Supporting Information

Novel *β*-carboline-quinazolinone hybrid as inhibitor of *Leishmania donovani* Trypanothione Reductase: Synthesis, molecular docking and bioevaluation

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EXPERIMENTAL SECTION

General Chemistry: All reagents were commercial and were used without further purification. Chromatography was carried on silica gel (100−200 mesh). All reactions were monitored by thin-layer chromatography (TLC), and silica gel plates with fluorescence F254 were used. Infrared spectra were recorded on a Perkin-Elmer AC-1 spectrometer. Melting points were taken in open capillaries on a Complab melting point apparatus and are presented uncorrected. 1H NMR and 13C NMR spectra were recorded using a BrukerSupercon Magnet DRX-300 spectrometer (operating at 300 and 400 MHz for ^{1}H and 75 and 100 MHz for ^{13}C) using $CDCl_3$ and DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in parts per million. Multiplicities are reported as follows: singlet (s), doublet (d), triplet (t), multiplet (m). Electrospray ionization mass spectra (ESIMS) were recorded on Thermo Lcq Advantage Max-IT. High resolution mass spectra (HRMS) were recorded on 6520 Agilent QTOF LC MS/MS (accurate mass).

Representative Procedure for the synthesis of 2-Amino-*N*-phenylbenzamide (3a)[1]: A stirred solution of isatoic anhydride (500 mg, 3 mmol) in acetonitrile (3mL) was heated to 80 °C. Then aniline (0.27 mL, 3 mmol) and catalytic amount of TEA was added to this solution. The reaction mixture was allowed to stir at the same temperature for an additional 4 h. After the completion of reaction (checked by TLC), the solvent was evaporated at reduced pressure. The corresponding solid product was further purified through column chromatography by using 100−200 mesh silica gels to obtain **3a** as a white solid (yield 81%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.77 (s, 1H), 7.60-7.58 (m, 2H), 7.50-7.48 (m, 1H), 7.41-7.37 (m, 2H), 7.30-7.26 (m, 1H), 7.19-7.15 (m, 1H), 6.76-6.73 (m, 2H), 5.51 (s, 1H); ^{13}C ($CDCl_3$, 100 MHz): 167.63, 148.97, 137.88, 132.76, 129.07, 127.24, 124.52, 120.62, 117.56, 116.86, 116.30.

Synthesis of 2-Aminobenzamides 3f, 3k and 3l [1-3]

The above procedure was followed for **3f**, **3k** and **3l**.

Representative Procedure for the synthesis of methyl-1-(4-oxo-3-phenyl-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8a): To the stirred solution of methyl 1-formyl-9H-pyrido[3,4-*b*]indole-3-carboxylate **7**[4] (200 mg, 0.787 mmol) and 2-amino-*N*-phenylbenzamide **3a** (167 mg, 0.787 mmol) in acetonitrile (3 mL) 10 mol % of cyanuric chloride was added. The mixture was stirred at room temperature and then transferred to heating at 60-70°C. The progress of the reaction was monitored by TLC. After completion, solvent was evaporated at reduced pressure. The corresponding solid products were obtained through column chromatography by using 100−200 mesh silica gels to obtain **8a** as a cream solid (yield 67%). mp 205-207 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.11(s, 1H), 8.83(s, 1H), 8.37(d, 1H, *J* = 8.3 Hz), 7.86(d, 1H, *J* = 7.6 Hz), 7.77(d, 1H, *J* = 8.2 Hz), 7.65(m, 2H), 7.55(s, 1H), 7.35-7.29(m, 4H), 7.15(t, 1H, *J* = 7.5 Hz), 7.04(t, 1H, *J* = 7.3 Hz), 6.88-6.76(m, 3H), 3.90(s, 3H); ^{13}C (DMSO- d_6, 75 MHz): 166.13, 163.89, 147.51, 141.46, 141.31, 140.51, 135.51, 134.61, 133.74, 129.43, 129.32, 128.44, 127.93, 126.75, 122.45, 121.05, 120.84, 118.53, 118.45, 116.97, 115.41, 112.96, 73.90, 52.49; IR(KBr) 3320, 3021, 1692, 1618, 1216, 1104 cm^{-1}. HRMS: calcd for [$C_{27}H_{20}N_4O_3 + H^+$] 449.1608, found 449.1607.

Synthesis of quinazolinone-*β*-carboline hybrid 8b-8o

The above procedure was followed for the synthesis of **8b−8o**.

