

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%. ^1H 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). ^1H NMR (400 MHz, CDCl_3): δ 3.95 (s, 3H); 4.20 (s, 3H); 7.44-7.56 (m, 2H); 7.82 (d, $J = 8\text{ Hz}$, 1H); 7.94 (d, $J = 8\text{ Hz}$, 1H). LRMS (ESI) calcd for $\text{C}_{11}\text{H}_{10}\text{O}_3\text{S}$ ($[\text{M}-\text{H}]^-$) 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) ^1H 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, $J = 8.4\text{ Hz}$, 2H). LRMS (ESI) calcd for $\text{C}_{17}\text{H}_{13}\text{ClO}_3\text{S}$ ($[\text{M}-\text{H}]^-$) 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) ^1H 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 $\text{C}_{18}\text{H}_{16}\text{O}_4\text{S}$ ($[\text{M}-\text{H}]^-$) 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) ^1H 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 $\text{C}_{17}\text{H}_{12}\text{F}_2\text{O}_3\text{S}$ ($[\text{M}-\text{H}]^-$) 333.05; found 333.3.

3-((4-Chlorobenzyl)oxy)benzo[b]thiophene-2-carboxylic acid (47): white powder, Yield 96%. ^1H 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 $\text{C}_{16}\text{H}_{11}\text{ClO}_3\text{S}$ ($[\text{M}-\text{H}]^-$) 317.01; found 317.3.

3-((4-Methoxybenzyl)oxy)benzo[b]thiophene-2-carboxylic acid (48): white powder, Yield 92%.

LRMS (ESI) calcd for C₁₇H₁₄O₄S ([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 C₁₆H₁₀F₂O₃S ([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%, dichloromethane/methanol (95:5). ¹H 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). ¹³C 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 C₁₅H₁₇ClN₂OS ([M+H]⁺) 310.0590; found 310.0666.

(3-Chlorobenzo[b]thiophen-2-yl)(*Z*-2,6-dimethylmorpholino)methanone (12): pale yellow oil, 39%, dichloromethane/methanol (95:5). ¹H 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). ¹³C 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 C₁₅H₁₇ClN₂OS ([M+H]⁺) 310.0590; found 310.0666.

(3-Chlorobenzo[b]thiophen-2-yl)(3,5-dimethylpiperazin-1-yl)methanone (13): pale yellow oil, 46%, dichloromethane/methanol (95:5). ¹H NMR (400 MHz, CDCl₃): 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). ¹³C NMR (100.6 MHz, CDCl₃): 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 C₁₅H₁₇ClN₂OS ([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). ¹H NMR (300 MHz, CDCl₃): δ 3.40-4.10 (m, 8H); 7.45-7.52 (m, 2H); 7.70-7.80 (m, 2H). ¹³C NMR (100.6 MHz, CDCl₃): 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 C₁₃H₁₂ClNO₂S ([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). ¹H NMR (300 MHz, CDCl₃): 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 $\text{C}_{14}\text{H}_{14}\text{ClNOS}$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{19}\text{H}_{20}\text{ClNOS}$ ($[\text{M}+\text{H}]^+$) 346.0954; found 346.1027.

3-Chloro-*N*-phenylbenzo[b]thiophene-2-carboxamide (17): white solid, 35%, petroleum ether/ethyl acetate (90:10). ^1H 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 $\text{C}_{15}\text{H}_{10}\text{ClNOS}$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{14}\text{H}_9\text{ClN}_2\text{OS}$ ($[\text{M}+\text{H}]^+$) 289.0124; found 289.0147

3-Chloro-*N*-(pyridin-4-yl)benzo[b]thiophene-2-carboxamide (19): white solid, 60%, dichloromethane/methanol (97:3). ^1H 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 $\text{C}_{14}\text{H}_9\text{ClN}_2\text{OS}$ ($[\text{M}+\text{H}]^+$) 289.0124; found 289.0210.

