Supplementary Information

Discovery of Novel and Ligand-Efficient Inhibitors of *Plasmodium falciparum* and *Plasmodium vivax N*-Myristoyltransferase

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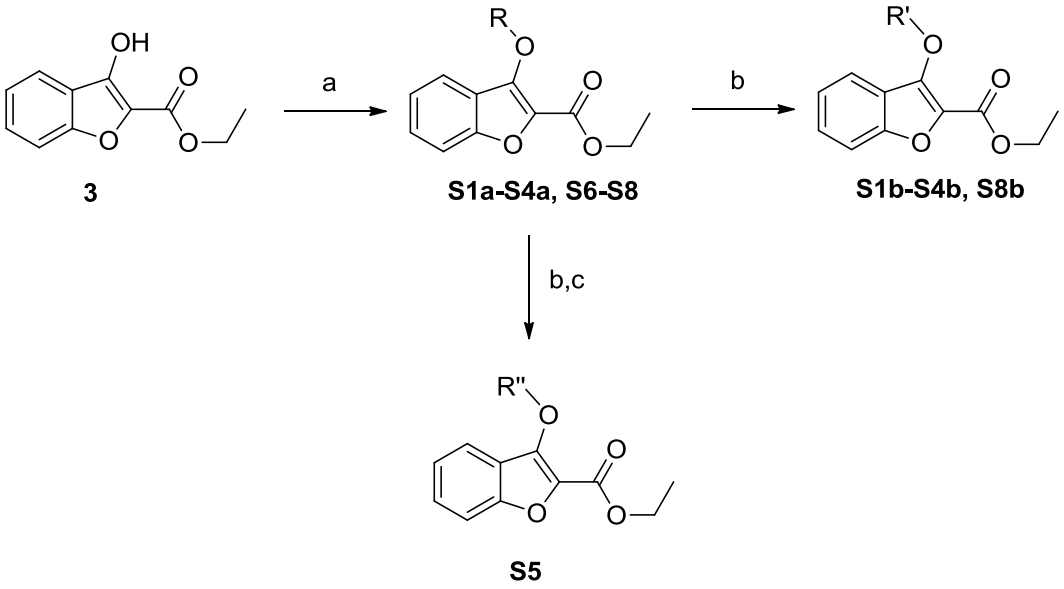
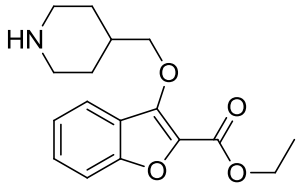
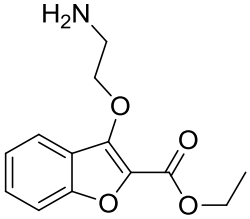
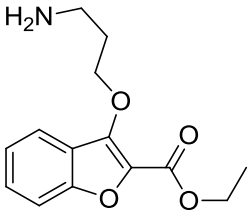
1. Additional Synthetic Schemes and Biological Results

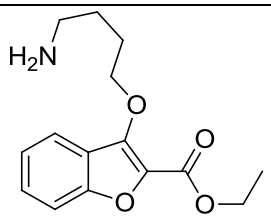
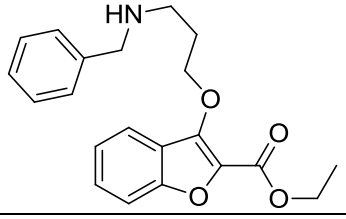
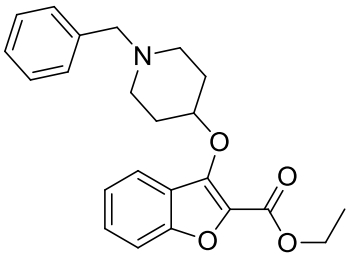
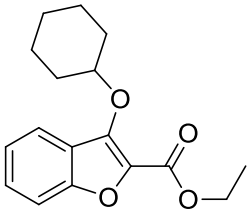
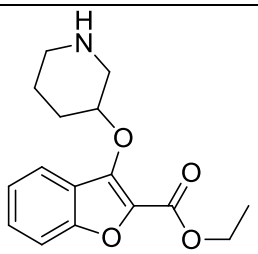


Scheme S1. **Variation of 3-Substituent of 2,3-Substituted Benzo[*b*]furans[a]**

[a] Reagents and Conditions: (a) R-OH, diisopropyl azodicarboxylate, PPh_3, THF, rt, 18 h; b) 10% TFA in DCM (*v/v*), rt, 2h; (c) Benzaldehyde, AcOH, $NaBH(OAc)_3$, rt, 18 h.

Table S1. **Enzyme Affinity of Various Piperidine Replacements**

No.	Structure	PfNMT[a] K_i (μM)	PvNMT[a] K_i (μM)	HsNMT[a] K_i (μM)
S1b		>100	>100	>100
S2b		>100	nd[b]	nd[b]
S3b		>100	>100	>100

S4b		>100	nd[b]	nd[b]
S5		>100	>100	nd[b]
S6		>100	>100	nd[b]
S7		>100	nd[b]	nd[b]
S8		>100	>100	nd[b]

[a] Enzyme K_i values are calculated from the IC_{50} values using the Cheng-Prusoff Equation (see Section 3.2). IC_{50} values are the mean value of two or more determinations. Standard deviation is typically within 20% of the IC_{50}.

[b] nd = not determined

2. Chemistry

2.1. General

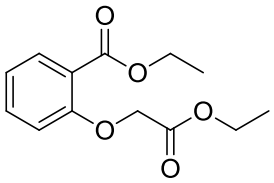
All chemicals were purchased from Sigma-Aldrich Ltd (Gillingham, UK), Acros Organics (Geel, Belgium) and Alfa Aesar (Heysham, UK) and used without further purification. Moisture sensitive reactions were performed under nitrogen atmosphere using dried glassware, anhydrous solvents, and standard syringe/septa techniques.

Silica gel normal phase column chromatography was performed on an Isolera (Biotage, UK) automated apparatus with SNAP silica cartridges (Biotage, UK). Final compounds were purified on a Gilson semi-preparative Reverse Phase-HPLC system equipped with a HICHROM C_{18} Column (250 x 21.2 mm), #306 pumps and a Gilson UV/Vis detector, detecting at 220 nm. The mobile phase consisted of H_2O + 0.1 % Formic acid (solvent A) and MeOH + 0.1% Formic Acid (solvent B), with an elution method of 0-2 min 50% B, 2-30 min 50%-98% B, 30-32 min 98%, 32-32.5 min 2% B at a flow rate of 12 mL/min. Following purification, the organic solvent was removed under reduced pressure and the compounds dried by lyophilisation. The purity of title compounds was verified by RP-HPLC/MS on a Waters 2767 system equipped with a photodiode array and an ESI mass spectrometer using a XBridge C18 (5 µm, 4.6 mm × 100 mm) column, equipped with an XBridge C18 guard column (5 µm, 4.6 mm × 20 mm). The following elution method was used: Gradient of solvent A and solvent B (as above): 0-10 min 5-98% B, 10-12 min 98% B, 12-13 min 98 to 5% B, 13-17 min 5% B. Flow rate: 1.2 mL/min. Purity of tested compounds was ≥ 95%, unless specified.

