Supporting Information

Accepting the Invitation to Open Innovation in Malaria Drug Discovery: Synthesis, Biological Evaluation and Investigation on the Structure–Activity Relationships of Benzo[*b*]thiophene-2-carboxamides as Antimalarial Agents.

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1. Analytical data for compounds 34, 36–38, 47–49.

3,6,7-Trichlorobenzo[b]thiophene-2-carbonyl chloride (10): pale yellow needles, 51%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 (d, *J*= 8.6, 1H); 7.70 (d, *J*= 8.6, 1H).

Methyl 3-methoxybenzo[b]thiophene-2-carboxylate (34): colourless oil, Yield 56%, petroleum ether/ethyl acetate (95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.95 (s, 3H); 4.20 (s, 3H); 7.44-7.56 (m, 2H); 7.82 (d, *J* = 8Hz, 1H); 7.94 (d, *J* = 8Hz, 1H). LRMS (ESI) calcd for $C_{11}H_{10}O_3S$ ($[M-H]^-$) 221.04; found 221.3.

Methyl 3-((4-chlorobenzyl)oxy)benzo[b]thiophene-2-carboxylate (36): colourless oil, Yield 58%, petroleum ether/ethyl acetate (95:5) ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.94 (s, 3H); 5.35 (s, 2H), 7.16 (bs, 1H); 7.35-7.55 (m, 6H); 7.79 (t, *J* = 8.4 Hz, 2H). LRMS (ESI) calcd for $C_{17}H_{13}ClO_3S$ ($[M-H]^-$) 331.03; found 331.4.

Methyl 3-((4-methoxybenzyl)oxy)benzo[b]thiophene-2-carboxylate (37): colourless oil, yield 35%, petroleum ether/ethyl acetate (95:5) ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.84 (s, 3H); 3.96 (s, 3H); 5.31 (s, 2H), 6.91-6.96 (m, 2H); 7.32-7.53 (m, 4H); 7.74-7.82 (m, 2H). LRMS (ESI) calcd for $C_{18}H_{16}O_4S$ ($[M-H]^-$) 327.07; found 327.3.

Methyl 3-((3,4-difluorobenzyl)oxy)benzo[b]thiophene-2-carboxylate (38): light grey powder, yield 41%, petroleum ether/ethyl acetate (95:5) ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.96 (s, 3H); 5.40 (s, 2H), 6.81-6.98 (m, 2H); 7.35-7.41 (m, 1H); 7.42-7.51 (m, 1H); 7.55-7.65 (m, 1H); 7.64-7.83 (m, 2H). LRMS (ESI) calcd for $C_{17}H_{12}F_2O_3S$ ($[M-H]^-$) 333.05; found 333.3.

3-((4-Chlorobenzyl)oxy)benzo[b]thiophene-2-carboxylic acid (47): white powder, Yield 96%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (s, 3H); 5.40 (s, 2H), 7.16 (bs, 1H); 7.28-7.52 (m, 7H); 7.83 (s, 2H). LRMS (ESI) calcd for $C_{16}H_{11}ClO_3S$ ($[M-H]^-$) 317.01; found 317.3.

3-((4-Methoxybenzyl)oxy)benzo[b]thiophene-2-carboxylic acid (48): white powder, Yield 92%. LRMS (ESI) calcd for $C_{17}H_{14}O_4S$ ($[M-H]^-$) 313.06; found 313.3.

3-((3,4-Difluorobenzyl)oxy)benzo[b]thiophene-2-carboxylic acid (49): white powder, Yield 97%. LRMS (ESI) calcd for $C_{16}H_{10}F_2O_3S$ ($[M-H]^-$) 319.03; found 319.3.

2. Analytical data for target compounds

(3-Chlorobenzo[b]thiophen-2-yl)(*E*-2,6-dimethylmorpholino)methanone (11): white solid, 42%, dichlomethane/methanol (95:5). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.08 (d, J = 60 Hz, 6H); 2.58 (bs, 1H); 2.89 (bs, 1H); 3.45-3.60 (m, 3H); 4.39 (bs, 1H); 7.50-7.62 (m, 1H); 7.85 (d, J = 8 Hz, 1H), 8.12 (d, J = 8 Hz, 1H). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 19.0, 52.5, 71.7, 118.3, 122.5, 123.9, 126.4, 127.3, 130.7, 135.4, 137.3, 160.7. HRMS (ESI) calcd for $C_{15}H_{17}ClN_2OS$ ($[M+H]^+$) 310.0590; found 310.0666.

(3-Chlorobenzo[b]thiophen-2-yl)(*Z*-2,6-dimethylmorpholino)methanone (12): pale yellow oil, 39%, dichlomethane/methanol (95:5). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.12 (d, J = 52 Hz, 6H); 3.10-3.20 (m, 1H); 3.40-3.71 (m, 3H); 3.90-4.05 (m, 2H); 7.50-7.63 (m, 1H); 7.86 (d, J = 8 Hz, 1H), 8.13 (d, J = 8 Hz, 1H). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 17.6, 64.1, 65.7, 118.3, 122.5, 124.0, 126.4, 127.3, 130.5, 135.4, 137.3, 161.4. HRMS (ESI) calcd for $C_{15}H_{17}ClN_2OS$ ($[M+H]^+$) 310.0590; found 310.0666.

(3-Chlorobenzo[b]thiophen-2-yl)(3,5-dimethylpiperazin-1-yl)methanone (13): pale yellow oil, 46%, dichlomethane/methanol (95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02 (s, 3H); 1.18 (s, 3H); 2.45-2.49 (m, 1H); 2.78-2.82 (m, 1H); 2.95-2.99 (m, 2H); 3.60 (d, J = 11 Hz, 1H), 4.67 (d, J = 11 Hz, 1H); 7.48-7.52 (m, 2H); 7.87-7.89 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.7, 51.0, 51.4, 122.0, 123.2, 123.8, 124.5, 129.0, 131.4, 138.8, 145.9, 162.0. HRMS (ESI) calcd for $C_{15}H_{17}ClN_2OS$ ($[M+H]^+$) 309.0750; found 309.0822.

(3-Chlorobenzo[b]thiophen-2-yl)(morpholino)methanone (14): pale yellow oil, 62%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.40-4.10 (m, 8H); 7.45-7.52 (m, 2H); 7.70-7.80 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 45.0, 71.8, 122.8, 123.4, 123.9, 124.9, 128.2, 131.9, 139.2, 146.0, 162.2. HRMS (ESI) calcd for $C_{13}H_{12}ClNO_2S$ ($[M+H]^+$) 282.0277; found 282.0345.

(3-Chlorobenzo[b]thiophen-2-yl)(piperidin-1-yl)methanone (15): pale yellow oil, 28%, petroleum ether/ethyl acetate (95:5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.75 (bs, 6H); 3.45 (bs, 2H); 3.76 (bs, 2H);

7.43-7.54 (m, 2H); 7.82-7.88 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 24.9, 25.8, 42.8, 119.4, 122.5, 122.8, 125.4, 126.8, 130.1, 135.8, 138.2, 160.9. HRMS (ESI) calcd for $C_{14}H_{14}ClNOS$ ($[M+H]^+$) 280.0485; found 280.0522.