3-Chloro-N,N-diethylbenzo[b]thiophene-2-carboxamide (20): white solid, 65%, petroleum ether/ethyl acetate (90:10). ¹H NMR (300 MHz, CDCl₃): 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). ¹³C NMR (100.6 MHz, CDCl₃): 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 C₁₄H₉ClN₂OS ([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). ¹H NMR (300 MHz, CDCl₃): 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). ¹³C NMR (100.6 MHz, CDCl₃): 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 C₁₆H₁₈ClN₂OS ([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). ¹H NMR (300 MHz, CDCl₃): 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). ¹³C NMR (100.6 MHz, CDCl₃): 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 C₁₅H₁₅Cl₂NO₂S ([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). ¹H NMR (300 MHz, CDCl₃): 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). ¹³C NMR (100.6 MHz, CDCl₃): 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 C₁₅H₁₅ClFNO₂S ([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). ^1H 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, $J = 8\text{Hz}$, 1H); 7.64 (s, 1H); 7.76 (d, $J = 8\text{Hz}$, 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 $\text{C}_{16}\text{H}_{18}\text{ClNO}_2\text{S}$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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, $J = 8\text{Hz}$, 1H); 7.71 (d, $J = 8\text{Hz}$, 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 $\text{C}_{15}\text{H}_{14}\text{Cl}_3\text{NO}_2\text{S}$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{15}\text{H}_{17}\text{NO}_2\text{S}$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{15}\text{H}_{18}\text{N}_2\text{OS}$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_2\text{S}$ ($[\text{M}+\text{H}]^+$) 289.0091; found 289.0157.

Benzo[b]thiophen-2-yl(4-methylpiperazin-1-yl)methanone (57): yellowish solid, 68%, dichloromethane/methanol (95:5). ^1H 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 $\text{C}_{14}\text{H}_{16}\text{N}_2\text{OS}$ ($[\text{M}+\text{H}]^+$) 261.0983; found 261.1047.

Benzo[b]thiophen-2-yl(morpholino)methanone (58): pale oil, 71%, petroleum ether/ethyl acetate (95:5). ^1H 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 $\text{C}_{13}\text{H}_{13}\text{NO}_2\text{S}$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{17}\text{H}_{21}\text{NO}_2\text{S}$ ($[\text{M}+\text{H}]^+$) 304.1293; found 304.1369.

Benzofuran-2-yl(*E*-2,6-dimethylmorpholino)methanone (60): white solid, 31%, petroleum ether/ethyl acetate (90:10). ^1H 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 $\text{C}_{15}\text{H}_{17}\text{NO}_3$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_2$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{16}\text{H}_{19}\text{NO}_3\text{S}$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{22}\text{H}_{23}\text{NO}_3\text{S}$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{22}\text{H}_{22}\text{ClNO}_3\text{S}$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{23}\text{H}_{25}\text{NO}_4\text{S}$ ($[\text{M}+\text{H}]^+$) 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). ^1H 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 $\text{C}_{22}\text{H}_{21}\text{F}_2\text{NO}_3\text{S}$ ($[\text{M}+\text{H}]^+$) 418.1210; found 418.1458.

(*E*-2,6-dimethylmorpholino)(1H-indol-2-yl)methanone (67): colourless oil, 13%,

dichloromethane/methanol (95:5). ^1H 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 $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_2$ ($[\text{M}+\text{H}]^+$) 259.1368; found 259.1891.

(3,5-dimethylpiperazin-1-yl)(1H-indol-2-yl)methanone (68): colourless oil, 44%,

dichloromethane/methanol (95:5). ^1H 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 $\text{C}_{15}\text{H}_{19}\text{N}_3\text{O}$ ($[\text{M}+\text{H}]^+$) 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). ¹H 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 C₁₅H₁₈N₂O₂S ([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). ¹H 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 C₂₂H₂₄N₂O₂S ([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). ¹H NMR (300 MHz, CDCl₃): 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). ¹³C NMR (100.6 MHz, CDCl₃): 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 C₁₆H₁₉NO₂S ([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). ¹H NMR (300 MHz, CDCl₃): 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). ¹³C NMR (100.6 MHz, CDCl₃): 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 C₁₄H₁₆N₂O₂S ([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). ¹H NMR (300 MHz, CDCl₃): 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 C₁₇H₂₁NO₄S ([M+H]⁺) 336.12; found 336.3.