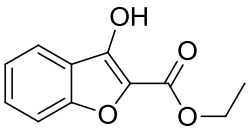
^{1}H and ^{13}C NMR spectra were recorded on 400 MHz and 101 MHz respectively Bruker AV instruments at room temperature and were referenced to residual solvent signals. Data are presented as follows: chemical shift in ppm, integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constants in Hz.

Mass spectra were obtained from the Mass Spectrometry Service of Department of Chemistry, Imperial College London.

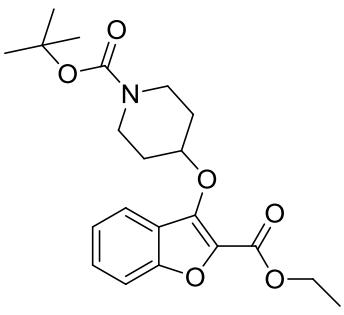
2.2. Synthesis and Characterization of Compounds **2-5**, **7-14b**, **S1-S8**



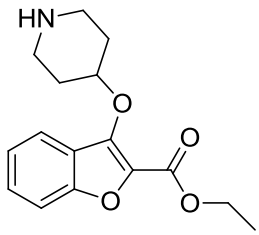
Ethyl-2-(2-ethoxy-2-oxoethoxy)benzoate **2** To a solution of ethyl-2-hydroxybenzoate (884 µL, 6.02 mmol) in acetone (11 mL) was added potassium carbonate (2.50 g, 18.1 mmol) and ethyl bromoacetate (667 µL, 6.02 mmol). The reaction mixture was stirred at 60 °C for 3 h. The white solid was removed by filtration and the filtrate concentrated under reduced pressure, yielding the desired product **2** as an orange oil (1.46 g, 96%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.83 (1H, dd, *J* = 7.7, 1.8), 7.45 (1H, ddd, *J* = 8.4, 7.6, 1.8), 7.06 (1H, ddd, *J* = 7.7, 7.6, 0.7), 6.90 (1H, dd, *J* = 8.4, 0.7), 4.71 (2H, s), 4.38 (2H, q, *J* = 7.0), 4.28 (2H, q, *J* = 7.2), 1.40 (3H, t, *J* = 7.0), 1.30 (3H, t, *J* = 7.2).



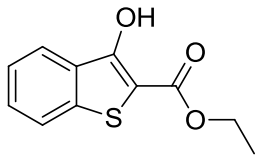
Ethyl-3-hydroxybenzofuran-2-carboxylate **3** To a suspension of potassium *tert*-butoxide (1.80 g, 16.0 mmol) in tetrahydrofuran (60 mL) was added **2** (2.02 g, 8.01 mmol) in tetrahydrofuran (40 mL) slowly over 2 mins. The reaction mixture was stirred at room temperature for 15 mins, then quenched with saturated ammonium chloride solution (100 mL). **3** was immediately extracted with 3 × 100 mL portions of ethyl acetate. The organic layers were combined, dried over magnesium sulfate and concentrated under reduced pressure to give desired product **3** as a yellow solid (1.56 g, 94%). ^{1}H NMR ($CDCl_3$, δ, ppm) 8.15 (1H, brs), 7.75 (1H, d, *J* = 7.9), 7.53–7.45 (2H, m), 7.31 (1H, ddd, *J* = 7.9, 6.1, 1.9), 4.50 (2H, q, *J* = 7.1), 1.47 (3H, t, *J* = 7.1).



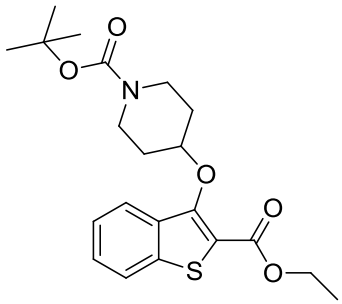
tert-Butyl-4-((2-(ethoxycarbonyl)benzofuran-3-yl)oxy)piperidine-1-carboxylate **4** To a solution of **3** (100 mg, 0.49 mmol) in tetrahydrofuran (3 mL) was added *tert*-butyl-4-hydroxypiperidine-1-carboxylate (108 mg, 0.53 mmol) and triphenylphosphine (140 mg, 0.53 mmol). The reaction mixture was stirred at room temperature under nitrogen for 15 mins, and then diisopropyl azodicarboxylate (105 µL, 0.53 mmol) in tetrahydrofuran (0.5 mL) was added dropwise over 2 mins. Reaction mixture was stirred at room temperature for 18 h, then concentrated under vacuum and crude product purified by flash chromatography to give **4** as a clear colourless oil (50 mg, 26%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.66 (1H, d, *J* = 7.9), 7.51 (1H, d, *J* = 8.4), 7.47–7.42 (1H, m), 7.31–7.24 (1H, m), 4.88–4.80 (1H, m), 4.44 (2H, q, *J* = 7.1), 3.83–3.74 (2H, m), 3.38–3.30 (2H, m), 2.00–1.80 (4H, m), 1.47 (9H, s), 1.43 (3H, t, *J* = 7.1).



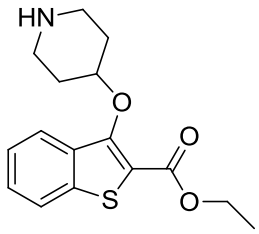
Ethyl 3-(piperidin-4-yloxy)benzofuran-2-carboxylate **5** To a solution of **4** (50 mg, 0.13 mmol) in dichloromethane (1 mL) was added trifluoroacetic acid (100 µL) and the solution stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and purified by HPLC yielding **5** as a pale yellow solid (36 mg, 97%). R_t = 8.5 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 9.07 (1H, s), 7.65 (1H, d, *J* = 7.9), 7.59–7.45 (2H, m), 7.32 (1H, apparent t, *J* = 7.9), 5.12–5.03 (1H, m), 4.46 (2H, q, *J* = 7.1), 3.71–3.54 (2H, m), 3.40–3.23 (2H, m), 2.35–2.14 (4H, m), 1.44 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$, δ, ppm) 159.39, 153.33, 146.22, 132.87, 128.97, 123.90, 122.62, 120.40, 113.25, 74.17, 61.58, 40.17, 27.56, 14.51; ESI HRMS, found 290.1399 ($C_{16}H_{20}NO_4$, $[M+H]^+$, requires 290.1392).



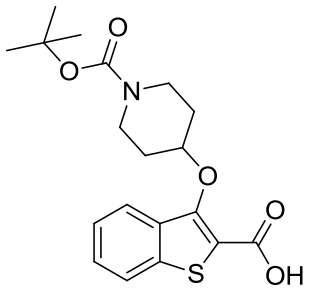
Ethyl-3-hydroxybenzo[*b*]thiophene-2-carboxylate **7** To a solution of methyl-2-mercaptobenzoate (1.63 mL, 11.9 mmol) and ethyl bromoacetate (1.32 mL, 11.9 mmol) in dry tetrahydrofuran (130 mL) at 0 °C was added potassium *tert*-butoxide (5.14 g, 71.3 mmol) gradually over 2 mins. The reaction mixture was stirred and allowed to warm to room temperature over 15 mins, quenched with 2 M hydrochloric acid to pH 2 and diluted with 75 mL water. **7** was immediately extracted with 3 × 75 mL portions of ethyl acetate. The organic layers were combined, washed with 75 mL brine, dried over magnesium sulfate and concentrated under reduced pressure to give desired product **7** as a yellow solid (2.32 g, 88%). ^{1}H NMR ($CDCl_3$, δ, ppm) 10.21 (1H, s), 7.94 (1H, d, *J* = 8.0), 7.74 (1H, d, *J* = 8.0), 7.50 (1H, ddd, *J* = 8.0, 7.5, 1.4), 7.44–7.37 (1H, m), 4.43 (2H, q, *J* = 7.1), 1.43 (3H, t, *J* = 7.1).