***N*-(Adamantan-1-yl)-3-chlorobenzo[b]thiophene-2-carboxamide (16):** colorless oil, 60%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.76 (bs, 6H); 2.19 (bs, 9H); 6.94 (bs, 1H); 7.45-7.53 (m, 2H); 7.83-7.89 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 29.5, 36.4, 41.7, 53.2, 117.5, 122.8, 123.0, 125.2, 127.1, 135.0, 137.1, 137.7, 159.6. HRMS (ESI) calcd for $C_{19}H_{20}ClNOS$ ($[M+H]^+$) 346.0954; found 346.1027.

3-Chloro-*N*-phenylbenzo[b]thiophene-2-carboxamide (17): white solid, 35%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.22 (t, J = 8 Hz, 1H); 7.43 (app t, J = 8 Hz, 2H); 7.52-7.58 (m, 2H); 7.69-7.73 (m, 2H); 7.82-7.98 (m, 2H); 8.95 (bs, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 120.4, 122.9, 123.3, 125.1, 125.6, 127.7, 129.2, 133.6, 137.0, 137.2, 138.2, 158.7. HRMS (ESI) calcd for $C_{15}H_{10}ClNOS$ ($[M+H]^+$) 288.0172; found 288.0201.

3-Chloro-N-(pyridin-2-yl)benzo[b]thiophene-2-carboxamide (18): white solid, 23%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.22 (t, J = 8 Hz, 1H); 7.42-7.60 (m, 2H); 7.75-8.00 (m, 3H); 8.26-8.45 (m, 2H); 9.62 (bs, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 114.7, 120.5, 122.9, 123.0, 123.5, 125.7, 127.9, 132.3, 137.0, 138.3, 138.6, 148.1, 150.9, 159.0. HRMS (ESI) calcd for $C_{14}H_9ClN_2OS$ ($[M+H]^+$) 289.0124; found 289.0147

3-Chloro-N-(pyridin-4-yl)benzo[b]thiophene-2-carboxamide (19): white solid, 60%, dichloromethane/methanol (97:3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.54-7.59 (m, 2H); 7.66 (d, J = 5.4 Hz, 2H); 7.90 (d, J = 8 Hz, 1H); 7.96 (d, J = 8 Hz, 1H); 8.61 (d, J = 5.4 Hz, 2H); 9.06 (bs, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 114.1, 119.6, 122.9, 123.4, 128.8, 128.1, 132.4, 136.7, 138.4, 144.3, 150.1, 159.2. HRMS (ESI) calcd for $C_{14}H_9ClN_2OS$ ($[M+H]^+$) 289.0124; found 289.0210.

3-Chloro-N,N-diethylbenzo[b]thiophene-2-carboxamide (20): white solid, 65%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10-1.35 (m, 6H); 3.40-3.63 (m, 4H); 7.45-7.53 (m, 2H); 7.81-7.88 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 12.5, 43.9, 122.5, 123.2, 123.8, 124.0, 128.6, 131.3, 138.5, 148.0, 161.8. HRMS (ESI) calcd for $C_{14}H_9ClN_2OS$ ($[M+H]^+$) 289.0124; found 289.0621.

(3-Chlorobenzo[b]thiophen-2-yl)(3,5-dimethylpiperidin-1-yl)methanone (21): white solid, 58%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80-1.02 (m, 7H); 1.70-1.92 (m, 3H); 2.25-2.35 (m, 1H); 2.61-2.69 (m, 1H); 3.65-3.70 (m, 1H); 4.71-4.76 (m, 1H); 7.45-7.55 (m, 2H); 7.81-7.89 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 19.0. 31.1, 32.3, 39.3, 42.3, 49.3, 54.4, 119.0, 122.5, 122.7, 125.4, 126.4, 130.4, 135.8, 137.4, 161.2. HRMS (ESI) calcd for $C_{16}H_{18}ClN_2OS$ ($[M+H]^+$) 308.0798; found 308.0873.

(3,6-Dichlorobenzo[b]thiophen-2-yl)(*E*-2,6-dimethylmorpholino)methanone (22): white solid, 71%, petroleum ether/ethyl acetate (80:20). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.12-1.30 (m, 6H); 2.64 (app bs, 1H); 2.96 (app bs, 1H); 3.48-3.73 (m, 3H); 4.60 (bs, 1H); 7.49 (d, J = 8Hz, 1H); 7.79 (d, J = 8Hz, 1H), 7.84 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 16.9, 43.7, 71.9, 119.1, 122.3, 123.6, 126.6, 130.1, 130.1, 133.1, 134.3, 138.5, 160.9. HRMS (ESI) calcd for $C_{15}H_{15}Cl_2NO_2S$ ($[M+H]^+$) 344.0201; found 344.1085.

(3-Chloro-6-fluorobenzo[b]thiophen-2-yl)(*E*-2,6-dimethylmorpholino)methanone (23): white solid, 68%, petroleum ether/ethyl acetate (80:20). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14-1.32 (m, 6H); 2.63 (app bs, 1H); 2.96 (app bs, 1H); 3.48-3.73 (m, 3H); 4.59 (app bs, 1H); 7.26-7.30 (m, 1H); 7.54 (d, J = 8Hz, 1H); 7.81-7.85 (m, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.6, 47.8, 72.1, 108.8, 114.7, 119.0, 124.0, 129.4, 132.3, 138.6, 149.3, 161.03. HRMS (ESI) calcd for $C_{15}H_{15}ClFNO_2S$ ($[M+H]^+$) 329.0496; found 329.0528.

(3-Chloro-6-methylbenzo[b]thiophen-2-yl)(*E*-2,6-dimethylmorpholino)methanone (24): white solid, 35%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14-1.30 (m, 6H); 2.53 (s, 3H); 2.63 (app bs, 1H); 2.94 (app bs, 1H); 3.55-3.73 (m, 3H); 4.60 (app bs, 1H); 7.34 (d, *J* = 8Hz, 1H); 7.64 (s, 1H); 7.76 (d, *J* = 8Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.7, 21.7, 48.2, 72.0, 119.2, 122.2, 122.5, 127.3, 128.3, 133.6, 137.1, 137.9, 161.6. HRMS (ESI) calcd for $C_{16}H_{18}ClNO_2S$ ($[M+H]^+$) 324.0747; found 324.0777.

(*E*-2,6-dimethylmorpholino)(3,6,7-trichlorobenzo[b]thiophen-2-yl)methanone (25): colourless solid, 41%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14-1.30 (m, 6H); 2.65 (app bs, 1H); 2.97 (app bs, 1H); 3.50-3.73 (m, 3H); 4.60 (app bs, 1H); 7.60 (d, *J* = 8Hz, 1H); 7.71 (d, *J* = 8Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.7, 47.8, 71.9, 119.5, 121.6, 126.2, 128.1, 131.0, 131.7, 135.3, 138.4, 160.5. HRMS (ESI) calcd for $C_{15}H_{14}Cl_3NO_2S$ ($[M+H]^+$) 377.9811; found 377.9884.