3. Inhibition of ClpB by selected compounds

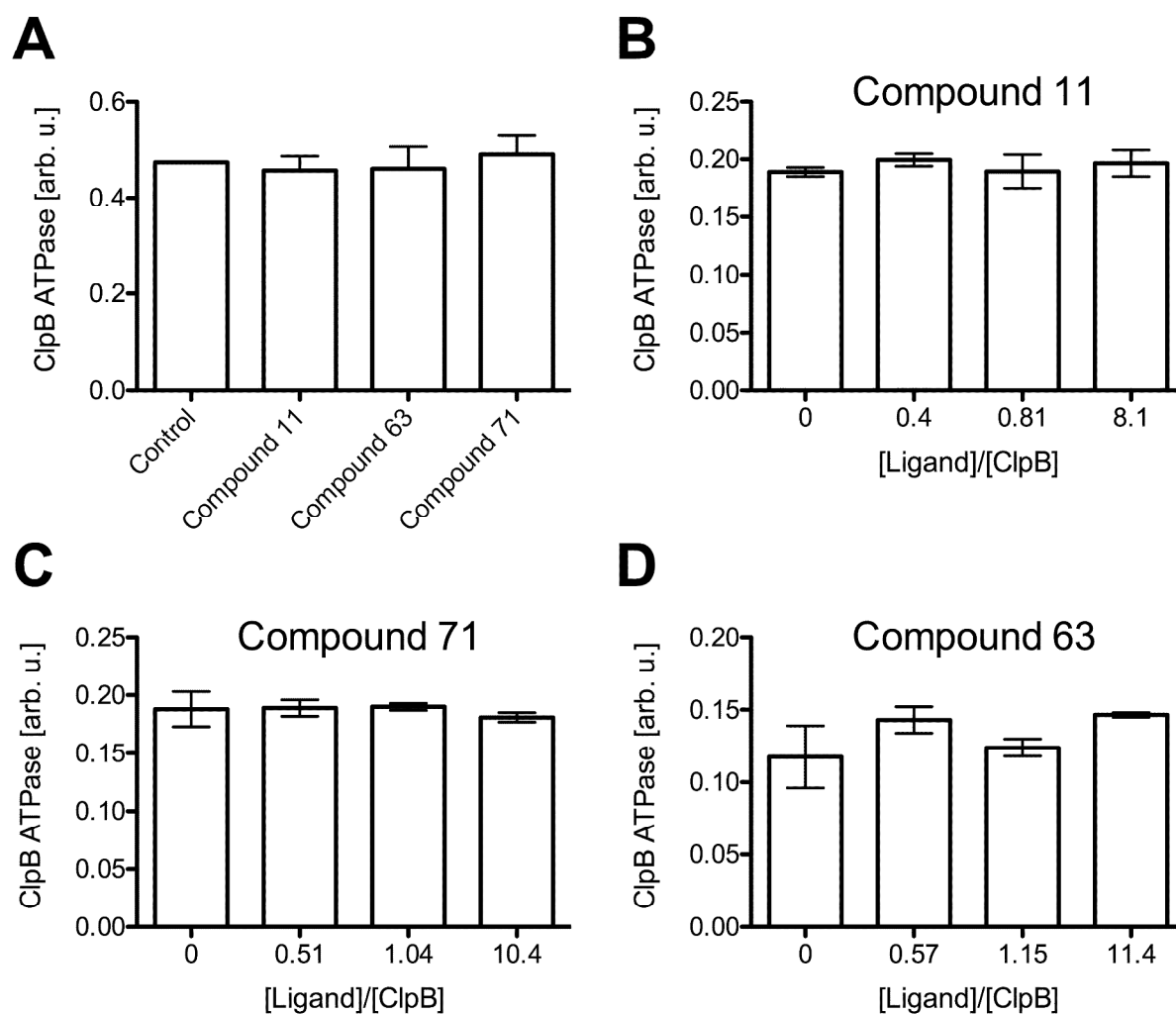


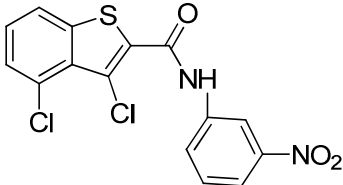
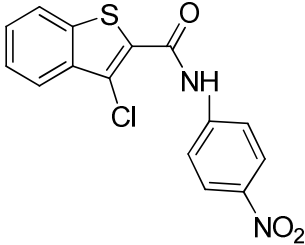
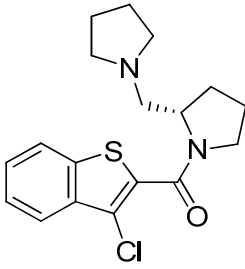
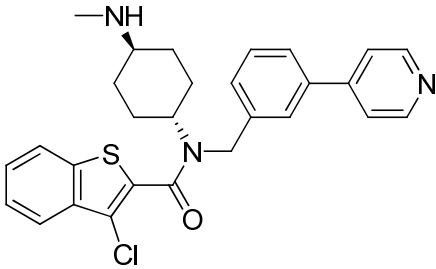
Figure S1. The ATPase activity of *E. coli* ClpB in the presence of the compounds 11, 63, and 71.