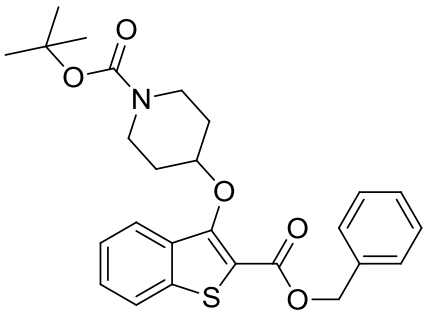
tert-Butyl-4-((2-(ethoxycarbonyl)benzo[*b*]thiophen-3-yl)oxy) piperidine-1-carboxylate **8** To a solution of **7** (2.70 g, 12.2 mmol) in tetrahydrofuran (30 mL) was added *tert*-butyl-4-hydroxypiperidine-1-carboxylate (4.89 g, 24.3 mmol) and triphenylphosphine (6.38 g, 24.3 mmol). The reaction mixture was stirred under nitrogen for 20 mins, and cooled to 0 °C and diisopropyl azodicarboxylate (4.79 mL, 24.3 mmol) in tetrahydrofuran (10 mL) was added dropwise over 5 min. Reaction mixture was allowed to warm to room temperature and stirred for 1.5 h, then concentrated under reduced pressure and the crude product purified by flash chromatography to give **8** as a pink oil (4.77 g, 97%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.86 (1H, d, *J* = 7.9), 7.74 (1H, d, *J* = 8.0), 7.47 (1H, ddd, *J* = 8.0, 7.8, 0.8), 7.39 (1H, dd, *J* = 7.9, 7.8), 4.79 – 4.69 (1H, m), 4.38 (2H, q, *J* = 7.2), 4.01–3.86 (2H, m), 3.20–3.07 (2H, m), 2.05–1.95 (2H, m), 1.91–1.79 (2H, m), 1.48 (9H, s), 1.41 (3H, t, *J* = 7.2).



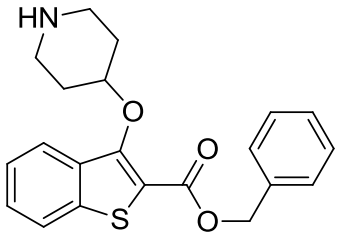
Ethyl-3-(piperidin-4-yloxy)benzo[*b*]thiophene-2-carboxylate **9** To a solution of **8** (47.0 mg, 0.12 mmol) in dichloromethane (1.00 mL) was added trifluoroacetic acid (100 µL) and the solution was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and purified by HPLC yielding **9** as a colourless oil (29 mg, 82%). R_t = 15.6 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 8.73–8.36 (1H, s), 7.83–7.75 (2H, m), 7.52 (1H, ddd, *J* = 7.9, 7.1, 1.0), 7.44 (1H, ddd, *J* = 8.2, 7.1, 1.0), 5.00–4.94 (1H, m), 4.38 (2H, q, *J* = 7.1), 3.77–3.65 (2H, m), 3.39–3.24 (2H, m), 2.36–2.17 (4H, m), 1.42 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$, δ, ppm) 161.9, 153.8, 138.2, 134.2, 128.4, 125.1, 123.3, 122.7, 116.3, 76.0, 61.6, 41.0, 28.2, 14.4; ESI HRMS, found 306.1173 ($C_{16}H_{20}NO_3S$, $[M+H]^+$, requires 306.1164).



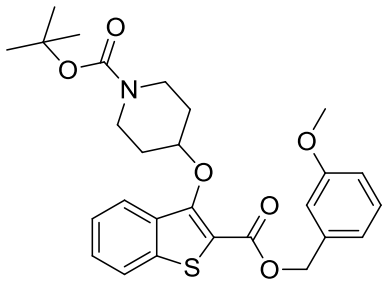
3-((1-(*tert*-butoxycarbonyl)piperidin-4-yl)oxy)benzo[*b*]thiophene-2-carboxylic acid **10** To a solution of **8** (2.50 g, 6.17 mmol) in methanol (30 mL) was added lithium hydroxide monohydrate (3.35 g, 79.8 mmol) and reaction mixture stirred at room temperature for 3 h. Reaction mixture was concentrated under reduced pressure, then dissolved in 100 mL water and acidifed to pH 2 with 2 M hydrochloric acid (30 mL). Precipitate removed by filtration and washed with 5 × 10 mL water, then dried overnight in a vacuum desiccator yielding **10** as an off-white solid (1.36 g, 58%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.87 (1H, d, *J* = 8.0), 7.81 (1H, d, *J* = 8.2), 7.53 (1H, ddd, *J* = 8.0, 7.0, 0.9), 7.44 (1H, dd, *J* = 8.2, 7.0), 4.82–4.73 (1H, m), 4.06–3.93 (2H, m), 3.15–3.03 (2H, m), 2.11–2.00 (2H, m), 1.94–1.80 (2H, m), 1.49 (9H, s).



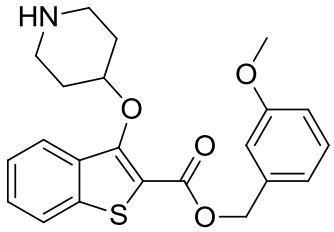
tert-butyl 4-((2-((benzyloxy)carbonyl)benzo[*b*]thiophen-3-yl)oxy)piperidine-1-carboxylate **11a** To a solution of **10** (50 mg, 0.13 mmol) in dry acetonitrile (2 mL) was added hydroxybenzotriazole (27 mg, 0.20 mmol), *N*,*N*-diisopropylethylamine (26 µL, 0.16 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (30 mg, 0.16 mmol) and reaction mixture stirred at room temperature for 15 mins. Phenylmethanol (17 µL, 0.16 mmol) was then added and reaction mixture stirred at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure, dissolved in 10 mL saturated ammonium chloride solution, and **11a** extracted with 3 × 10 mL ethyl acetate. Combined organic layers were then washed with brine (10 mL), dried over magnesium sulphate, concentrated under reduced pressure and crude product purified by flash chromatography to give **11a** as a colourless oil (41 mg, 66%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.85 (1H, d, *J* = 8.0), 7.74 (1H, d, *J* = 8.2), 7.51 – 7.35 (7H, m), 5.37 (2H, s), 4.72 – 4.63 (1H, m), 3.96 – 3.83 (2H, m), 3.05 – 2.96 (2H, m), 1.97 – 1.86 (2H, m), 1.83 – 1.71 (2H, m), 1.48 (9H, s).