Benzo[b]thiophen-2-yl(*E*-2,6-dimethylmorpholino)methanone (54): white solid, yield 25%, petroleum ether/ethyl acetate (80:20). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (s, 3H); 1.24 (s, 3H); 2.78 (bs, 2H), 3.62-3.70 (m, 2H), 4.33 (bs, 2H), 7.41-7.44 (m, 2H), 7.49 (s, 1H), 7.83-7.89 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.7, 43.8, 72.0, 122.4, 124.7, 124.9, 125.3, 125.9, 136.4, 138.6, 140.2, 163.6. HRMS (ESI) calcd for $C_{15}H_{17}NO_2S$ ($[M+H]^+$) 276.0980; found 276.0289.

Benzo[b]thiophen-2-yl(3,5-dimethylpiperazin-1-yl)methanone (55): yellowish solid, yield 42%, dichloromethane/methanol (95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (s, 3H); 1.14 (s, 3H); 2.41-2.98 (m, 5H), 4.89 (bs, 2H), 7.41-7.44 (m, 2H), 7.49 (s, 1H), 7.83-7.89 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 19.2, 51.2, 52.8, 122.2, 124.8, 125.0, 125.3, 125.9, 137.2, 138.8, 141.0, 162.9. HRMS (ESI) calcd for $C_{15}H_{18}N_2OS$ ($[M+H]^+$) 275.1140; found 275.1152.

1-(4-(Benzo[b]thiophene-2-carbonyl)piperazin-1-yl)ethan-1-one (56): yellowish solid, 35%, dichloromethane/methanol (95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.17 (s, 3H); 3.55-3.60 (m, 2H);

3.70-3.84 (m, 6H), 7.42-7.46 (m, 2H), 7.53 (s, 1H), 7.83-7.90 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 28.8, 48.9, 49.2, 119.9, 122.1, 122.6, 123.5, 126.2, 132.0, 133.4, 146.6, 160.8, 168.2. HRMS (ESI) calcd for $C_{15}H_{16}N_2O_2S$ ($[M+H]^+$) 289.0091; found 289.0157.

Benzo[b]thiophen-2-yl(4-methylpiperazin-1-yl)methanone (57): yellowish solid, 68%, dichloromethane/methanol (95:5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H); 2.49 (t, J = 4.9 Hz, 4H), 3.81 (t, J = 4.9 Hz, 4H); 7.38-7.44 (m, 2H), 7.49 (s, 1H), 7.80-7.89 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 45.4, 48.6, 51.0, 118.7, 122.2, 122.8, 123.9, 125.2, 130.9, 133.5, 146.6, 162.5. HRMS (ESI) calcd for $C_{14}H_{16}N_2OS$ ($[M+H]^+$) 261.0983; found 261.1047.

Benzo[b]thiophen-2-yl(morpholino)methanone (58): pale oil, 71%, petroleum ether/ethyl acetate (95:5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.76-3.84 (m, 8H); 7.38-7.46 (m, 2H), 7.51 (s, 1H), 7.80-7.89 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 52.5, 71.8, 122.0, 124.4, 125.2, 125.5, 126.0, 136.4, 138.9, 139.8, 162.4. HRMS (ESI) calcd for $C_{13}H_{13}NO_2S$ ($[M+H]^+$) 248.0667; found 248.0924.

(2,6-Dimethylmorpholino)(3-ethylbenzo[b]thiophen-2-yl)methanone (59): white solid, 27%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.15-1.30 (m, 6H); 1.31 (t, J = 7.6 Hz, 3H); 2.68-2.72 (m, 2H); 2.90 (q, J = 7.6 Hz, 2H); 3.55-3.65 (m, 2H); 7.40-7.45 (m, 2H), 7.79-7.86 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 14.5, 18.7, 20.8, 51.2, 72.2, 122.7, 124.5, 123.4, 129.4, 137.9, 138.6, 139.5, 164.4. HRMS (ESI) calcd for $C_{17}H_{21}NO_2S$ ($[M+H]^+$) 304.1293; found 304.1369.

Benzofuran-2-yl(*E*-2,6-dimethylmorpholino)methanone (60): white solid, 31%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (s, 3H); 1.27 (s, 3H); 2.60-3.05 (m, 2H); 3.64-3.75 (m, 2H); 4.10-4.60 (m, 2H); 7.29-7.34 (m, 2H); 7.43 (dt, J_1 = 1.3 Hz, J_2 = 7.2 Hz, 1H); 7.55 (dd, J_1 = 1.3 Hz, J_2 = 8.3 Hz, 1H); 7.65-7.70 (m, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 19.7, 51.2, 72.2, 111.7, 111.9, 122.5, 124.1, 125.9, 127.0, 149.2, 155.3, 159.9. HRMS (ESI) calcd for $C_{15}H_{17}NO_3$ ($[M+H]^+$) 260.1208; found 260.1526.

Benzofuran-2-yl(*E*-3,5-dimethylpiperazin-1-yl)methanone (61): white solid, 60%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13 (s, 3H); 1.15 (s, 3H); 2.45-3.05 (m, 4H); 4.12-4.62 (m, 2H); 7.28-7.34 (m, 2H); 7.43 (dt, J_1 = 1.3 Hz, J_2 = 7.2 Hz, 1H); 7.55 (dd, J_1 = 1.3 Hz, J_2 = 8.3 Hz, 1H); 7.65-7.70 (m, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 19.3, 50.8, 51.6, 111.8, 111.9, 122.2, 123.6, 126.4, 127.0, 149.0, 154.6, 159.7. HRMS (ESI) calcd for $C_{15}H_{18}N_2O_2$ ($[M+H]^+$) 259.1368; found 259.1758.

(*E*-2,6-Dimethylmorpholino)(3-methoxybenzo[b]thiophen-2-yl)methanone (62): white solid, 46%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13-1.30 (m, 6H); 2.50-3.01 (m, 2H); 3.60-3.85 (m, 3H); 4.05 (s, 3H); 4.60 (bs, 1H); 7.40-7.44 (m, 2H); 7.74-7.81 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.7, 52.0, 61.0, 72.0, 115.1, 121.9, 122.5, 124.5, 126.4, 132.7, 136.9, 148.8, 163.0. HRMS (ESI) calcd for $C_{16}H_{19}NO_3S$ ($[M+H]^+$) 306.1086; found 306.1157.

(3-(Benzyloxy)benzo[b]thiophen-2-yl)(*E*-2,6-dimethylmorpholino)methanone (63): white solid, 42%, petroleum ether/ethyl acetate (99:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13-1.30 (m, 6H); 2.45-2.89 (m, 2H); 3.50-3.80 (m, 3H); 4.52 (bs, 1H); 5.23 (s, 2H); 7.37-7.47 (m, 7H); 7.77-7.81 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.7, 36.9, 72.1, 75.7, 117.3, 122.1, 122.9, 124.6, 126.4, 128.0, 128.5, 128.7, 133.0, 136.6, 137.1, 147.6, 162.8. HRMS (ESI) calcd for $C_{22}H_{23}NO_3S$ ($[M+H]^+$) 382.1399; found 382.1471.