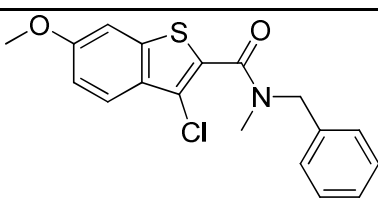
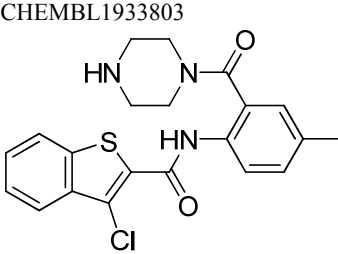
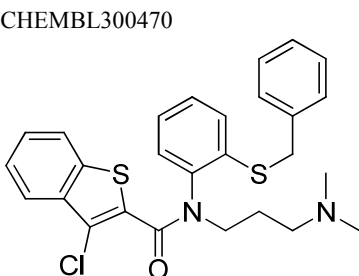
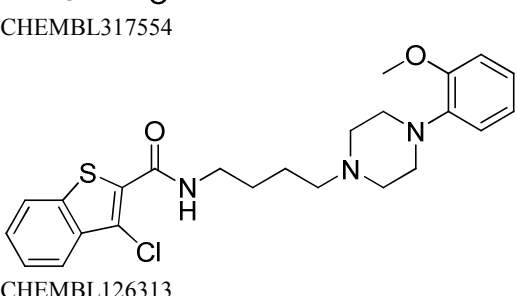
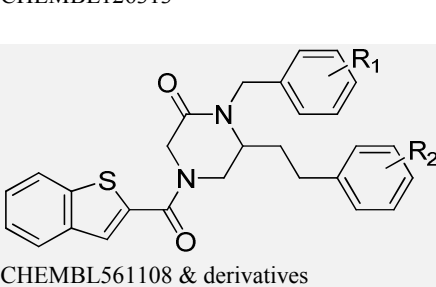
The ATP hydrolysis reaction was initiated by adding ClpB (A, B, C) or ATP (D) to the assay buffer

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 biological activity. (ID: identical residues, SS: total positive matches)

HIT	COMPOUNDS CHEMBL ID	TARGET IN CHEMBL	Sequence similarities with Pl. Falciparum proteome
a	 CHEMBL2424784	Inhibitor HSP bacteria: ClpB, bacterial representative HSP100 family (5)	HSP101 Thermus thermophilus (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		Inhibition of GST-tagged human DYRK1A (dual specificity tyrosine phosphorylation regulated kinase 1 A) (22)	
f	CHEMBL1933803 	Coagulation Factor X Some derivatives also inhibit thrombin (23)	
g	CHEMBL300470 	Neuropeptide Y receptor type 2 (24)	
h	CHEMBL317554 	Dopamine receptors D2 and D3 (25)	
i	CHEMBL126313 	Junin virus inhibition Lassa virus inhibition(13)	