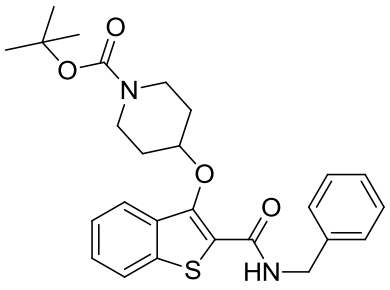
Benzyl-3-(piperidin-4-yloxy)benzo[*b*]thiophene-2-carboxylate **12a** **12a** was prepared as in **9**, replacing **8** with **11a** (40 mg, 0.09 mmol) and purified by HPLC yielding **12a** as a colourless oil (15 mg, 47%). R_t = 10.5 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 7.81 (1H, d, *J* = 8.0), 7.76 (1H, d, *J* = 8.1), 7.54 – 7.34 (7H, m), 5.36 (2H, s), 4.90 – 4.82 (1H, m), 3.58 – 3.48 (2H, m), 3.10 (2H, dd, *J* = 12.0, 6.0), 2.18 (4H, dd, *J* = 10.5, 5.3); ^{13}C NMR ($CDCl_3$, δ, ppm); 161.64, 154.12, 138.33, 135.61, 134.35, 128.87, 128.68, 128.44, 125.17, 123.22, 122.82, 116.00, 76.68, 67.13, 40.81, 28.24. ESI HRMS, found 368.1308 ($C_{21}H_{22}NO_3S$, $[M+H]^+$, requires 368.1320).



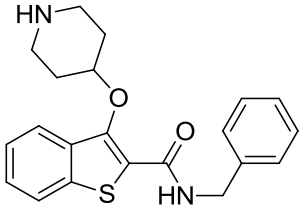
tert-Butyl-4-((2-(((3-methoxybenzyl)oxy)carbonyl)benzo[*b*]thiophen-3-yl)oxy)piperidine-1-carboxylate **11b** was prepared as in **11a**, replacing phenylmethanol with (3-methoxyphenyl)methanol (20 μL, 0.16 mmol) and purified by flash chromatography yielding **11b** as a colourless oil (37 mg, 60%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.86 (1H, d, *J* = 8.0), 7.75 (1H, d, *J* = 8.2), 7.51 – 7.46 (1H, m), 7.43 – 7.37 (1H, m), 7.32 (1H, apparent t, *J* = 7.9), 7.04 (1H, d, *J* = 7.8), 7.02 – 7.00 (1H, m), 6.90 (1H, dd, *J* = 8.2, 2.3), 5.35 (2H, s), 4.74 – 4.66 (1H, m), 3.94 – 3.86 (2H, m), 3.84 (3H, s), 3.07 – 2.98 (2H, m), 1.98 – 1.88 (2H, m), 1.85 – 1.73 (2H, m), 1.48 (9H, s).



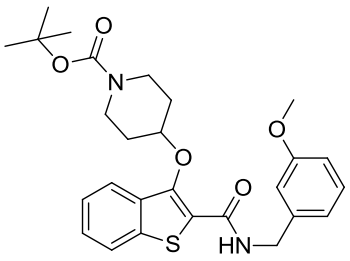
3-Methoxybenzyl-3-(piperidin-4-yloxy)benzo[b]thiophene-2-carboxylate **12b** was prepared as in **9**, replacing **8** with **11b** (34 mg, 0.07 mmol) and purified by HPLC, yielding **12b** as a colourless oil (5 mg, 18%). R_t = 12.3 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 7.82 (1H, d, *J* = 8.0), 7.76 (1H, d, *J* = 8.1), 7.56 – 7.48 (1H, m), 7.47 – 7.40 (1H, m), 7.33 (1H, apparent t, *J* = 7.9), 7.04 (1H, d, *J* = 7.4), 7.01 – 6.98 (1H, m), 6.91 (1H, dd, *J* = 8.2, 2.4), 5.34 (2H, s,), 4.88 – 4.81 (1H, m), 3.84 (3H, s), 3.54 – 3.44 (2H, m), 3.11 – 3.01 (2H, m), 2.22 – 2.11 (4H, m).^{13}C NMR ($CDCl_3$, δ, ppm); 161.48, 159.84, 153.90, 138.23, 136.98, 134.06, 129.82, 128.39, 125.07, 123.14, 122.61, 120.34, 115.87, 113.81, 113.79, 76.10, 66.82, 55.29, 41.01, 28.07. ESI HRMS, found 398.1425 ($C_{22}H_{24}NO_4S$, $[M+H]^+$, requires 398.1426).



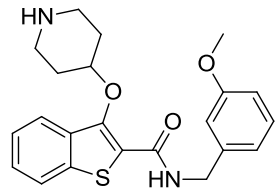
tert-butyl 4-((2-(benzylcarbamoyl)benzo[*b*]thiophen-3-yl)oxy)piperidine-1-carboxylate **13a** To a solution of **10** (43 mg, 0.11 mmol) in dichloromethane (2 mL) was added *N*,*N*-diisopropylethylamine (22 μL, 0.13 mmol) followed by benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (65 mg, 0.13 mmol). After 30 mins stirring at room temperature, benzylamine (14 μL, 0.13 mmol) was added and reaction mixture left stirring for 20 h. Reaction mixture was then diluted in 10 mL water, and **13a** was extracted with 3 × 10 mL dichloromethane. Combined organic layers were then washed with brine (10 mL), dried over magnesium sulphate and concentrated under reduced pressure and crude product purified by flash chromatography to give **13a** as a pale yellow oil (39 mg, 74%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.81 (1H, d, *J* = 7.5 Hz), 7.71 (1H, d, *J* = 7.3 Hz), 7.65–7.58 (1H, m), 7.47–7.29 (6H, m), 4.66 (2H, d, *J* = 5.5 Hz), 4.48–4.39 (1H, m), 4.00–3.82 (2H, m), 2.77–2.65 (2H, m), 1.94–1.82 (2H, m), 1.54–1.42 (11H, m).



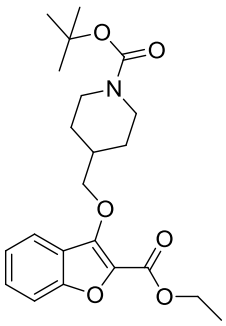
N-Benzyl-3-(piperidin-4-yloxy)benzo[*b*]thiophene-2-carboxamide **14a** **14a** was prepared as in **9**, replacing **8** with **13a** (38 mg, 0.08 mmol) and purified by HPLC yielding **14a** as a colourless oil (12 mg, 40%). R_t = 11.2 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 8.32 (1H, s), 7.82 (1H, d, *J* = 7.8 Hz), 7.69 (1H, d, *J* = 7.5 Hz), 7.50–7.28 (8H, m), 4.68 (2H, d, *J* = 5.9), 4.56–4.48 (1H, m), 3.38–3.27 (2H, m), 2.89–2.79 (2H, m), 2.16–1.99 (4H, m); ^{13}C NMR ($CDCl_3$, δ, ppm) 161.66, 147.24, 138.21, 137.69, 133.21, 129.09, 128.40, 128.02, 127.23, 125.06, 123.85, 121.96, 77.37, 43.90, 41.61, 28.66, one aromatic sp^2 C not observed; ESI HRMS, found 367.1477 ($C_{21}H_{23}N_2O_2S$, $[M+H]^+$, requires 367.1480).