Methyl-1-(3-benzyl-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8b): Cream Solid (yield 68%). mp 204-206 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.93 (s, 1H), 8.87 (s, 1H), 8.37 (d, 1H, *J* = 7.8 Hz), 7.82 (d, 1H, *J* = 7.74 Hz), 7.67-7.57 (m, 2H), 7.32-7.21 (m, 3H), 7.05-7.12 (m, 5H), 6.79-6.69 (m, 2H), 6.40 (s, 1H), 4.98 (d, 1H, *J* = 15.6

Hz), 4.06 (d, 1H, *J* = 15.3 Hz), 3.87 (s, 3H); ^{13}C (DMSO- d_6, 75 MHz): 166.19, 164.24, 147.07, 141.50, 141.25, 137.96, 135.81, 135.08, 133.35, 129.51, 129.24, 128.30, 128.17, 127.84, 127.05, 122.41, 121.17, 120.78, 118.54, 118.15, 116.49, 115.10, 112.96, 70.89, 52.47, 47.52; IR(KBr) 3413, 2925, 1710, 1639, 1216, 1153, cm^{-1}. HRMS: calcd for [$C_{28}H_{22}N_4O_3 + H^+$] 463.1765, found 463.1765.

Methyl-1-(4-oxo-3-propyl-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8c): Cream Solid (yield 71%). mp 234-236 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.30 (s, 1H), 8.84 (s, 1H), 8.19 (d, 1H, *J* = 7.9 Hz), 7.99-7.96 (m, 1H), 7.63-7.51 (m, 2H), 7.39-7.35 (m, 1H), 7.32-7.28 (m, 1H), 6.90-6.86 (m, 1H), 6.67 (d, 1H, *J* = 8.0 Hz), 6.36 (s, 1H), 5.15 (s, 1H), 3.99 (s, 3H), 3.92-3.85 (m, 2H), 2.71-2.64 (m, 2H), 0.72 (t, 3H, *J* = 7.4 Hz); ^{13}C (DMSO-d_6, 75 MHz): 165.66, 164.87, 145.37, 142.17, 140.89, 135.53, 133.92, 132.53, 128.81, 127.23, 122.10, 120.86, 120.44, 117.68, 117.35, 116.72, 114.46, 112.39, 69.36, 51.97, 28.05, 8.99; IR(KBr) 3279, 3020, 1724, 1640, 1216, 1069 cm^{-1}. HRMS: calcd for [$C_{24}H_{22}N_4O_3 + H^+$] 415.1765, found 415.1765.

Methyl-1-(3-butyl-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8d): Cream Solid (yield 69%). mp 230-232 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.05 (s, 1H), 8.96 (s, 1H), 8.44 (d, 1H, *J* = 7.80 Hz), 7.76-7.72 (m, 2H), 7.65 (t, 1H, *J* = 7.14 Hz), 7.36-7.30(m, 2H), 7.25-7.13 (m, 1H), 6.77-6.68 (m, 2H), 6.39 (s, 1H), 3.87 (s, 3H), 3.75-3.65 (m, 1H), 2.63-2.57 (m, 1H), 1.24-1.20 (m, 4H), 0.64(t, 3H, *J* = 7.2 Hz); ^{13}C ($CDCl_3$+ DMSO-d_6, 75 MHz): 165.12, 163.37, 146.34, 140.59, 139.35, 134.63, 134.25, 132.07, 128.82, 127.96, 127.14, 120.45, 119.96, 119.64, 117.48, 115.54, 113.84, 111.97, 72.68, 51.31, 42.52, 28.20, 18.93, 12.57; IR(KBr) 3247, 2926, 1721, 1637, 1218, 1102 cm^{-1}. HRMS: calcd for [$C_{25}H_{24}N_4O_3$+ H^+] 429.1921, found 429.1922.

Methyl-1-(3-cyclohexyl-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8e): Cream Solid (yield 67%). mp >240 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.54 (s, 1H), 8.79 (s, 1H), 8.39 (d, 1H, J = 7.7 Hz), 7.76-7.62 (m, 3H), 7.35 (t, 1H, J = 7.4 Hz), 7.20 (s, 1H), 7.08 (t, 1H, J = 6.8 Hz), 6.67-6.65 (m, 2H), 6.53 (d, 1H, J = 7.6 Hz), 4.42-4.40 (m, 1H), 3.77 (s, 3H), 1.80-1.76 (m, 4H), 1.49-1.47 (m, 2H), 1.27-1.24 (m, 4H); ^{13}C (DMSO-d_6, 75 MHz): 165.60, 162.53, 144.78, 143.29, 140.79, 135.33, 132.84, 131.88, 128.59, 127.30, 122.05, 121.00, 120.37, 118.30, 117.40, 117.27, 114.18, 112.30, 63.21, 51.94, 51.83, 30.56, 30.00, 25.51, 24.99; IR (KBr) 3324, 3021, 1725, 1618, 1216, 1136 cm^{-1}. HRMS: calcd for [$C_{27}H_{26}N_4O_3$ + H^+] 455.2078, found 455.2077.