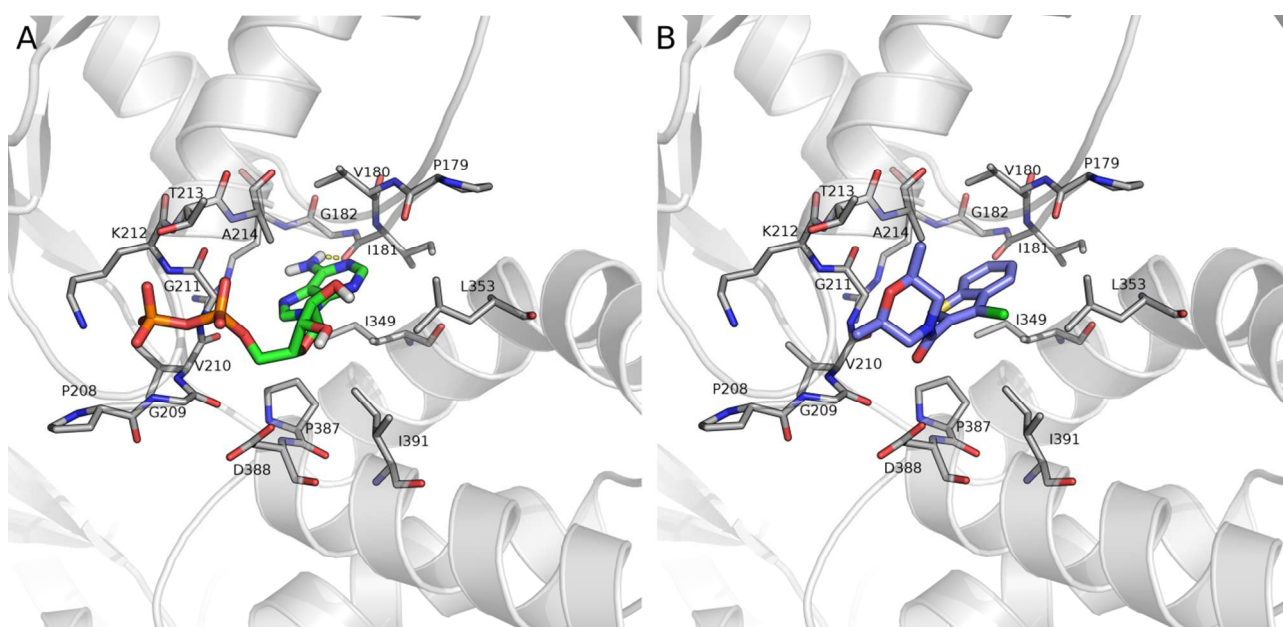
5. Figure S2

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.