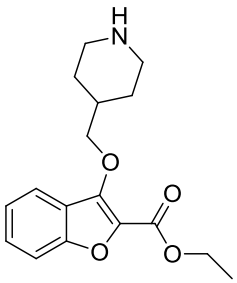
tert-butyl 4-((2-((3-methoxybenzyl)carbamoyl)benzo[*b*]thiophen-3-yl)oxy)piperidine-1-carboxylate **13b** **13b** was prepared as in **13a**, replacing benzylamine with 3-methoxybenzylamine (17 μL, 0.13 mmol) and purified by flash chromatography yielding **13b** as a colourless oil (39 mg, 66%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.81 (1H, d, *J* = 7.8 Hz), 7.75 – 7.69 (1H, m), 7.62 (1H, t, *J* = 5.6 Hz), 7.47 – 7.35 (2H, m), 7.32 – 7.27 (1H, m), 6.96 (1H, d, *J* = 7.6 Hz), 6.94 – 6.91 (1H, m), 6.85 (1H, dd, *J* = 8.3, 2.3 Hz), 4.64 (2H, d, *J* = 5.6 Hz), 4.49 – 4.38 (1H, m), 4.04 – 3.85 (2H, m), 3.81 (3H, s), 2.80 – 2.68 (2H, m), 1.98 – 1.85 (2H, m), 1.60 – 1.50 (2H, m), 1.47 (9H, s).



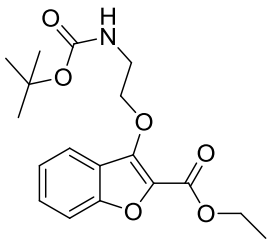
N-(3-Methoxybenzyl-3-(piperidin-4-yloxy)benzo[*b*]thiophene-2-carboxamide **14b** **14b** was prepared as in **9**, replacing **8** with **13b** (38 mg, 0.08 mmol) and purified by HPLC yielding **14b** as a colourless oil (25 mg, 81%). R_t = 9.6 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 9.55 (1H, brs), 7.81 (1H, d, *J* = 7.6 Hz), 7.74 – 7.65 (1H, m), 7.51 – 7.38 (2H, m), 7.35 – 7.26 (2H, m), 6.98 (1H, d, *J* = 7.6 Hz), 6.96 – 6.91 (1H, m), 6.84 (1H, dd, *J* = 8.1, 2.4 Hz), 4.65 (2H, d, *J* = 5.9 Hz), 4.58 – 4.47 (1H, m), 3.80 (3H, s), 3.46 – 3.29 (2H, m), 2.98 – 2.78 (2H, m), 2.21 – 2.02 (4H, m); ^{13}C NMR ($CDCl_3$, δ, ppm) 161.61, 160.11, 147.24, 139.76, 137.61, 133.29, 130.16, 127.25, 125.09, 124.70, 123.80, 121.98, 120.48, 113.98, 113.19, 76.10, 55.40, 43.74, 41.66, 28.59; ESI HRMS, found 397.1581 ($C_{22}H_{25}N_2O_3S$, $[M+H]^+$, requires 397.1586).



tert-Butyl 4-(((2-(ethoxycarbonyl)benzofuran-3-yl)oxy)methyl)piperidine-1-carboxylate **S1a** **S1a** was prepared as in **4**, replacing *tert*-butyl-4-hydroxypiperidine-1-carboxylate with *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate (115 mg, 0.53 mmol), resulting in **S1a** as a clear colourless oil (65 mg, 33%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.71 (1H, d, *J* = 7.9), 7.50 (1H, d, *J* = 8.4), 7.47 – 7.41 (1H, m), 7.26 (1H, t, *J* = 7.9), 4.43 (2H, q, *J* = 7.1), 4.28 (2H, d, *J* = 6.3), 4.23 – 4.08 (2H, m), 2.82 – 2.68 (2H, m), 2.09 – 1.96 (1H, m), 1.92 – 1.84 (2H, m), 1.46 (9H, s), 1.41 (3H, t, *J* = 7.1), 1.39 – 1.27 (2H, m).

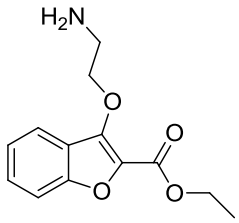


Ethyl 3-(piperidin-4-ylmethoxy)benzofuran-2-carboxylate **S1b** **S1b** was prepared as in **5**, replacing **4** with **S1a** (65 mg, 0.16 mmol), resulting in **S1b** as a clear colourless oil (48 mg, 71%). R_t = 8.3 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 9.04 (1H, s), 8.61 (1H, s), 7.70 (1H, d, *J* = 7.9), 7.56 – 7.43 (2H, m), 7.34 – 7.25 (1H, m), 4.43 (2H, q, *J* = 7.1), 4.35 (2H, d, *J* = 5.4), 3.66 – 3.44 (2H, m), 3.11 – 2.92 (2H, m), 2.29 – 2.06 (3H, m), 1.91 – 1.72 (2H, m), 1.43 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$, δ, ppm) 159.58, 153.26, 148.64, 131.49, 128.81, 123.53, 122.25, 120.79, 113.07, 77.34, 61.35, 44.11, 34.85, 25.42, 14.49; ESI HRMS, found 304.1551 ($C_{17}H_{22}NO_4$, $[M+H]^+$, requires 304.1549).

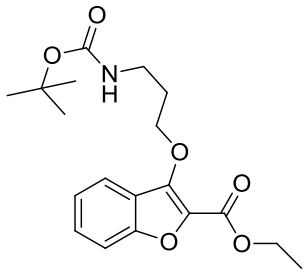


Ethyl 3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)benzofuran-2-carboxylate **S2a** **S2a** was prepared as in **4**, replacing *tert*-butyl-4-hydroxypiperidine-1-carboxylate with *tert*-butyl (2-hydroxyethyl)carbamate (86 mg, 0.53 mmol), resulting in **S2a** as a clear colourless oil (60 mg, 35%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.72 – 7.68 (1H, d, *J* = 7.9 Hz), 7.53 – 7.47 (1H, m), 7.47 – 7.42

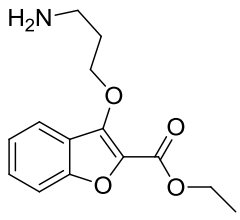
(1H, m), 7.31 – 7.24 (1H, m), 5.61 – 5.49 (1H, brs), 4.50 – 4.43 (4H, m), 3.58 – 3.48 (2H, q, *J* = 5.2 Hz), 1.48 – 1.43 (12H, m).