Methyl-1-(3-(4-methoxyphenyl)-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8f): Cream Solid (yield 65%). mp 238-239 °C;^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.07 (s, 1H), 8.82 (s, 1H), 8.35 (d, 1H, J = 7.8 Hz 7.81 (d, 1H, J = 6.90 Hz), 7.74 (d, 1H, J = 8.10 Hz), 7.62 (m, 1H), 7.48 (s, 1H), 7.32 (t, 2H, J = 7.5 Hz), 7.20 (d, 2H, J = 8.7 Hz), 6.82-6.78 (m, 3H), 6.66 (d, 2H, J = 8.8 Hz), 3.88 (s, 3H), 3.57 (s, 3H); ^{13}C (DMSO-d_6, 75 MHz): 166.16, 164.13, 157.82, 147.56, 141.49, 135.59, 134.72, 133.61, 133.29, 129.42, 129.25, 128.42, 122.44, 121.09, 120.83, 118.49, 118.35, 116.95, 115.33, 113.75, 113.08, 74.17, 55.49, 52.47; IR(KBr) 3352, 3021, 1723, 1630, 1216, 1134 cm^{-1}. HRMS: calcd for [$C_{28}H_{22}N_4O_4$ + H^+] 479.1714, found 479.1714.

Methyl-1-(3-cyclopropyl-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8g): Cream Solid (yield 71%). mp >240°C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.14 (s, 1H), 8.86 (s, 1H), 8.41 (d, 1H, J = 7.8 Hz), 7.76-7.62 (m, 3H), 7.36-7.28 (m, 2H), 7.15 (t, 1H, J = 7.7 Hz), 6.69-6.62 (m, 2H), 6.46 (s, 1H), 3.83 (s, 3H), 2.57-2.54 (m, 1H), 0.85-0.73

(m, 2H), 0.61-0.47 (m, 2H); IR(KBr) 3433, 3019, 1721, 1615, 1216, 1103 cm^{-1}. HRMS: calcd for $[C_{24}H_{20}N_4O_3 + H^+]$ 413.1608, found 413.1608.

Methyl-1-(3-cycloheptyl-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8h): Cream Solid (yield 67%). mp >240 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.20 (s, 1H), 8.81(s, 1H), 8.39 (d, 1H, J = 7.7 Hz), 7.76-7.61 (m, 3H), 7.35 (t, 1H, J = 7.1), 7.15-7.11(m, 2H), 6.69 (t, 1H, J = 7.5 Hz), 6.57-6.53 (m, 2H), 4.24 (s, 1H), 3.79 (s, 3H), 2.07-1.85 (m, 4H), 1.62-1.23 (m, 8H) ; ^{13}C (DMSO-d_6, 75 MHz): 166.13, 162.82, 145.70, 143.49, 141.41, 135.93, 133.67, 132.42, 129.25, 127.79, 122.54, 121.45, 120.87, 118.75, 118.00, 117.83, 114.71, 112.88, 65.74, 55.83, 52.33, 33.73, 31.84, 27.52, 27.38, 25.34, 25.21; IR (KBr) 3438, 3020, 1719, 1615, 1216, 1130 cm^{-1}. HRMS: calcd for $[C_{28}H_{28}N_4O_3 + H^+]$ 469.2234, found 469.2234.

Methyl-1-(3-cyclopentyl-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8i): Cream Solid (yield 69%). mp >240 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.18 (s, 1H), 8.82 (s, 1H), 8.39 (d, 1H, J = 7.8 Hz), 7.75-7.71 (m, 2H), 7.66-7.62 (m, 1H), 7.35 (m, 1H), 7.21 (d, 1H, J = 3.00 Hz), 7.11-7.06 (m, 1H), 6.68-6.64 (m, 1H), 6.58-6.55 (m, 2H), 4.60-4.58 (m, 1H), 3.80 (s, 3H), 1.89-1.64 (m, 3H), 1.49-1.33 (m, 5H); ^{13}C (DMSO-d_6, 100 MHz): 166.12, 163.57, 145.49, 143.44, 141.34, 135.93, 133.65, 132.52, 129.27, 129.19, 127.78, 122.56, 121.46, 120.90, 118.58, 118.05, 117.88, 114.77, 112.85, 65.54, 55.33, 52.36, 29.74, 29.11, 24.03, 23.46; IR (KBr) 3317, 3021, 1727, 1620, 1216, 1136 cm^{-1}. HRMS: calcd for $[C_{26}H_{24}N_4O_3 + H^+]$ 441.1921, found 441.1921.