PlClpB2	1	MTRRYLKYIIFVTLLFF	VQVINNVLCAPDNKQ	-EQGKYLNRITINILNAGKNIAKSYGHNKLKPIHLSALAK	71
PlClpB1	1	MVNSFFFCFVIIIGLIYV [86]	VNYDNNMTSGINKKR [32]	INSDDYTEKAWEAISLNLKIGEKYDSAYVEAEMLLALLN	190
EcClpB	1	-----	-----	MRLDRLTNKFQLALADAQSLALGHDNQFIEPLHLSALLN	40
TthClpB	1	-----	-----	MNLERWTQAAREALAAQVLAQRMKHQAIDLPHLWAVLLK	40
PlClpB2	72	SD---YGSTLFKENNVNAAANLKEYIDIALEQTRAGAPLDNKSIVNSAEVKETLALAEAAANKYKSPKVDVEHLLSGL--			146
PlClpB1	191	DSPDGLAERILKESGIDTQLLVQEIDDYLLKKQPK-MPSGFGEQKILGRITQLTVLSTSKRLKKEFNDEYISIEHLLLSITS			269
EcClpB	41	QE-GGSVSPLLTSAGINAGQLRDTINQALNRLPQ-V-EGTGGDVQPSQDLVRVLNLCDLKQAKRGDNFISSELFVLALE			117
TthClpB	41	DE-RSLAWRLLEKAGADPKALKELQERELARLPK-V-EGAEVGYQLTSRLSGAINRAEALMEELKDRYVAVDTLVLALAE			117
PlClpB2	147	SNDELVNEIFNEVYLTDEAIK-AILKRKFEKTKKDKDGKTGTLYIEQFGSNMNEKVRNGK	LGGIYGRD	EIRRAIESLLR	225
PlClpB1	270	EDSKFTRFWLLKYNVYKVKKAVEKIRGKKKVTSKTPEMTYQALEKYSRDLTALARAGK	LDPVIGRD	EIRRAIQILSR	349
EcClpB	118	SRGTLA-DILKAAGATTANTITQAIEMRGGESVNDQGAEDQRQALKKYITDLTERAEQCK	LDPVIGRD	EIRRTIQVLQR	196
TthClpB	118	ATFGLP-GL-----EADKGALKELRGGRTVQTEHAESTYNALQYIGIDLTLALAEK	LDPVIGRD	EIRRVIQILLR	188
PlClpB2	226	YNKNSPV	LVGNPOTGKTTIVEGL	LVRIEKGDPVKELQSYTVISLNRKFTSGTSYRGEFETRMKNIKELKNKKNKIIL-	304
PlClpB1	350	RTKNNP	ILLGDPGVGKTAIVEGL	AIKIVQGDVPSLKGKRLVSLDMSLIAGAKYRGDFEERLSILKEVQDAEGQ-VVm	428
EcClpB	197	RTKNNPV	LIGEPGVGKTAIVEGL	QRIINGEVPEGLKGRRLALDMGALVAGAKYRGEFEERLKGVLNDLAKQEGNVIL-	275
TthClpB	189	RTKNNPV	LIGEPGVGKTAIVEGL	QRIIVKGDVPEGLKGRIVSLQMGSLLAGAKYRGEFEERLKAIVQEVVQSQSEVIL-	267
PlClpB2	305	FVDE IHLLLGAGK-AEGGTDAAANLLKPVLSKGEIKLIG ATT IAEYRKFIESCASFERRFEKIL VEPPSVDMT VKILRS			383
PlClpB1	429	FIDE IHTVVGAGAvAEGALDAGNLKPMPLARGELRCIG ATT VSEYRQFIEKDKALERRFQQL VEQPSVDETS SILRGL			508
EcClpB	276	FIDE LHTMVGAGK-ADGAMDAGNMLKPALARGELHCVG ATT LDEYROYIEKDAALERRFQKV VAEPSVEDT IAILRGL			354
TthClpB	268	FIDE LHTVVGAGK-AEGAVDAGNMLKPALARGELRLIG ATT LDEYRE-IEKDPALERRFQPV VDEPTVEET SILRGL			345
PlClpB2	384	SK YENFYGINITDKALVAAAKISDRFIK RYLPDKAID LLN KAC SFLQVLQSGKPRIIDVTERDIERLSYEIST		LE	459
PlClpB1	509	ER YEVHHGVRIILDSALVQAAVLSDRYIS RYLPDKAID LIDEAASNLKQLSSKPIQLENIEKQLILEMEKIS [53] LK			637
EcClpB	355	ER YELHHVQITDPAIVAAATLSHRYIA RYLPDKAID LIDEAASSIRMQIDSKEEELDRDRRIQLKLEQQA		LM	430
TthClpB	346	EK YEVHHGVRIISDAIAAATLSHRYITE RYLPDKAID LIDEAAARLMALESAPAEIDALERKKLQLEIEREA		LK	421