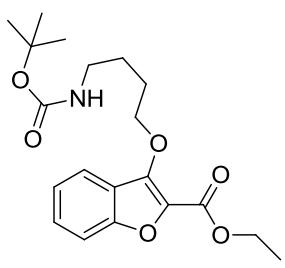
Ethyl 3-(2-aminoethoxy)benzofuran-2-carboxylate **S2b** **S2b** was prepared as in **5**, replacing **4** with **S2a** (58 mg, 0.17 mmol), resulting in **S2b** as a clear colourless oil (30 mg, 72%). R_t = 8.0 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 8.57 (2H, brs), 7.67 (1H, d, *J* = 7.9), 7.57 – 7.45 (2H, m), 7.36 – 7.28 (1H, m), 4.62 (2H, m), 4.41 (2H, q, *J* = 7.1), 3.39 (2H, m), 1.41 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$, δ, ppm) 160.35, 153.42, 147.91, 133.11, 129.25, 124.01, 121.68, 120.30, 113.26, 70.53, 62.15, 39.41, 14.26; ESI HRMS, found 250.1083 ($C_{13}H_{16}NO_4$, $[M+H]^+$, requires 250.1079).



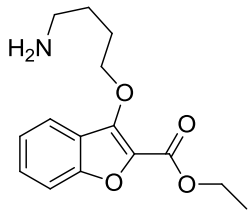
Ethyl 3-(3-((*tert*-butoxycarbonyl)amino)propoxy)benzofuran-2-carboxylate **S3a** **S3a** was prepared as in **4**, replacing *tert*-butyl-4-hydroxypiperidine-1-carboxylate with *tert*-butyl (2-hydroxypropyl)carbamate (93 mg, 0.53 mmol), resulting in **S2a** as a clear yellow oil (77 mg, 44%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.77 – 7.71 (1H, d, *J* = 7.9 Hz), 7.54 – 7.49 (1H, d, *J* = 8.4 Hz), 7.48 – 7.42 (1H, m), 7.30 – 7.24 (1H, m), 5.36 – 5.27 (1H, m), 4.54 – 4.49 (2H, t, *J* = 5.8 Hz), 4.49 – 4.42 (2H, q, *J* = 7.2 Hz), 3.47 – 3.38 (2H, dt, *J* = 5.6 Hz), 2.08 – 2.00 (2H, m), 1.47 – 1.40 (12H, m).



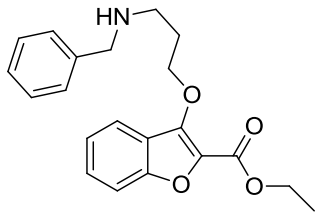
Ethyl 3-(2-aminopropoxy)benzofuran-2-carboxylate **S3b** **S3b** was prepared as in **5**, replacing **4** with **S3a** (73 mg, 0.20 mmol), resulting in **S3b** as a clear pale green oil (44 mg, 83%). R_t = 7.9 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 8.31 (2H, brs), 7.73 (1H, d, *J* = 7.9), 7.59 – 7.45 (2H, m), 7.36 – 7.28 (1H, m), 4.62 (2H, t, *J* = 5.3), 4.42 (2H, q, *J* = 7.1), 3.41 (2H, m), 2.43 – 2.24 (2H, m), 1.41 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$, δ, ppm) 160.29, 153.41, 148.84, 131.21, 129.09, 123.77, 121.19, 121.00, 113.35, 73.11, 61.91, 39.20, 26.92, 14.34; ESI HRMS, found 264.1228 ($C_{14}H_{18}NO_4$, $[M+H]^+$, requires 264.1236).



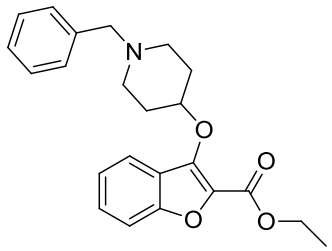
Ethyl 3-(3-((*tert*-butoxycarbonyl)amino)butoxy)benzofuran-2-carboxylate **S4a** **S4a** was prepared as in **4**, replacing *tert*-butyl-4-hydroxypiperidine-1-carboxylate with *tert*-butyl (2-hydroxybutyl)carbamate (101 mg, 0.53 mmol), resulting in **S4a** as a clear yellow oil (130 mg, 72%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.74 – 7.69 (1H, d, *J* = 7.9 Hz), 7.51 – 7.46 (1H, d, *J* = 8.5 Hz), 7.46 – 7.40 (1H, m), 7.28 – 7.22 (1H, m), 4.81 – 4.72 (1H, m), 4.48 – 4.39 (4H, m), 3.26 – 3.17 (2H, m), 1.91 – 1.81 (2H, m), 1.79 – 1.70 (2H, m), 1.45 – 1.38 (12H, m).



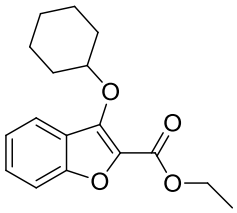
Ethyl 3-(2-aminobutoxy)benzofuran-2-carboxylate **S4b** **S4b** was prepared as in **5**, replacing **4** with **S4a** (135 mg, 0.358 mmol), resulting in **S4b** as a clear colourless oil (22 mg, 22%). R_t = 8.0 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 7.98 (2H, brs), 7.72 (1H, d, *J* = 7.9), 7.55 – 7.41 (2H, m), 7.33 – 7.24 (1H, m), 4.58 – 4.46 (2H, m), 4.39 (2H, q, *J* = 7.1), 3.19 (2H, m), 2.09 – 1.92 (4H, m), 1.40 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$, δ, ppm) 160.06, 153.35, 148.89, 130.85, 128.87, 123.55, 121.59, 121.23, 113.20, 73.71, 61.54, 39.76, 26.55, 24.65, 14.36; ESI HRMS, found 278.1392 ($C_{15}H_{20}NO_4$, $[M+H]^+$, requires 278.1392).



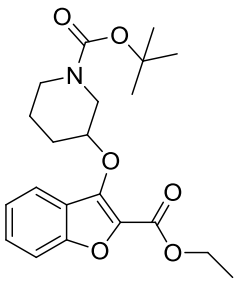
Ethyl 3-(3-(benzylamino)propoxy)benzofuran-2-carboxylate **S5** To a solution of **S3b** (20 mg, 0.08 mmol) in anhydrous tetrahydrofuran (1 mL) was added acid acetic (9 μL, 0.15 mmol) and benzaldehyde (9 μL, 0.09 mmol) and reaction mixture stirred at room temperature for 1.5 h. $NaBH(OAc)_3$ was then added (97 mg, 0.46 mmol) and suspension was stirred at room temperature for a further 18 h. Reaction mixture was diluted with 5% $NaHCO_3$ (aq) (5 mL) then extracted with 3 x 5 mL EtOAc. Combined organic layers were then washed with brine (10 mL), dried over sodium sulphate, concentrated under reduced pressure and crude product purified by HPLC to give **S5** as a white solid (18 mg, 67%). R_t = 8.4 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 9.96 (1H, brs), 7.71 (1H, d, *J* = 8.0), 7.56 – 7.47 (4H, m), 7.41 – 7.35 (3H, m), 7.34 – 7.28 (1H, m), 4.55 (2H, t, *J* = 5.5), 4.40 (2H, q, *J* = 7.1), 4.33 (2H, brs), 3.41 – 3.32 (2H, m), 2.43 – 2.32 (2H, m), 1.43 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$, δ, ppm) 160.43, 153.49, 149.11, 131.29, 130.98, 130.39, 130.22, 129.56, 129.25, 129.22, 123.87, 120.95, 113.36, 72.88, 61.93, 52.00, 45.92, 25.99, 14.47; ESI HRMS, found 354.1708 ($C_{21}H_{24}NO_4$, $[M+H]^+$, requires 354.1705).