(3-((4-Chlorobenzyl)oxy)benzo[b]thiophen-2-yl)(*E*-2,6-dimethylmorpholino)methanone (64): white solid, 42%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13-1.30 (m, 6H); 2.50-2.79 (m, 2H); 3.50-3.80 (m, 3H); 4.53 (bs, 1H); 5.19 (s, 2H); 7.31-7.48 (m, 6H); 7.78 (d, J = 8 Hz, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.6, 37.2, 73.9, 74.9, 117.4, 121.9, 122.9, 124.6, 126.5, 128.8, 129.4, 132.9, 134.4, 135.1, 137.0, 147.5, 162.8. HRMS (ESI) calcd for $C_{22}H_{22}ClNO_3S$ ($[M+H]^+$) 416.1009; found 416.1089.

(*E*-2,6-Dimethylmorpholino)(3-((4-methoxybenzyl)oxy)benzo[b]thiophen-2-yl)methanone (65): white solid, 30%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13-1.30 (m, 6H); 2.51-2.80 (m, 2H); 3.50-3.82 (m, 3H); 3.84 (s, 3H); 4.53 (bs, 1H); 5.13 (s, 2H); 6.92 (d, *J* = 8 Hz, 2H); 7.34 (d, *J* = 8 Hz, 2H); 7.36-7.44 (m, 2H); 7.76-7.80 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.7, 39.2, 55.3, 72.0, 75.8, 114.0, 117.7, 122.1, 122.9, 124.5, 126.4, 128.7, 129.9, 133.1, 137.1, 147.7, 159.9, 162.9. HRMS (ESI) calcd for $C_{23}H_{25}NO_4S$ ($[M+H]^+$) 412.1504; found 412.1578.

(3-((3,4-Difluorobenzyl)oxy)benzo[b]thiophen-2-yl)(*E*-2,6-dimethylmorpholino)methanone (66): white solid, 38%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14-1.30 (m, 6H); 2.50-2.88 (m, 2H); 3.54-3.82 (m, 3H); 4.53 (bs, 1H); 5.20 (s, 2H); 6.84-6.92 (m, 2H); 7.30-7.45 (m, 3H); 7.76-7.80 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.7, 40.2, 69.2, 72.0, 104.1, 111.4, 118.8, 119.7, 121.8, 122.9, 124.7, 126.5, 132.0, 132.8, 137.1, 147.1, 162.5. HRMS (ESI) calcd for $C_{22}H_{21}F_2NO_3S$ ($[M+H]^+$) 418.1210; found 418.1458.

(*E*-2,6-dimethylmorpholino)(1H-indol-2-yl)methanone (67): colourless oil, 13%, dichloromethane/methanol (95:5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (s, 3H); 1.30 (s, 3H); 3.65 (bs, 2H); 4.02-4.17 (m, 4H); 6.75-6.80 (m, 1H); 7.16 (app t, *J* = 10 Hz, 1H); 7.26-7.33 (m, 1H); 7.46 (app d, *J* = 10.6 Hz, 1H); 7.68 (app d, *J* = 10.6 Hz, 1H); 9.50 (bs, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.8, 50.3, 72.0, 105.3, 111.8, 120.7, 121.9, 124.5, 127.4, 129.1, 135.7, 162.2. HRMS (ESI) calcd for $C_{15}H_{18}N_2O_2$ ($[M+H]^+$) 259.1368; found 259.1891.

(3,5-dimethylpiperazin-1-yl)(1H-indol-2-yl)methanone (68): colourless oil, 44%, dichloromethane/methanol (95:5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.15 (s, 3H); 1.17 (s, 3H); 2.50-3.00 (m, 4H); 4.63 (d, *J* = 11.5 Hz, 2H); 6.78-6.80 (m, 1H); 7.16 (app t, *J* = 8 Hz, 1H); 7.26-7.33 (m, 1H); 7.45 (d, *J* = 8 Hz, 1H); 7.68 (d, *J* = 8 Hz, 1H); 9.47 (bs, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 19.4, 51.2, 105.1, 111.9, 120.5, 121.8, 124.2, 127.4, 129.5, 135.9, 162.5. HRMS (ESI) calcd for $C_{15}H_{19}N_3O$ ($[M+H]^+$) 258.1528; found 258.1798.

(3-Aminobenzo[b]thiophen-2-yl)(*E*-2,6-dimethylmorpholino)methanone (69): white solid, 28%, petroleum ether/ethyl acetate (60:40). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.09 (s, 3H); 1.12 (s, 3H); 2.47-2.74 (m, 2H); 3.50-3.55 (m, 2H); 4.10-4.16 (m, 2H); 6.44 (s, 2H); 7.36-7.44 (m, 2H); 7.81 (d, J = 7.5 Hz, 1H); 8.03 (d, J = 7.5 Hz, 1H). δ 18.6, 42.8, 72.0, 122.4, 123.4, 126.3, 128.2, 132.6, 135.6, 138.5, 140.6, 160.9. HRMS (ESI) calcd for $C_{15}H_{18}N_2O_2S$ ($[M+H]^+$) 291.1089; found 291.2411.

(3-(Benzylamino)benzo[b]thiophen-2-yl)(*E*-2,6-dimethylmorpholino)methanone (70): yellow solid, 31%, petroleum ether/ethyl acetate (80:20). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.17 (s, 3H); 1.18 (s, 3H); 2.48-2.55 (m, 2H); 3.47-3.55 (m, 2H); 4.16-4.24 (m, 2H); 4.62 (s, 2H); 7.25-7.46 (m, 7H); 7.75 (d, J = 8 Hz, 1H); 7.83 (d, J = 8 Hz, 1H). δ 18.7, 36.9, 43.8, 72.1, 121.9, 122.0, 123.2, 125.6, 126.4, 128.2, 128.4, 128.7, 136.2, 136.9, 139.1, 148.0, 162.7. HRMS (ESI) calcd for $C_{22}H_{24}N_2O_2S$ ($[M+H]^+$) 381.1558; found 381.1920.

(*E*-2,6-Dimethylmorpholino)(3-methylbenzo[b]thiophen-2-yl)methanone (71): white powder, 61%, petroleum ether/ethyl acetate (90:10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (bs, 6H); 2.36 (s, 3H); 2.50-2.70 (m, 2H); 3.45-3.50 (m, 2H); 4.16-4.24 (m, 2H); 7.28-7.32 (m, 2H); 7.75 (d, J = 8 Hz, 1H); 7.83 (d, J = 8 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 10.9, 18.6, 46.0, 71.1, 122.0, 123.0, 127.2, 128.2, 131.8, 135.6, 138.2, 140.2, 162.8. HRMS (ESI) calcd for $C_{16}H_{19}NO_2S$ ($[M+H]^+$) 290.1136; found 290.1850.

