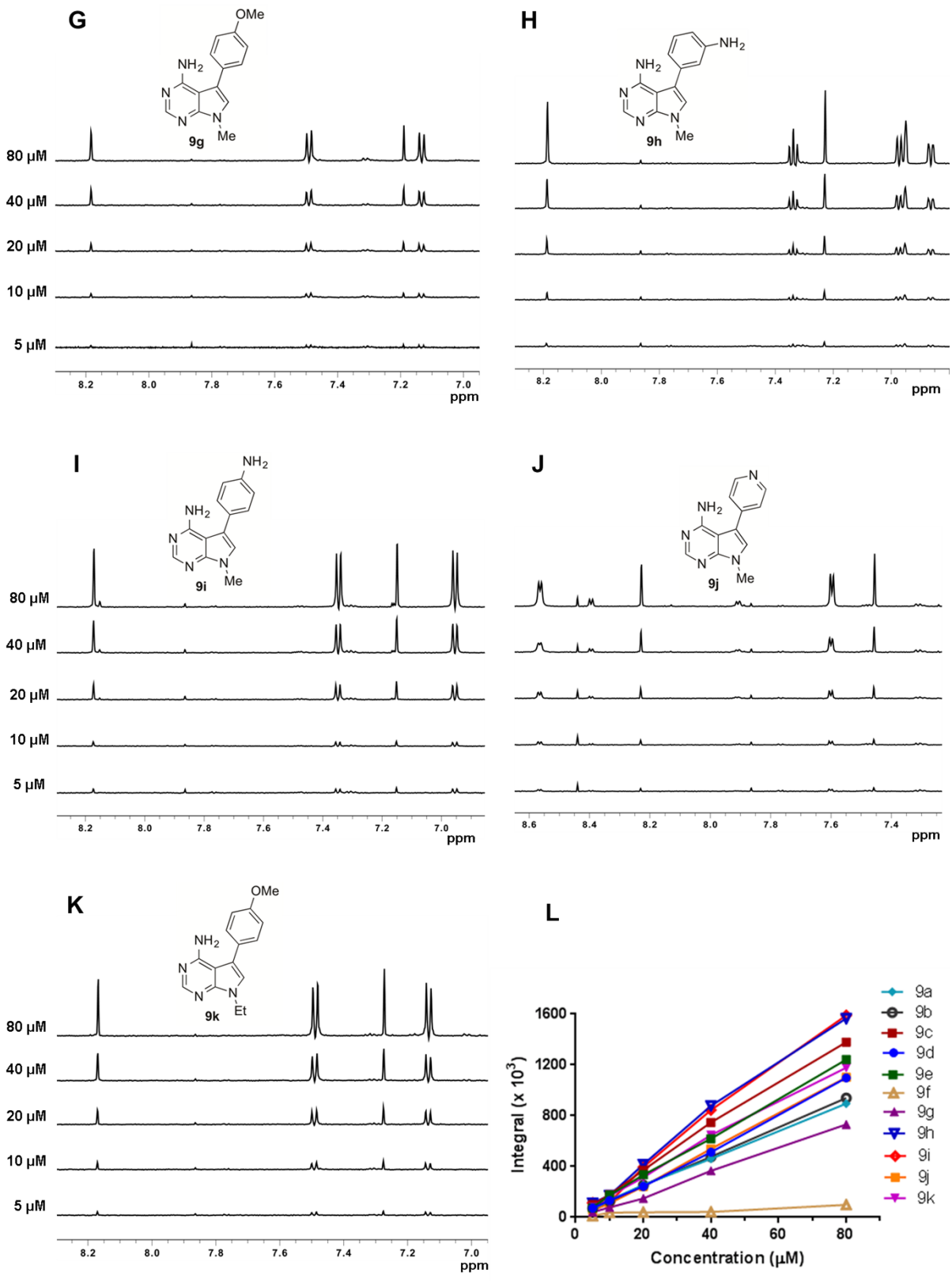


Figure S2. (A-K) 1D ^{1}H NMR spectra of pyrrolo[2,3-*d*]pyrimidine-4-amines (**9a**–**k**) at different concentrations. (L) Peak intensities of proton signals (~8.2 ppm (H2)) of **9a**–**k** at increasing compound concentrations. Note that **9f** precipitated out of solution, which caused the reduction in signal.