Methyl-1-(3-ethyl-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8j): Cream Solid (yield 71%). mp 237-238 °C;^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.08 (s, 1H), 9.02 (s, 1H), 8.48 (d, 1H, J = 7.9 Hz), 7.81-7.76 (m, 2H), 7.69 (t, 1H, J = 7.2 Hz),

7.40-7.25 (m, 3H), 6.82-6.72 (m, 2H), 6.44 (s, 1H), 3.92 (s, 3H), 3.76-3.64 (m, 1H), 2.80-2.68 (m, 1H), 0.94 (t, 3H, J = 6.9 Hz); ^{13}C (DMSO-d_6, 75 MHz): 165.86, 163.53, 147.16, 141.33, 141.10, 135.67, 134.75, 132.93, 129.36, 129.11, 127.67, 122.18, 120.87, 120.62, 118.40, 117.78, 116.25, 114.63, 112.84, 71.33, 52.18, 12.79; IR(KBr) 3321, 3021, 1729, 1688, 1215, 1100 cm^{-1}. HRMS: calcd for [$C_{23}H_{20}N_4O_3 + H^+$] 401.1608, found 401.1608.

Methyl-1-(3-(4-chlorophenyl)-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8k): Cream Solid (yield 62%). mp 226-228 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.08 (s, 1H), 8.83 (s, 1H), 8.36 (d, 1H, J = 8.0 Hz), 7.83 (d, 1H, J = 8.0 Hz), 7.74 (d, 1H, J = 8.1 Hz), 7.63-7.56 (m, 2H), 7.35-7.28 (m, 4H), 7.19 (d, 2H, J = 8.4 Hz), 6.87-6.79 (m, 3H), 3.89 (s, 3H); ^{13}C (DMSO-d_6, 75 MHz): 166.14, 163.94, 147.50, 141.52, 141.10, 139.41, 135.58, 134.61, 133.96, 131.02, 129.78, 129.59, 129.44, 128.47, 122.50, 121.06, 120.96, 118.64, 116.79, 115.59, 113.75, 113.11, 73.85, 52.55; IR(KBr) 3322, 2922, 1707, 1620, 1253, 1100 cm^{-1}. HRMS: calcd for [$C_{27}H_{19}ClN_4O_3 + H^+$] 483.1218, 485.1201, found 483.1217, 485.1206.

Methyl-1-(3-(4-fluorophenyl)-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8l): Cream Solid (yield 65%). mp 215-217 °C; ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ 12.07 (s, 1H), 8.83 (s, 1H), 8.35 (d, 1H, J = 8.2 Hz), 7.85 (d, 1H, J = 7.4 Hz), 7.76 (d, 1H, J = 8.2 Hz), 7.64 (t, 1H, J = 7.4 Hz), 7.55 (s, 1H), 7.35-7.30 (m, 4H), 6.98 (t, 2H, J = 8.5 Hz), 6.84-6.82 (m, 3H), 3.89 (s, 3H); ^{13}C (DMSO- d$_6$, 75 MHz): 166.12, 160.51 (d, J_{1CF} = 241.5 Hz), 158.90, 147.62, 141.50, 141.16, 136.69, 135.57, 134.68, 133.84, 130.18, 130.07, 129.52, 129.37, 128.48, 122.46, 121.05, 120.88, 118.55 (d, J_{3CF} = 4.5 Hz), 116.79, 115.38, 115.28 (d, J_{2CF} = 30.7 Hz), 113.09, 74.17, 52.50; IR(KBr) 3322, 3020, 1725, 1621, 1216, 1103 cm^{-1}. HRMS: calcd for [$C_{27}H_{19}FN_4O_3 + H^+$] 467.1514, found 467.1513.

Methyl-1-(3-(4-methoxybenzyl)-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8m): Cream Solid (yield 64%). mp 231-233 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 11.85 (s, 1H), 8.86 (s, 1H), 8.36 (d, 1H, *J* = 7.9 Hz), 7.82-7.80 (m, 1H), 7.66-7.57 (m, 2H), 7.32-7.28 (m, 2H), 7.25-7.21 (m, 1H), 6.92 (d, 2H, *J* = 8.5 Hz), 6.79 (t, 1H, *J* = 7.2 Hz), 6.71 (d, 1H, *J* = 8.0 Hz), 6.57 (d, 2H, *J* = 8.6 Hz), 6.34 (s, 1H), 4.81 (d, 1H, *J* = 15.2 Hz), 4.06 (d, 1H, *J* = 15.2 Hz), 3.88 (s, 3H)**,** 3.51 (s, 3H); ^{13}C (DMSO- d_6, 100 MHz): 166.22, 164.17, 158.46, 147.13, 141.53, 141.22, 135.75, 135.16, 133.32, 129.72, 129.49, 129.23, 128.82, 128.17, 127.84, 122.35, 121.15, 120.70, 118.56, 118.12, 116.49, 115.04, 113.84, 112.96, 70.97, 55.28, 52.47, 46.76; HRMS: calcd for [$C_{29}H_{24}N_4O_4 + H^+$] 493.1870, found 493.1870.