Benzo[d]thiazol-2-yl(*E*-2,6-dimethylmorpholino)methanone (73): white solid, 73%, petroleum ether/ethyl acetate (80:20). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (s, 3H); 1.29 (s, 3H); 2.66 (t, J = 9 Hz, 1H); 3.01 (t, J = 9 Hz, 1H); 3.73-3.77 (m, 2H); 4.61 (d, J = 12 Hz, 1H); 5.44 (d, J = 12 Hz, 1H); 7.49-7.60 (m, 2H); 7.99 (d, J = 6 Hz, 1H); 8.12 (d, J = 8 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.7, 18.9, 48.8, 52.0, 71.9, 72.4, 121.9, 124.7, 126.6, 126.8, 136.2, 153.0, 159.5, 164.6. HRMS (ESI) calcd for $C_{14}H_{16}N_2O_2S$ ($[M+H]^+$) 277.0932; found 277.1078.

(*E*-2,6-dimethylmorpholino)(3-(methoxymethoxy)benzo[b]thiophen-2-yl)methanone (75): white solid, 41%, petroleum ether/ethyl acetate (70:30). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10-1.40 (m, 6H); 2.50-2.80 (m, 2H); 3.50 (s, 3H); 3.55-3.80 (m, 2H); 4.50-4.70 (m, 1H); 5.25 (s, 2H); 7.38-7.50 (m, 2H); 7.70-7.90 (m, 2H). LRMS (ESI) calcd for $C_{17}H_{21}NO_4S$ ($[M+H]^+$) 336.12; found 336.3.

3. Inhibition of ClpB by selected compounds

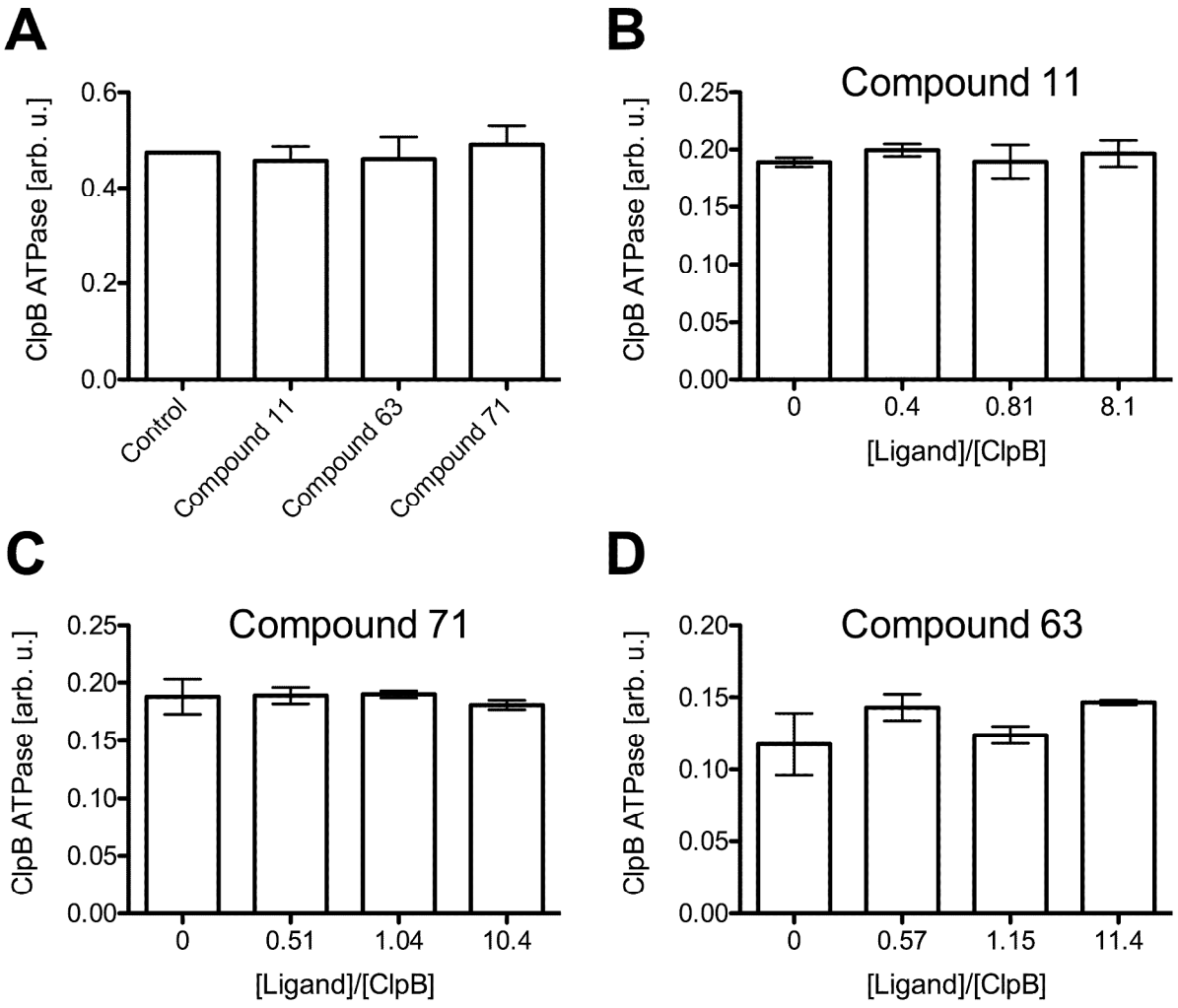
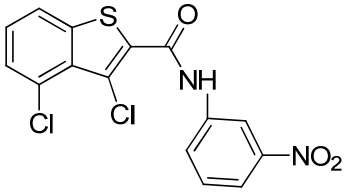
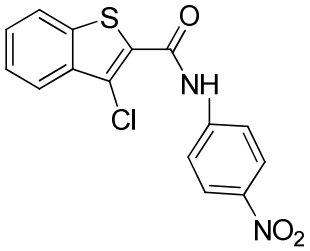
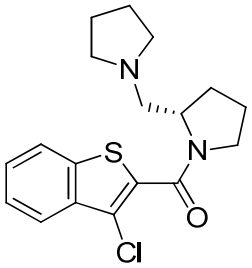
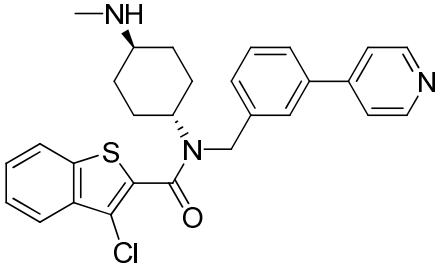