Parasite growth inhibition assays

Flow cytometry based growth inhibition assays were performed as described in detail elsewhere.[37,46,47] Compounds **5a**–**5c** and **9a**–**9k** were dissolved in 100% DMSO to make a 100 mM stock. These stocks and DMSO controls were then diluted in PBS to make the dilution series shown in Figure S3. Synchronised early trophozoite stage parasites (3D7 strain) were grown in the presence of each compound and DMSO controls. After two invasion cycles, early trophozoite stage parasites were stained, and parasitaemia measured by flow cytometry. Each dilution series was run in duplicate with the percent growth inhibition = (% parasitaemia in test well/mean % parasitaemia of DMSO control wells x100) -100) x-1).

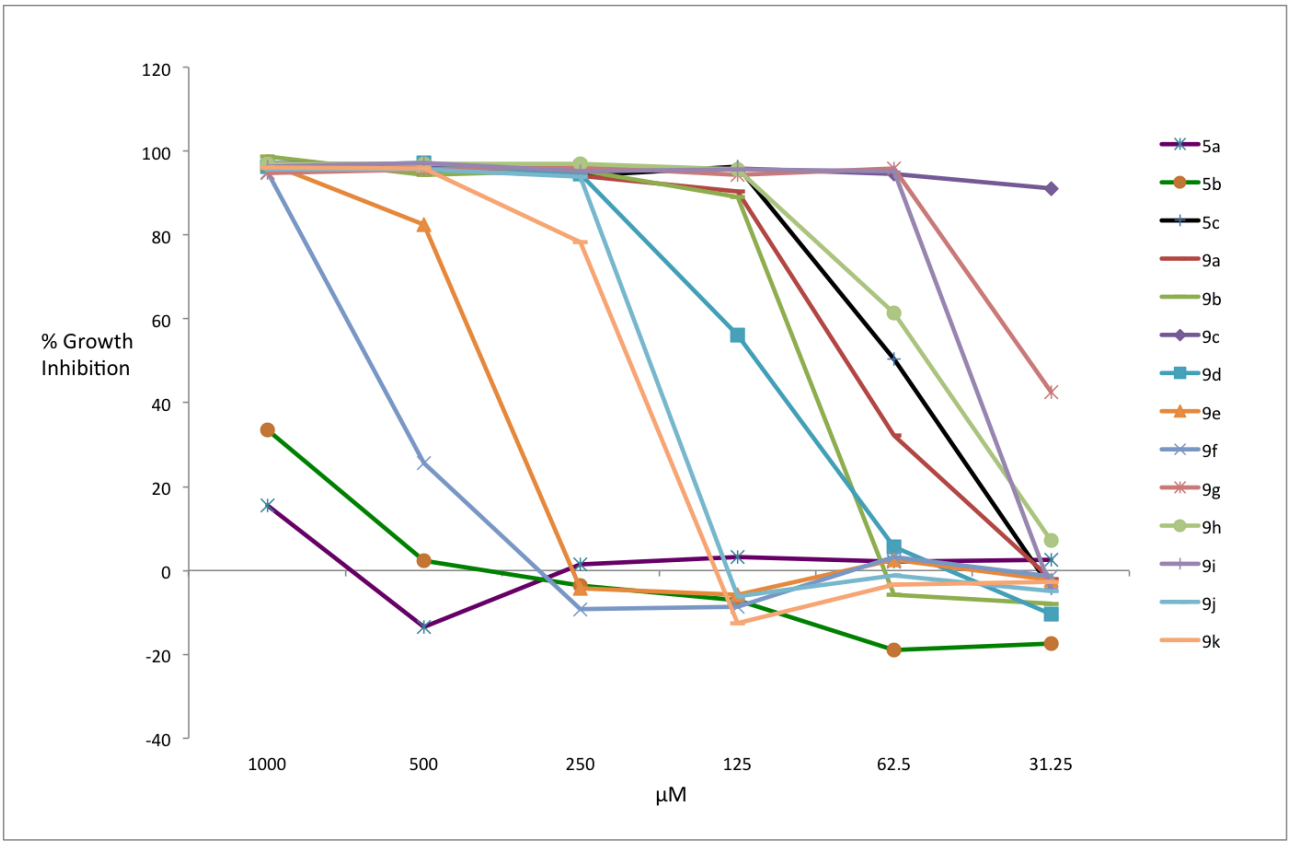


Figure S3. Growth inhibition assay of **9a**–**k**. *Pf*RON2 gave 100% inhibition in this assay over this concentration range.

References

45. H. E. Gottlieb, V. Kotlyar, and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512–7515.

46. D. W. Wilson, B. S. Crabb, and J. G. Beeson, *Malar. J.*, 2010, **9**, 152.

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