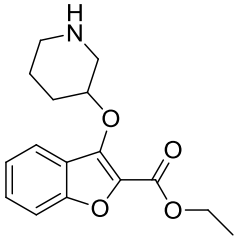
Ethyl 3-((1-benzylpiperidin-4-yl)oxy)benzofuran-2-carboxylate **S6** **S6** was prepared as in **4**, replacing *tert*-butyl-4-hydroxypiperidine-1-carboxylate with 1-benzylpiperidin-4-ol (102 mg, 0.53 mmol) and purifying by HPLC, resulting in **S6** as an orange oil (19 mg, 10%). R_t = 8.6 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 7.58 – 7.53 (2H, m), 7.52 – 7.37 (6H, m), 7.34 – 7.28 (1H, m), 5.11 (1H, s), 4.46 (2H, q, *J* = 7.1), 4.26 (2H, s), 3.51 – 3.36 (4H, m), 2.45 – 2.27 (2H, m), 2.26 – 2.12 (2H, m), 1.47 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$, δ, ppm) 159.33, 153.34, 146.07, 132.86, 131.18, 130.30, 129.52, 129.02, 128.48, 123.91, 121.98, 120.41, 113.44, 72.54, 61.47, 61.44, 47.09, 27.55, 14.63; ESI HRMS, found 380.1860 ($C_{23}H_{26}NO_4$, $[M+H]^+$, requires 380.1862).



Ethyl 3-(cyclohexyloxy)benzofuran-2-carboxylate **S7** **S7** was prepared as in **4**, replacing *tert*-butyl-4-hydroxypiperidine-1-carboxylate with cyclohexanol (56 μL, 0.53 mmol) and purifying by normal phase silica gel column chromatography, resulting in **S7** as a colourless oil (28 mg, 20%). 50g SNAP column, 1 column volume 2% EtOAc in *n*-hexane, 10 column volumes 2%-10% EtOAc, 2 column volume 10% EtOAc. R_t = 5.1 column volumes; ^{1}H NMR ($CDCl_3$, δ, ppm) 7.70 (1H, d, *J* = 7.8), 7.51 (1H, d, *J* = 8.5), 7.48 – 7.41 (1H, m), 7.31 – 7.24 (1H, m), 4.66 – 4.55 (1H, m), 4.45 (2H, q, *J* = 7.1), 2.11 – 1.98 (2H, m), 1.92 – 1.79 (2H, m), 1.76 – 1.63 (2H, m), 1.61 – 1.51 (1H, m), 1.44 (3H, t, *J* = 7.1), 1.41 – 1.31 (3H, m); ^{13}C NMR ($CDCl_3$, δ, ppm) 159.67, 153.44, 147.55, 133.04, 128.31, 123.78, 123.26, 121.16, 112.92, 82.43, 61.10, 32.54, 25.59, 23.67, 14.55; ESI HRMS, found 289.1431 ($C_{17}H_{21}O_4$, $[M+H]^+$, requires 289.1440).



(±)-*tert*-Butyl 3-((2-(ethoxycarbonyl)benzofuran-3-yl)oxy)piperidine-1-carboxylate **S8a** **S8a** was prepared as in **4**, replacing *tert*-butyl-4-hydroxypiperidine-1-carboxylate with *tert*-butyl-3-hydroxypiperidine-1-carboxylate (107 mg, 0.53 mmol) resulting in **S8a** as a clear yellow oil (31 mg, 16%). **S8a** was carried through to **S8b** without further purificaton.



Ethyl 3-(piperidin-3-yloxy)benzofuran-2-carboxylate **S8b** **S8b** was prepared as in **5**, replacing **4** with **S8a** (30 mg, 0.08 mmol), resulting in **S8b** as a clear colourless oil (2.6 mg, 12%). R_t = 8.3 min; ^{1}H NMR ($CDCl_3$, δ, ppm) 7.95 (1H, brs), 7.65 (1H, d, *J* = 7.8), 7.60 – 7.50 (2H, m), 7.35 (1H, dd, *J* = 7.8, 7.2), 4.94 (1H, brs), 4.47 (2H, q, *J* = 7.1), 3.62 – 3.47 (1H, m), 3.47 – 3.38 (1H, m), 3.37 – 3.17 (2H, m), 2.49 – 2.30 (2H, m), 2.09 – 1.85 (2H, m), 1.46 (3H, t, *J* = 7.1); ^{13}C NMR ($CDCl_3$, δ, ppm) 160.72, 153.59, 146.02, 133.87, 129.41, 124.13, 121.78, 120.18, 113.50, 74.04, 62.48, 45.80, 43.94, 27.63, 17.92, 14.33; ESI HRMS, found 290.1389 ($C_{16}H_{20}NO_4$, $[M+H]^+$, requires 290.1392).

3. Biochemical Data

3.1. Assay Information

All IC_{50} determinations were carried out using a 7-diethylamine-3-(4'maleimidylphenyl)-4-methylcoumarin (CPM) fluorescence assay, as described by Goncalves *et al*.[1,2] The HsNMT1 and PvNMT assays are as described in the literature, for PfNMT the final enzyme concentration and peptide substrates are modified, see below:

PfNMT Final Concentration: 400 ng/mL

PfNMT Peptide Substrate: *Homo sapiens* $p60^{src}$ (2-16), final concentration 4.0 μM, Sequence: GSNKSKPKDASQRRR-NH_2.

IC_{50} values are the mean value of two or more determinations, standard deviation is typically within 20% of the IC_{50}. Data were elaborated using Microsoft Office Excel 2010 and IC_{50} values were determined using GraFit 7.0 (Erithacus Software Ltd, UK) by non-linear regression fitting.

3.2. K_i Calculations

K_i values were calculated from the experimentally determined IC_{50} values, the substrate concentration ([S]) and the Michaelis-Menten constant (K_m) as described by the Cheng-Prusoff equation:[3]

***Equation 1*. Cheng-Prusoff Equation for Determination of K_i from IC_{50}**

$$K_i = \frac{IC_{50}}{1+\frac{[S]}{K_m}}$$

For example, **9** had an experimentally determined IC_{50} of 3.9 ± 0.7 μM. The Michaelis Constant (K_m) was 2.0 μM and the substrate concentration was 4.0 μM, giving a K_i of 1.3 μM.

3.3 *P. falciparum* Inhibition Assay

Measurement of the ability of compounds to kill parasites was performed in 96-well plates using a modification of the fluorescence-activated cell sorting (FACS) assay platform:[4,5] each well (in total 100 μL medium) contained synchronous cultures of late trophozoite-stage parasites (1% parasitemia and 2% hematocrit) and variable concentrations of an inhibitor in DMSO diluent or diluent alone at 0.5% final concentration. The mixture was incubated at 37 ˚C for a full 48-hour growth cycle. Aliquots of 50 μL were removed from each well, added to 500 μL freshly diluted hydroethidine (HE, 1:200 dilution of 10 mg/mL DMSO stock in PBS) and incubated for 20 min at

37°C. Samples were then diluted with 1 mL PBS to enable appropriate cell counts (50,000) and stored on ice. Parasites cultured in the absence of an inhibitor and non-infected red blood cells were used as positive and negative controls respectively. Parasitemia was measured using a FACS-Calibur flow cytometer. Growth inhibition at each concentration was calculated as % inhibition = [1-(readout-negative control) / (positive-negative control)] x 100%. To determine the EC_{50} of an inhibitor, its activity at a range of concentrations from 10 µM down to 0.31 µM was measured and the 50% inhibitory concentration (EC_{50}) of an inhibitor was calculated by a nonlinear regression analysis using GraFit 7.0.1 version (Erithacus Software Limited, UK). All assays were carried out in triplicate.