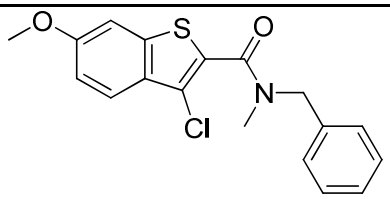
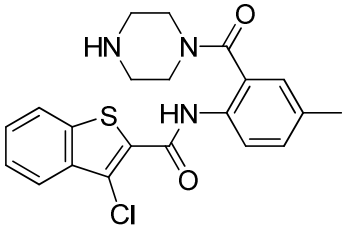
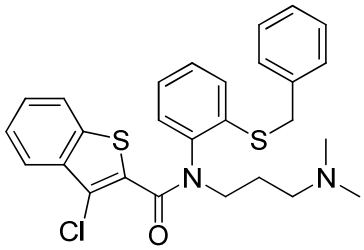
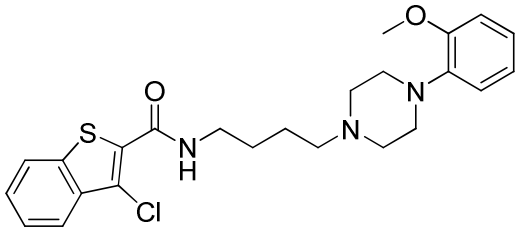
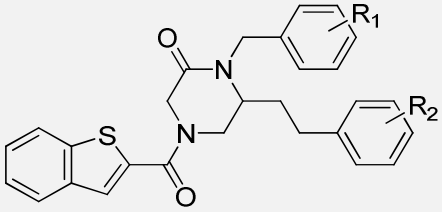
Figure S1. The ATPase activity of *E. coli* ClpB in the presence of the compounds 11, 63, and 71. The ATP hydrolysis reaction was initiated by adding ClpB (A, B, C) or ATP (D) to the assay buffer

containing the remaining components (see Methods). The ClpB concentration was 0.5 μM (A) or 0.2 μM (B, C, D). In panel A, the compound concentration was 2.0 μM (**11**), 1.5 μM (**63**), and 1.7 μM (**71**). The inorganic phosphate concentration was measured after incubation at 37 ˚C for 15 min (A) or 21 min (B, C, D).

4. Table S1: hits from the Target fishing studies

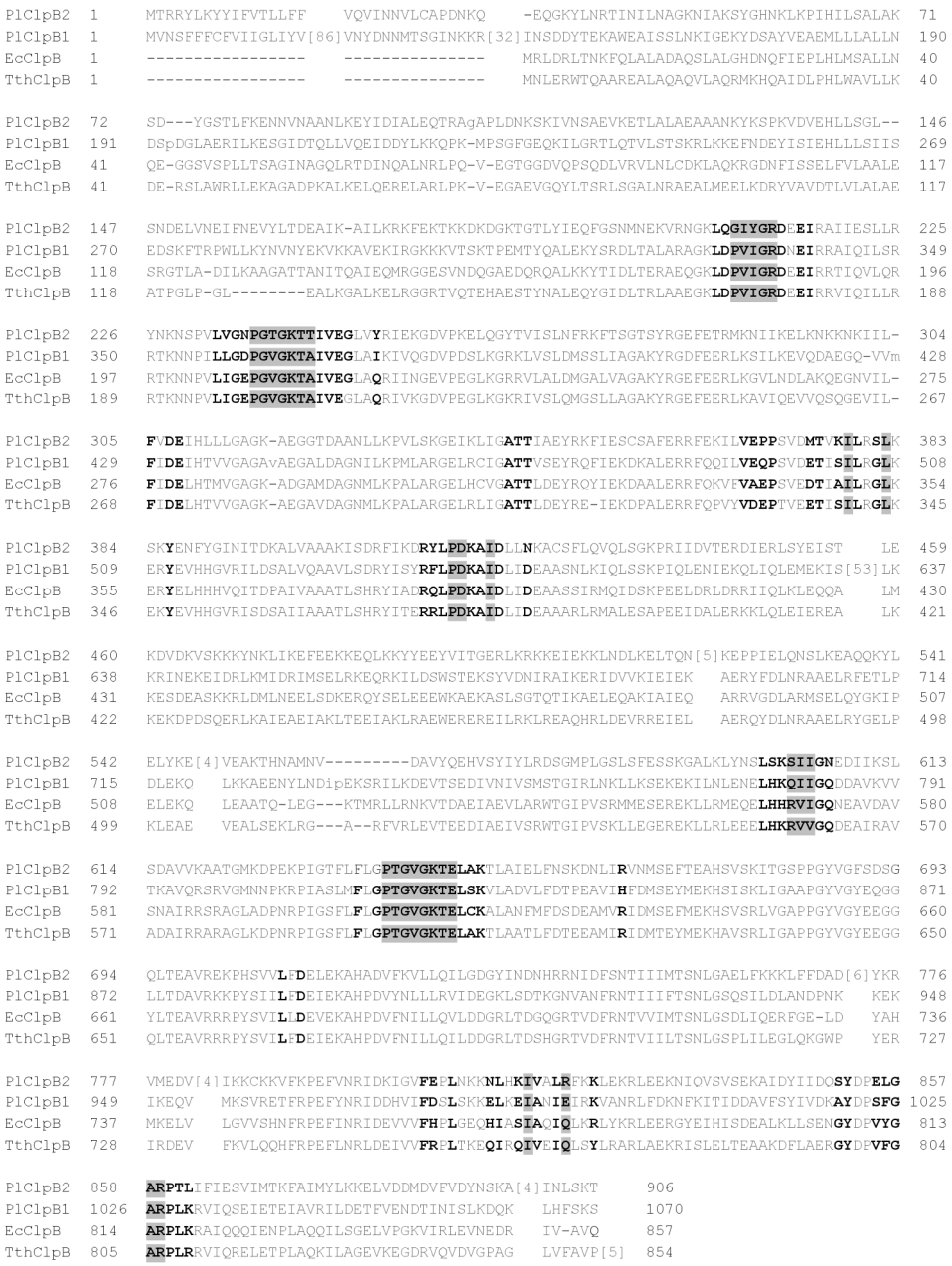
Final list of compounds retrieved after similarity search and analysis of preliminary results. In the last column are reported plasmodium proteins with the highest degree of similarity with the target of the ChEMBL compounds (2nd column). In the last two entries (9-10) are reported ChEMBL compounds with similar similar biological activity. (ID: identical residues, SS: total positive matches)

HIT	COMPOUNDS CHEMBL ID	TARGET IN CHEMBL	Sequence similarities with Pl. Falciparum proteome
a	 CHEMBL2424784	Inhbitor HSP bacteria: ClpB, bacterial representative HSP100 family (5)	HSP101 Thermus termophilus (crystal 1QVR): -ClpB1 plasmodium falciparum 3D7 (ID: 47%, SS:67%) -ClpB2 plasmodium falciparum 3D7 (ID: 43%, SS:64%) HSP101 E. coli(crystal 4CIU): -ClpB1 plasmodium falciparum 3D7 (ID: 47%, SS:69%) -ClpB2 plasmodium falciparum 3D7 (ID: 40%, SS:63%)
b	 CHEMBL3105506	DNA topoisomerase II alpha(6)	Pl. falciparum topoisomerase 2 (ID: 50%, Pos: 69%)
c	 CHEMBL376957	Histamine H3 receptor (20)	No similarities
d	 CHEMBL1221983	Sonic hedgehog protein (activation of shh pathway in mouse) (21)	Phosphatidylinositol 4-kinase (putative) (ID:28%, SS: 54%) Erythrocyte membrane protein 1(ID:25%, SS:50%)

e	 CHEMBL1933803	Inhibition of GST-tagged human DYRK1A (dual specificity tyrosine phosphorilation regualted kinase 1 A) (22)	
f	 CHEMBL300470	Coagulation Factor X Some derivatives also inhibit thrombin (23)	Cysteine repeat modulator protein 2 (ID:25%, SS:38%) Herzoile surface protein 5 (ID:41%, SS=47%) no crystals Erythrocyte membrane protein 1(ID:31%, SS:54%)
g	 CHEMBL317554	Neuropeptide Y receptor type 2 (24)	No similarities
h	 CHEMBL126313	Dopamine receptors D2 and D3 (25)	Rifin(RIF) (ID:30%, SS:57%)
i	 CHEMBL561108 & derivatives	Junin virus inhibition Lassa virus inhibition(13)	