PlClpB2	460	KDVKVSKKKYNKLIKFEFEKKEQLKKYEEYVITGERLKRKKEIEKLNLDKELTON [5] KEPP IELQNSLKEAQKYL			541
PlClpB1	638	KRINEKIDRLKMI DRIMSELKKEQRKILDSWSTEKSYVDNIRAIKERIDVVKIEIEK	AERYFDLNRAELRFETLP		714
EcClpB	431	KESDEASKRRLDMLNEELSDKERQYSELEENKAEKASLSGTQTIKAELEQAKIAIEQ	ARRVGD LARMSSELQYGIKIP		507
TthClpB	422	KEKDPDSQERLKAIEAETIAKLTEEIAKLAEWEREREILRLKREAQHRLDEVREIEL	AERQYDLNRAELRYGELP		498
PlClpB2	542	ELYKE [4] VEAK THNAMNV-----DAVYQEHVSYIYLRDSGMPLGSLSFESSKGALKLYNS LSKSIIGN EDIISKSL			613
PlClpB1	715	DLEKO	LKKAENYLNIDipeKSRILKDEVTSEDIVNIVSMSTGIRLNLKKEKEKILNLENE LHKQIIGQ DDAVKVV		791
EcClpB	508	ELEKQ	LEAATQ-LEG---KTMRLLRNKVTDAEIAEVLARWTGIPVSRMMESEREKLLRMEQE LHHRVIGQ NEAVDAV		580
TthClpB	499	KLEAE	VEALSEKLRG---A--RFVRLEVTEEDIAEIVSRWTGIPVSKLLEGEREKLLRLEE LHKRVVGG QDEAIRAV		570
PlClpB2	614	SDAVVKAATGMKDPEKPIGTFTFLG PTGVGKTELAK TLAIELFNSKDNLIRVNMSEFTEAHSVSKITGSPPGYVGFSDSG			693
PlClpB1	792	TKAVQSRVGMNPKRPIASLM FI LOPTGVGKTELSK V/LADVLEDTPEAVIH DF MSEYMEKHSISKLIGAAPGYVGYEQGG			871
EcClpB	581	SNAIRRSRAGLADPNRPISGFI FI LOPTGVGKTELSK ALANFMFDSDEAMVRIDMSEFMKHSVSRVLGAPPGYVGYEEGG			660
TthClpB	571	ADAIRRARAGLKDPNRPISGFI FI LOPTGVGKTELSK LAATLFDTEAMIRIDMTEYMEKHAVSRVLGAPPGYVGYEEGG			650
PlClpB2	694	QLTEAVREKPHSV VL LD DELEKAHADVEKVLQLQILGDGYINDNHRNIDFSNTIIIMTSNLGAELEFKKKLFFPDAD [6] YKR			776
PlClpB1	872	LLTDAVRKKPY SII LD DEIEKAHPDVYNLLLRVIDEGKLSDTKGNVANFRNTIIIFTSNLGSQSILDLANDPNK	KEK		948
EcClpB	661	YLTEAVRRRPYS VII LD DEVEKAHPDVFNILLQVLDDGRLTDGQGRVDFERNVTVMITSNLGSDLIQERFGE-LD	YAH		736
TthClpB	651	QLTEAVRRRPYS VII LD DEIEKAHPDVFNILLQILDDGRLTDSHGRTVDFERNVTIILTSNLGSPILILEGLQKGWP	YER		727
PlClpB2	777	VMEDV [4] IKKCK VFKPEFVNRIDKIG VF FLNKHNLKIVLR FKLEKRLEEKNIOVSSEKAIDYIID SYDPELG		857	
PlClpB1	949	IKELV	MKSVRTFRPEFYNRIDHDV FD LSKELKELIANIE IRKANRLFDKNFKITIDDAVFSYIVDK AYDPSFG		1025
EcClpB	737	MKELV	LGVSHNFRPEFYNRIDEVV VF FLGECHISIAQ QLKRLYKRLEERGYYEIHISDEALKLSEN GYDVFYG		813
TthClpB	728	IRDEV	FKVLQQHFRPEFLNRLDEIV VF FLTKELQIV IEQLSYLRARLAEKRISLELTAAKDFLAER GYDVFVG		804
PlClpB2	050	ARPT LIFIESVIMTKFAMYLKKELVDDMDVFDYNSKA [4] INLSKT		906	
PlClpB1	1026	ARPL KRVIQSEIETETIAVRILDETfVENDTINISLKDQK	LHFSKS	1070	
EcClpB	814	ARPL KRAIQQIENPLAQQLSGELVPGKVRILEVNEDR	IV-AVQ	857	
TthClpB	805	ARPL KRVIQRELETPLAQKILAGEVKEGDRVGVVDVGPAG	LVFAVP [5]	854	