Methyl-1-(3-(3,4-dichlorobenzyl)-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8n): Cream Solid (yield 66%). mp 199-200 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 11.84 (s, 1H), 8.86 (s, 1H), 8.34 (d, 1H, *J* = 8.0 Hz), 7.84 (m, 1H), 7.64 (d, 1H, *J* = 8.2 Hz), 7.60-7.56 (m, 1H), 7.43 (s, 1H), 7.31-7.26 (m, 2H), 7.09 (d, 1H, *J* = 8.2 Hz), 7.02-7.01 (m, 1H), 6.85-6.79 (m, 2H), 6.76 (d, 1H, *J* = 8.1 Hz), 6.37 (s, 1H), 4.51 (d, 1H, *J* = 15.6 Hz), 4.39 (d, 1H, *J* = 15.6 Hz), 3.90 (s, 3H); ^{13}C (DMSO- d_6, 75 MHz): 166.17, 164.61, 147.65, 141.49, 140.70, 139.10, 135.35, 133.64, 130.68, 129.92, 129.65, 129.61, 129.49, 129.27, 128.26, 127.88, 122.26, 120.95, 120.77, 118.66, 118.32, 116.07, 115.23, 112.97; IR(KBr) 3250, 3020, 1719, 1631, 1216, 1133 cm^{-1}. HRMS: calcd for [$C_{28}H_{20}Cl_2N_4O_3 + H^+$] 531.0985, 533.0963, found 531.0984, 533.0964.

Methyl-1-(3-pentyl-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (8o): Cream Solid (yield 70%). mp 224-225 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.05 (s, 1H), 8.97 (s, 1H), 8.44 (d, 1H, *J* = 7.9 Hz), 7.76-7.73 (m, 2H), 7.65-7.61 (m, 1H), 7.36-7.31(m, 2H), 7.25-7.21 (m, 1H), 6.78-6.69 (m, 2H), 6.39 (s, 1H), 3.87 (s, 3H), 3.69-3.62 (m, 1H),

2.67-2.60 (m, 1H), 1.26-1.23 (m, 3H), 1.05-1.01 (m, 3H), 0.67 (t, 3H, J = 6.9 Hz) ; ^{13}C (DMSO-d_6, 75 MHz): 165.37, 163.61, 146.64, 140.91, 139.82, 134.49, 132.28, 129.01, 128.23, 127.36, 120.84, 120.29, 119.92, 117.60, 115.88, 114.18, 112.27, 72.43, 51.53, 43.14, 28.16, 26.05, 21.23, 13.21; IR(KBr) 3427, 3019, 1722, 1637, 1216, 1104 cm^{-1}. HRMS: calcd for [$C_{26}H_{26}N_4O_3$ + H^+] 443.2078, found 443.2078.

Experimental section for docking analysis

Material and Method:

The sequence of enzyme Trypanothione reductase of *L .donovani* (LdTR) was retrieved from Uniprot (ID-P39050). Blastp was performed to find out probable templates for the given protein. The X-ray structure of the *Leishmania infantum* trypanothione reductase in complex with auranofin was used as the template [PDB Identifier: 2YAU].[5] Ten *L. donovani* TR models were built with the Modeller-9.9 package.[6] For molecular visualization Chimera software was used.[7] The compounds were drawn using sketch module of Syby7.1 and energy minimized using MMFF94 force field with 1000 iterations.[8] All docking studies were performed with the use of the Autodock 4.2.[9]

The LdTR has 98% of sequence identity with template and the superimposition of the modelled protein with template shows a RMSD of 0.269Å (Fig 1). Validation of the resulting model was done with the SAVS server.[10] The majority of the residues (91.7%) occupy the most favored region of Ramachandran Plot generated by PROCHECK [11] and 7.6%, 0.7% and 0.0% residues lie in additional allowed region, generously allowed and disallowed region respectively (Fig 2).

The enzyme in its active form is a homo dimer consisting of two identical chains each consists of 491 residues.[12] The catalytic site of the enzyme include two redox active cysteine

residues (Cys52 and Cys57) and one histidine residue (His461) that are involved in anchoring of trypanothionedisulfide at the active site[5,12] The trypanothione binding site that is involved in the binding of an inhibitor also[12] was selected as the active site for docking of these molecules. The active site residues selected for docking studies include Glu18, Cys52, Val53, Cys57, Lys61 and Tyr110 from chain A and Ser394, Met400, Asn402, His461, Ser464, Glu466, Glu467 and Ser470 from B chain. The top scoring docked conformations of all the ligands in the active site of homology model of LdTR were analyzed in terms of key residues involved in the interaction and their preferred mode of binding. The docking energies of docked conformation are given in supplementary table 1. Fig 3a shows all compounds in the binding pocket of protein. It can be seen from the figure that all the ligands are docked bound inside cavity which is formed at the interface of chain A and chain B. Fig 3b, 3c and 3d show the binding mode of compound **8k**, **8l** and **8m** respectively. As it can be seen from the figures all the compounds are involved in hydrogen bond formation with His461 and Glu466. Both of these residues play important role in catalytic activity of the enzyme. All these interactions are known to play important role in substrate binding; therefore they might contribute to inhibition activity of these compounds.