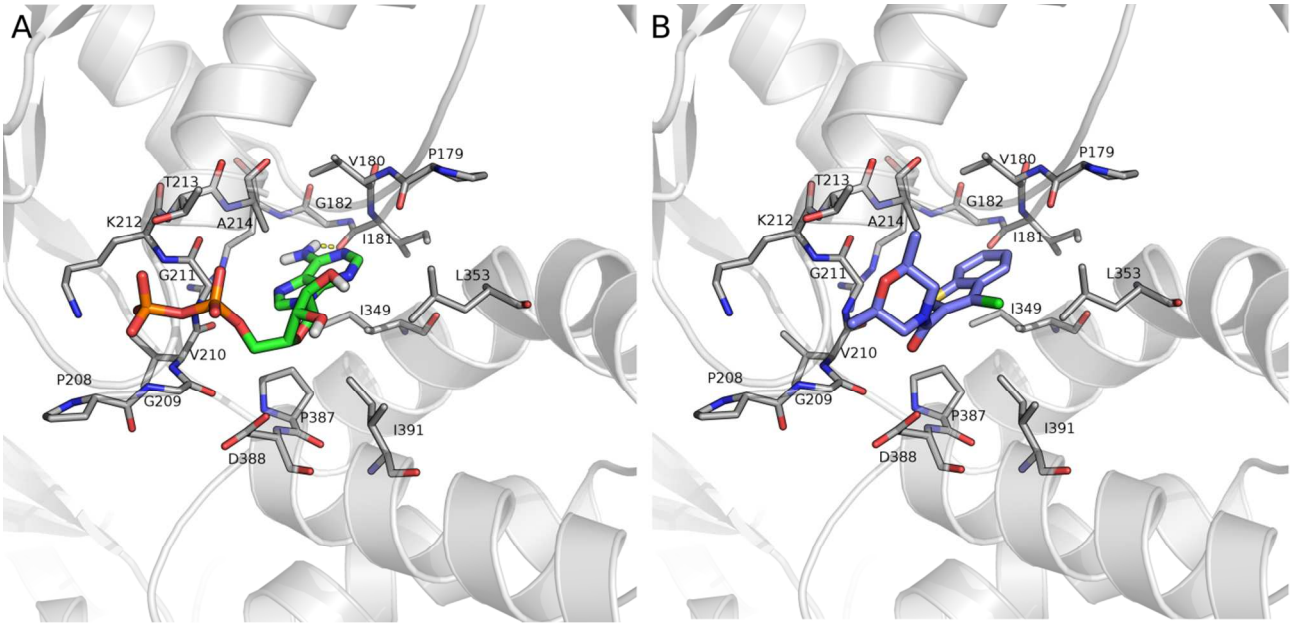
5. Figure S2

Alignment between PlClpB1, PlClpB2, *E. Coli* ClpB and *T. Th* ClpB. Using *E.coli* sequence as reference, in black, bold and with gray background are highlighted residues within 4Å of the co-crystallized ligands in 4CIU crystal structure, whereas only in black and bold those within 6Å.



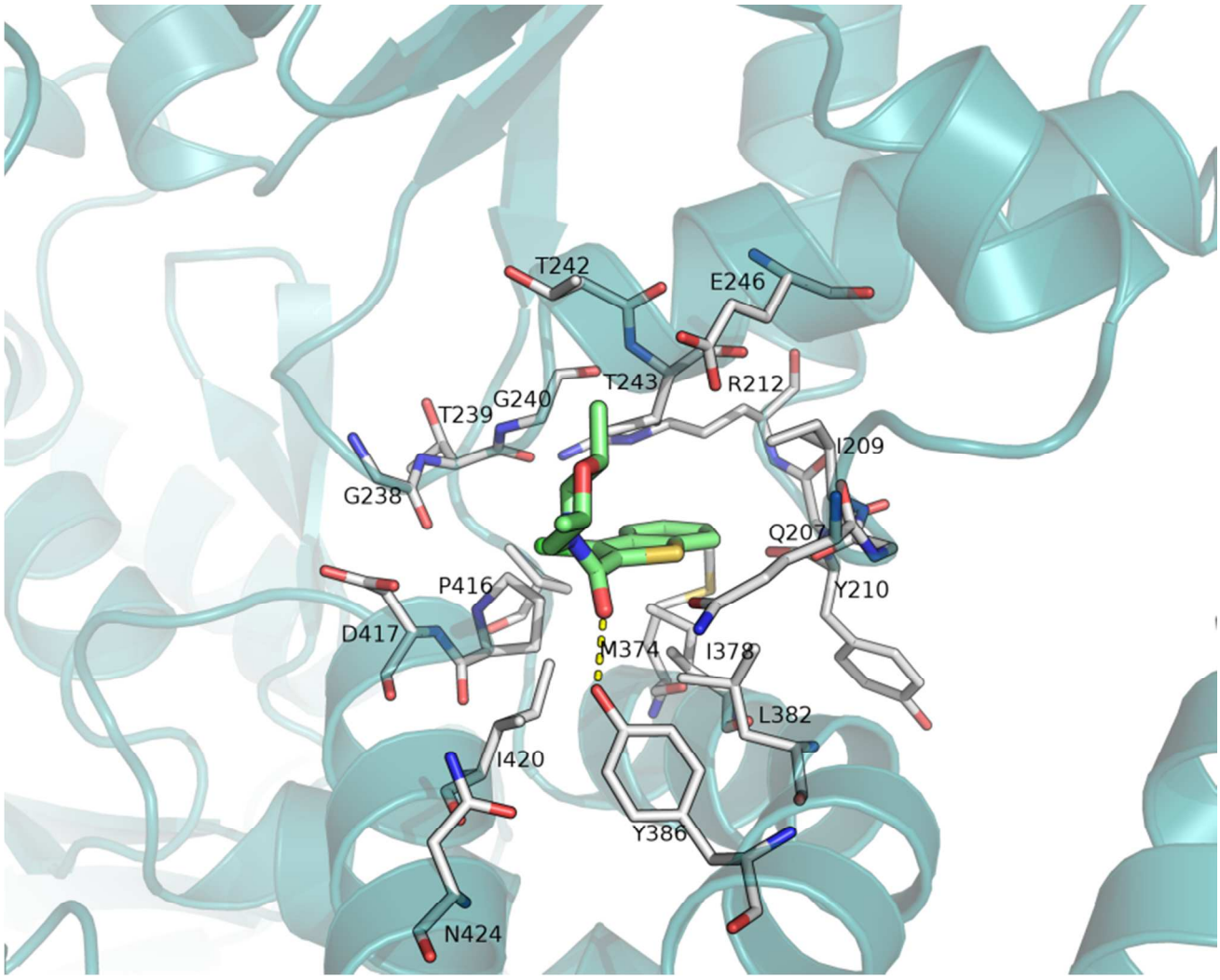
6. Figure S3

In figure S3A is reported 4CIU crystallographic complex with ADP, whereas in figure S3B the docking pose of **11** in 4CIU crystal structure. The benzothiophene core is accommodated in the binding pocket surrounded by small hydrophobic residues, whereas the morpholine ring points towards the more solvent exposed region occupied by the ribosylphosphate portion of ADP in the crystal structure.