4. Protein crystallization, X-ray data collection, processing and refinement

4.1 Experimental Procedure

Crystals of the ternary complex of the non-hydrolysable co-factor and compound **12b** bound to PvNMT were obtained essentially as described previously.[1] X-ray diffraction data were collected on beamline ID14-1[6] at the European Synchrotron Research Facility, Grenoble and processed using XDS[7] and SCALA[8] implemented within *xia2*.[9] Data collection and refinement statistics are summarized in Supplementary Table S2. The data set is essentially complete (98%) to 1.9 Å resolution, tapering to 43 % completeness in the outer resolution shell at 1.63 Å spacing. For R_{free} calculations, 5% of the data were excluded. Rigid body refinement using maximum likelihood methods implemented in REFMAC[10] using the protein chains of 4TST.pdb[6] as a starting model was followed by refinement using anisotropic temperature factors interspersed with cycles of model building and adjustment using COOT.[11] Complete chains (corresponding to residues 27-410, numbering as in full-length protein) can be traced for two of the three molecules in the asymmetric unit. N-terminal residues (derived from the purification tag) in all three chains and loop residues 227-238 in chain C have not been modeled and these are assumed to be disordered. Electron density maps in the vicinity of the bound ligand are well-defined for the piperidine ring and the benzothiophene nucleus, but are less clear around the phenoxymethyl portion of the molecule. There appears to be alternate binding conformations for this part of the ligand, as reflected by the refined atomic B-factors (Table S3). The best fit to the electron density for this part of the molecule has been modeled in all three chains in the asymmetric unit and for purposes of illustration, the form bound to protein chain B is referred to in the main text. The final refined protein structure model displays good geometry with 96.2% of the residues in the preferred region of the Ramachandran plot and only 0.3% (corresponding to amino acid residue Phe336 in all three chains) as outliers. The coordinates and structure factor files have been deposited in the Protein Data Bank under the accession code 4BBH.

4.2. X-Ray Data Collection and Refinement Statistics

Table S2. **X-ray data collection and refinement statistics**

	PvNMT-NHM-**12b**
PDB accession code	**4BBH**
Cell dimensions *a*, *b*, *c*	57.47, 121.87, 178.34
Space Group	$P2_12_12_1$
Data collection	
Beamline / Wavelength	ESRF ID14-4 / 0.9393
Detector type	ADSC Q315r CCD
Images x oscillation (°)	360 x 0.5 & 180 x 1
Resolution (Å)	72–1.63 (1.72–1.63) [a]
R_{sym} (%) [b]	10.0 (34.7)
$I/\sigma I$	15.4 (2.5)
Completeness (%)	83.7 (43.9) [c]
Redundancy	8.3 (2.8)
Refinement	
No. unique reflections	130696
R_{work} / R_{free} [d]	21.4 / 26.7
No. atoms	10684
Protein	9597
Ligand	84
Co-factor	192
Water	788
B-factors (Å^2)	
All atoms	13.5
Protein (A/B/C)	13.2/12.3/13.2
Ligand (A/B/C)	26.4/28.5/26.9
Co-factor (A/B/C)	9.7/9.0/9.7
Water	19.5
R.m.s.deviations [e]	
Bond lengths (Å)	0.020
Bond angles (°)	2.179

[a]Highest resolution shell is shown in parentheses.

[b]$R_{sym} = \Sigma_{\mathbf{h}}\Sigma_l |I_{\mathbf{h}l} - \langle I_{\mathbf{h}}\rangle| / \Sigma_{\mathbf{h}}\Sigma_l\langle I_{\mathbf{h}}\rangle$, where I_l is the l^{th} observation of reflection h and $\langle I_{\mathbf{h}}\rangle$ is the weighted average intensity for all observations *l* of reflection h.

[c] Data is 98% complete to 1.9 Å resolution

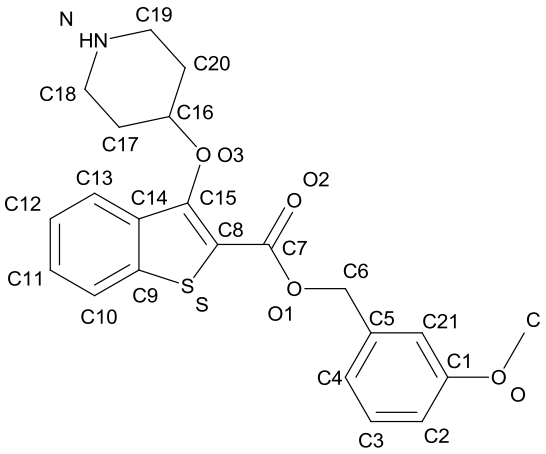
[d] $R_{cryst} = \sum||F_o| - |F_c||/\sum|F_o|$ where F_o and F_c are the observed and calculated structure factor amplitudes, respectively.R_{free} is the R_{cryst} calculated with 5% of the reflections omitted from refinement.

[e] Root-mean-square deviation of bond lengths or bond angles from ideal geometry.

4.3 B-Factor Values of Compound 12b Ligand Atoms Bound to PvNMT Chains A-C

Table S3. **B-factor values of compound 12b ligand atoms bound to PvNMT chains A-C**

Atom	A	B	C
O2	31.65	21.79	26.24
C7	25.96	23.54	26.80
O1	32.04	27.64	36.34
C6	40.00	37.58	43.33
C5	47.00	48.70	42.75
C4	50.65	53.14	40.81
C3	47.04	51.59	44.65
C2	44.45	54.40	44.79
C21	48.42	57.91	46.92
C1	44.57	56.26	45.16
O	38.14	53.56	39.90
C	32.93	46.49	28.33
C8	21.23	19.53	22.32
C15	18.07	17.29	19.92
C14	15.48	15.71	19.82
C13	14.08	14.99	17.54
C12	13.42	13.77	17.64
C11	13.00	13.96	14.82
C10	14.40	14.71	16.26
C9	16.01	16.07	17.68
S	18.88	19.48	21.80
O3	16.13	16.71	18.42
C16	16.21	17.16	16.71
C20	15.79	16.70	17.01
C19	15.32	15.66	16.85
N	15.29	17.05	17.20
C18	15.43	19.41	15.87
C17	17.31	15.91	16.38
Average	26.39	28.45	26.87



The B-factor ($Å^2$) is given by $B = 8\pi^2\langle\mu^2\rangle$ where $\langle\mu^2\rangle$ is the mean square displacement of the atom position. The higher values for atoms in the phenoxmethyl ring indicate that they are in a flexible portion of the molecule

5. References

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