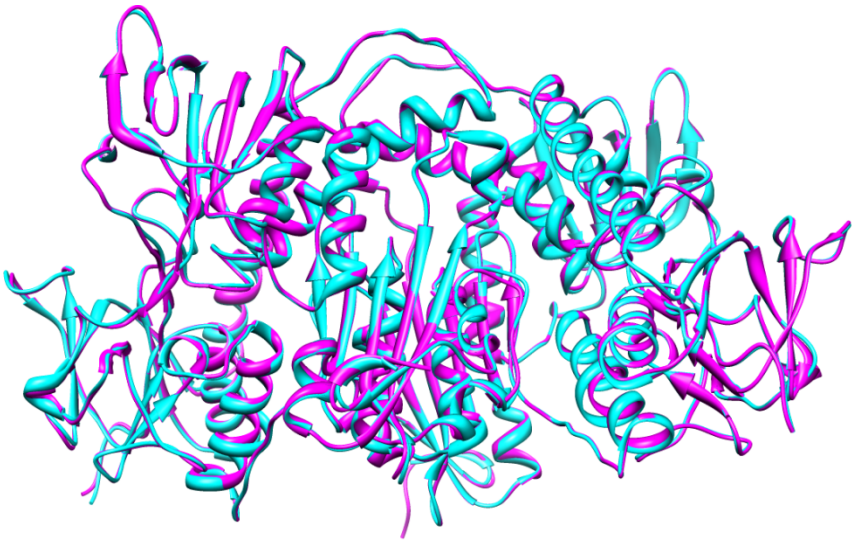


Fig 1: Superimposed structures of LdTR (*cyan*) and template 2YAU (*magenta*)

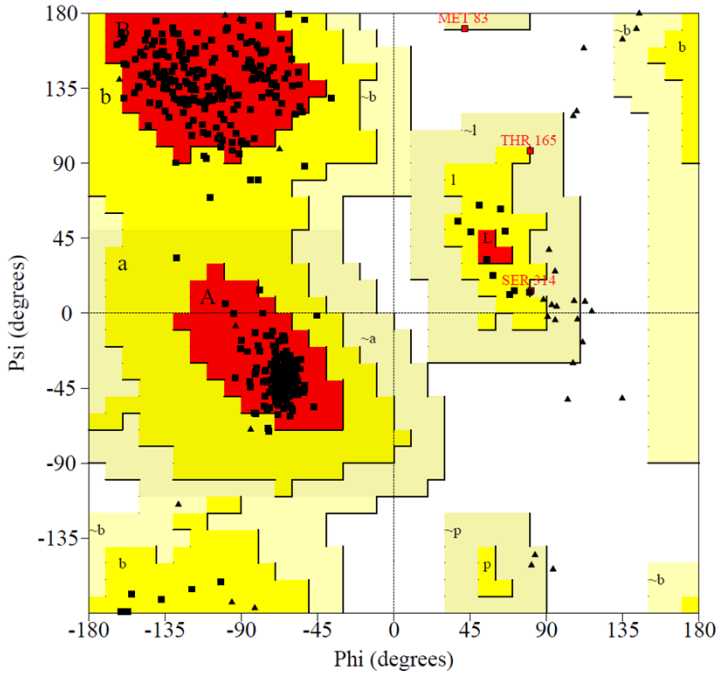


Fig 2: Ramachandran Map of modeled protein. The different colored areas indicate "disallowed" (*white*), "generously allowed" (*light yellow*), "additional allowed" (*yellow*), and "most favored" (*red*) regions.