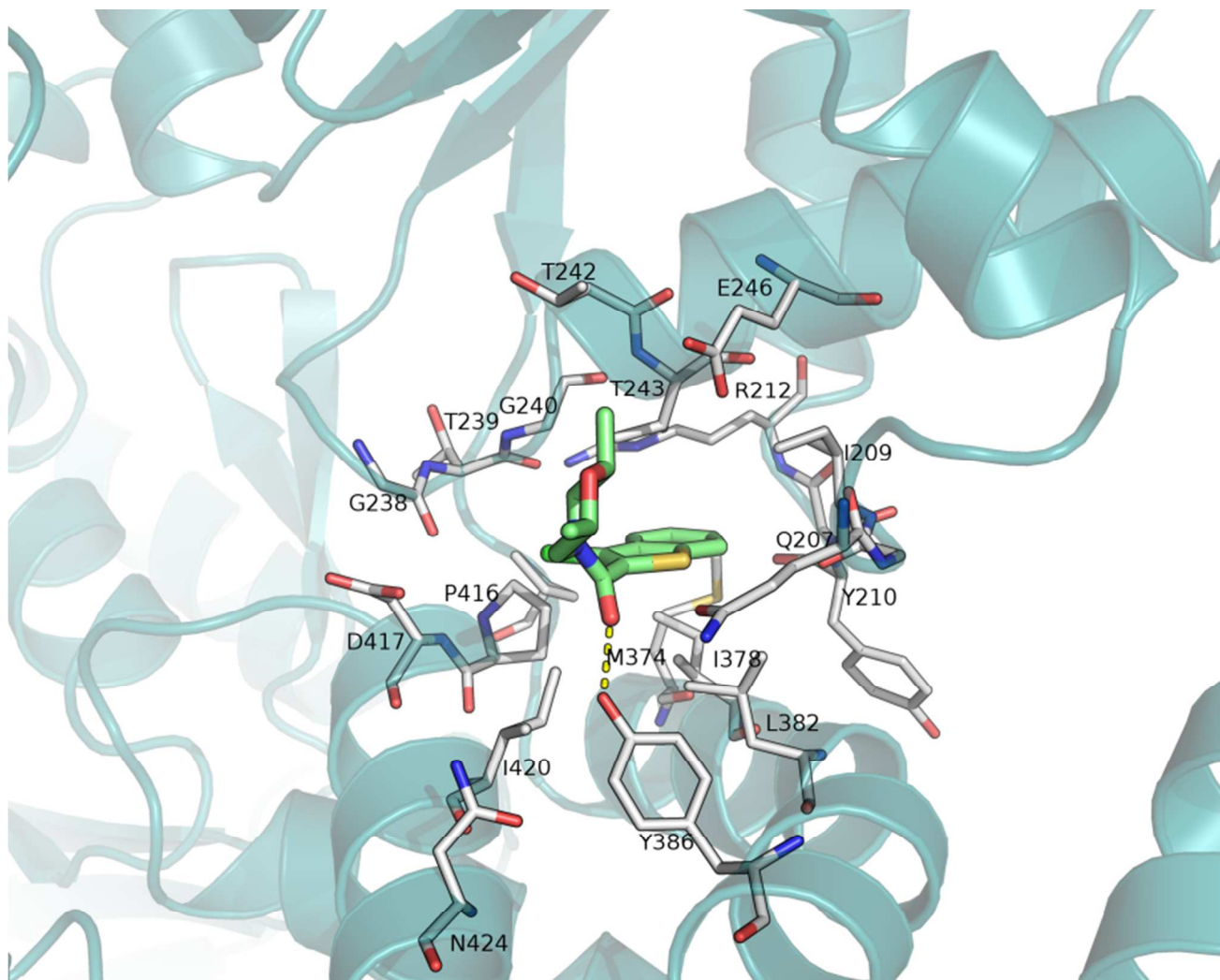
6. Figure S3

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 PIClpB2 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.

```

tr|Q8IM71|Q8IM71_PLAF7      ---MESKICDPIGLDKNKFRKVEEE-NEENSKIIVENGQMTYNDDLEMTTIQLNQEIDIR
tr|Q8I406|Q8I406_PLAF7      NEEFKKKNCIKIIR--LLWGELFEEKKRIRKVSPIYGLKTWD-L---KCVIIKGGDDLK
      :.*.*.*      : :.*.*.:. *: *:. * * . : : : *:*

tr|Q8IM71|Q8IM71_PLAF7      KNPFFL-----HMINQNDIPLCAQEFSLTDPYIKKICLEKVHDSRCNEDDVCVNQ
tr|Q8I406|Q8I406_PLAF7      QELLASQLIKQFKII FENAGLPLWLRPYEILVTGSNSG--IIIEY-----VNDTCSVD
      : :      :.* : : :.* : : .*.      :*      :.*.* :

tr|Q8IM71|Q8IM71_PLAF7      ILSGLTNQLFEVSIKEDTAIEYRITRRHVLFRITYGKDV-DALYNPLSE---FE----VYK
tr|Q8I406|Q8I406_PLAF7      -----SLKRRKFCADSI STIFNIVFSDYIFEAKKNPIE
      * * : * * . : : * : . * * . :

tr|Q8IM71|Q8IM71_PLAF7      TMSKYRIAPLLLNTFDGGRIEEWLYGDPLSIDDLKNKSILVGIANVLGKFHTLSRKRHLP
tr|Q8I406|Q8I406_PLAF7      SHAAYSLISYLLQV-----KDRHNGNLLDSDGHLIHIIDYGFMLTNSP
      : : * : * : .      . * : * : * : . * : : : *

tr|Q8IM71|Q8IM71_PLAF7      EHWDKT PCVFKMMDRWRLA-----VSNYKNLDKVT---LDINKYIQESHKFLKFIKIYT
tr|Q8I406|Q8I406_PLAF7      GNVNFETSPFKLTQEYLDIMDGEKSDNYEYFRRLIVSGFLEARKHSEEIILFVELMMPAL
      : : . * * : : : . * * : : : * : . * : * * : : :

tr|Q8IM71|Q8IM71_PLAF7      QIENIANDIVFCHNDLQENNIMNT--NKCLRLIDFEYSGYNFLSADIANFFIETTIDYSY
tr|Q8I406|Q8I406_PLAF7      KIPCFANGTQFCIESLKERFMTNLTVDVCIQRINAL-----IEASINNF-----RS
      :* :*.* * * :.*.* : * : * : : * : : . * . * *

tr|Q8IM71|Q8IM71_PLAF7      NAYPFFIINKKNYISYESRILFVTYLSKYLDDSTAASDQDIIDQFLEAIEVQALGLHLI
tr|Q8I406|Q8I406_PLAF7      VQYDYFQRITNGIM-----
      * : * . : :

tr|Q8IM71|Q8IM71_PLAF7      WAFWSIIRGYQTKSYNEFDFFLYAKERLKMYDEQKQYLMSKNIIKDYYDD
tr|Q8I406|Q8I406_PLAF7      -----

```

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 $[M+H]^+ \rightarrow m/z$ 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 $[M+H]^+ \rightarrow m/z$ 141.1 + m/z 184.9 + m/z 212.9 (TL 82 V; CE 54, 40, 22 eV); **11**: m/z 310.0 $[M+H]^+ \rightarrow m/z$ 123.1 + m/z 167.0 + m/z 194.9 (TL 80 V; CE 54, 40, 23 eV).