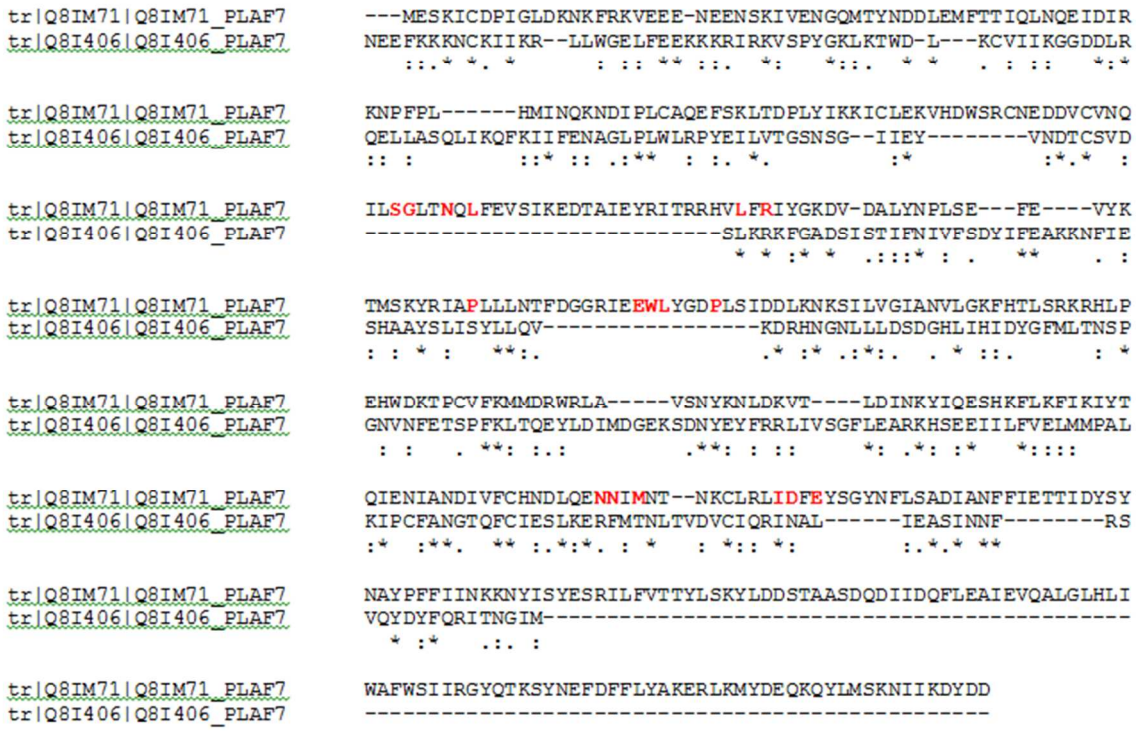
7. Figure S4

Docking pose of **11** in PlClpB2 comparative model. Tyr386 is located closer to the binding site and therefore could establish hydrogen bond with the carbonyl oxygen of the amidic bridge.



8. Figure S5

The overall similarity between choline kinase and PI4K catalytic domain (N1261- -M1559) is quite low, with only 21.5% of identity. Alignment of the catalytic portion of plasmodium falciparum PI4K (Uniprot ID: Q8I406) and plasmodium falciparum choline kinase sequence (Uniprot ID: Q8IM71) was performed with Clustal Omega web service and is reported here. The similarity in the region of choline kinase ADP binding site (binding site residues derived from *pl. falciparum* choline kinase crystal structure 3FI8 are highlighted in red) is very low.



9. Evaluation of the stability of compounds 11, 19 and 23.

Time (hours)	% cpd 11[a]	% cpd 19[a]	% cpd 23[a]
0	100	100	100
1	99 ± 8	99 ± 3	100 ± 8
2	96 ± 8	95 ± 1	98 ± 10
20	86 ± 3	90 ± 5	93 ± 1
24	81 ± 1	96 ± 10	91 ± 5

[a] Percent of compound remaining at stated time points in RPMI 1640 medium, 37°C. Means ± SD (n=3).

Experimental

The chemical stability of compounds **11**,**19** and **23** was tested in the RPMI 1640 medium employed in the drug susceptibility assay, starting from freshly prepared DMSO stock solutions of the compounds. Briefly, samples were prepared by adding compounds to the medium (final compound concentration: 1 μM; final DMSO concentration: 1%) and were kept in a thermostated water-bath at 37°C. At stated time points (t=0 h; 1 h; 2 h; 20 h; 24 h), aliquots of the buffered solution were withdrawn, diluted 1:1 with internal standard and injected into the HPLC-MS/MS system for quantification of remaining compound. A Thermo Accela UHPLC gradient system coupled to a Thermo TSQ Quantum Access Max triple quadrupole mass spectrometer (Thermo Italia, Milan, Italy) equipped with a heated electrospray ionization (H-ESI) ion source was employed for compound separation and analysis. Chromatographic separation occurred on a Phenomenex Synergi Fusion column (100 x 2.0 mm, 4 μm particle size) employing a gradient elution. Eluent A: acetonitrile + 0.1% formic acid; eluent B: water + 0.1% formic acid. Gradient was as follows: T(0 min): 10%A:90%B; T(7 min): 95%A:5%B; T(8 min): 95%A:5%B; T(9 min): 10%A:90%B. MS/MS analyses were done in positive ion and in multiple reaction monitoring mode (MRM). H-ESI interface parameters were set as follows: probe middle (D) position; capillary temperature 270 °C; spray voltage 3.5 kV. Nitrogen was used as nebulizing gas at the following pressure: sheath gas: 35 psi; auxiliary gas 15 arbitrary units (a.u.). Argon was used as the

collision gas at a pressure of approximately 1.5 mtorr (1 torr = 133.3 Pa). For the quantitative analysis, the following parent→product ion transitions were selected: **19**: m/z 289.0 $[M+H]^+$ → m/z 96.2 + m/z 166.9 + m/z 194.9 (tube lens (TL) 77 V; collision energies (CE) 18, 37, 26 eV, respectively); **23**: m/z 328.0 $[M+H]^+$→ m/z 141.1 + m/z 184.9 + m/z 212.9 (TL 82 V; CE 54, 40, 22 eV); **11**: m/z 310.0 $[M+H]^+$→ m/z 123.1 + m/z 167.0 + m/z 194.9 (TL 80 V; CE 54, 40, 23 eV).