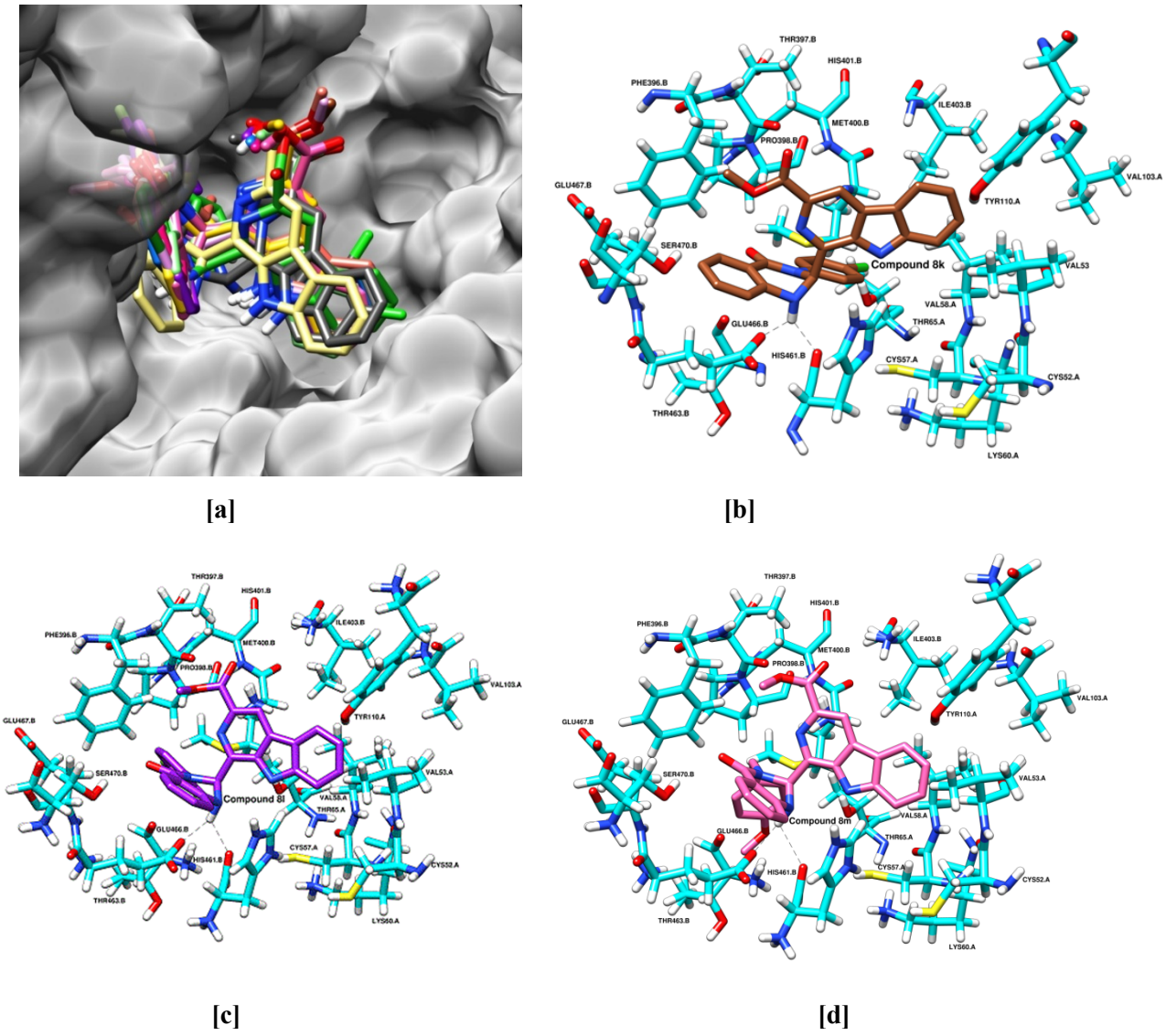


Fig 3: [a] Overlay of inhibitors in the binding site of modelled LdTR protein [b] Interaction of Compound **8k** (*Brown*) [c] Interaction of Compound **8l** (*Purple*) [d] Interaction of Compound **8m** (*Pink*)

Table 1: Docking energies of compounds **8j-8o**

Compound	Docking Energy(kcal/mol)
8a	-9.01
8b	-9.18
8c	-8.31
8d	-8.38
8e	-9.24
8f	-9.09
8g	-8.42
8h	-9.33
8i	-9.33
8j	-8.83
8k	-8.12
8l	-8.57
8m	-8.75
8n	-8.52
8o	-9.15

Experimental Procedures for TR inhibition assays

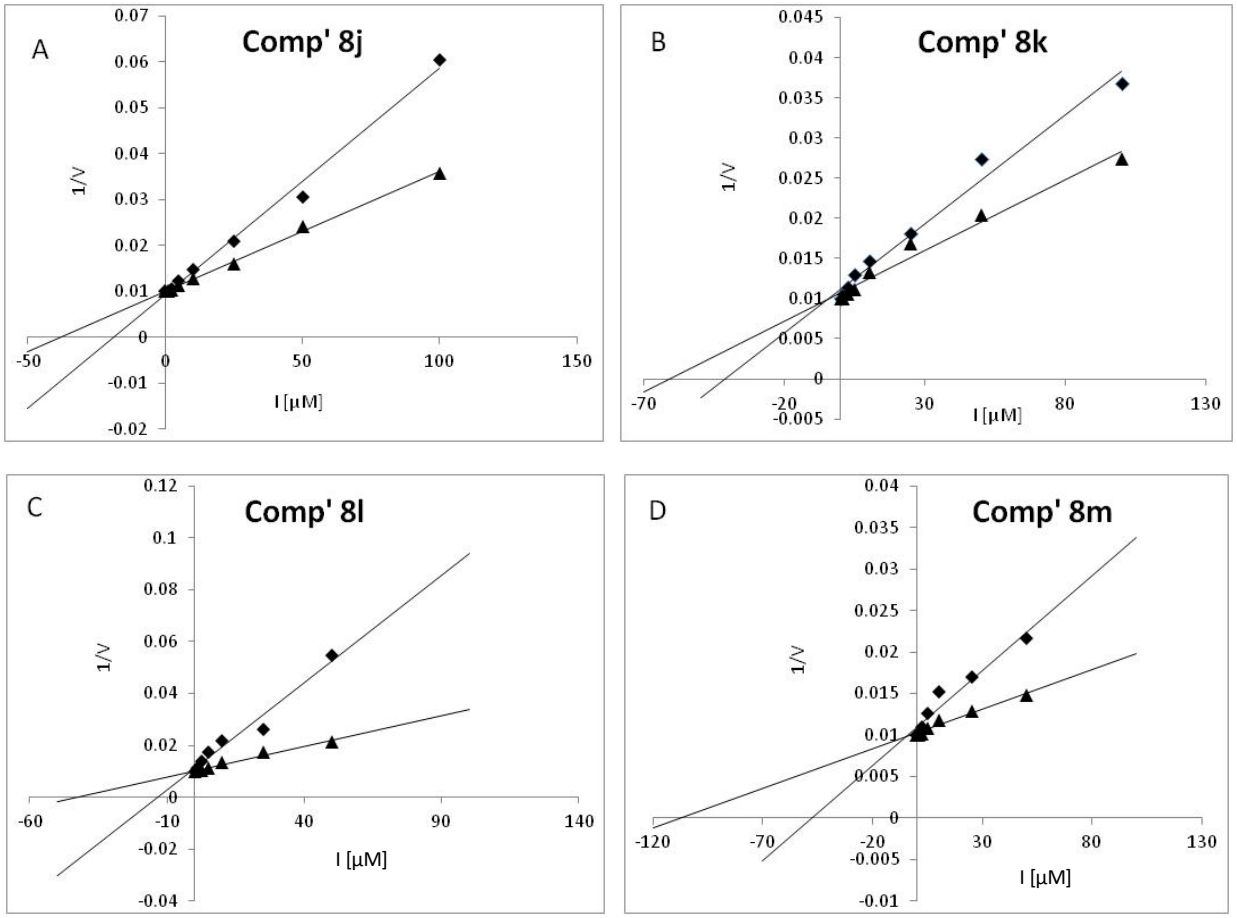
Materials and Methods

Trypanothione (TS_2) was purchased from Bachem, Switzerland. Glutathione sepharose-4B resin and thrombin were purchased from GE Healthcare Bio-Sciences Ltd. All other chemicals of the highest purity were purchased from Sigma Chemical Co. Stock solutions (10 mM) of the inhibitors were made in DMSO and stored at -20 °C.

Recombinant *Leishmania donovani* trypanothione reductase (LdTR) was expressed in *E. coli* BL21 (DE3) and purified to homogeneity as described earlier[13] and purity was checked by SDS-PAGE.[14] Enzyme activity was measured according to the method of Hamilton et al.[15] in presence of oxidized trypanothione (TS_2). Briefly, the reaction mixture contained *L.donovani* TR (1 m-unit), 40 mM HEPES pH 7.5, 1 mM EDTA, 0.15 mM NADPH, 25μM DTNB and 5μM TS_2. The reaction was initiated by the addition of oxidized trypanothione and the change in O.D. was monitored at 412 nm using Spectramx M2 (Molecular Devices). The protein concentration was determined by the Bradford method[16] using bovine serum albumin (BSA) as the standard. Primary screening of compounds for their inhibitory activity against LdTR activity was performed at varied concentration (10 - 100μM) of the compound(s). The compounds were added to enzyme in reaction mixture 5 min prior to the addition of the substrate. Control assays containing the respective amount of DMSO were carried out where appropriate. The compounds exhibiting dose dependent inhibition were evaluated further for their type of inhibition and inhibitor constant.

Determination of Inhibitor Constants

Inhibition of LdTR activity was determined in presence of varying concentrations of inhibitor and two substrate ($T[S]_2$) concentration (50 and 100μM). The type of inhibition and inhibitor constant (K_i) was derived from Dixon plot. Dixon plot is a graphical method for determination of the type of enzyme inhibition and the dissociation constant (K_i) for an enzyme-inhibitor complex.[17] The effect on the enzyme activity (v) is determined at two substrate concentrations (50 and 100 μM) and over a range of inhibitor concentrations (I). From the Dixon plots (figure 5) it is clear that all the six potential inhibitors are competitive type of inhibitors as the lines intersect on the y-axis, illustrating that such inhibitors do not affect V_{max}.



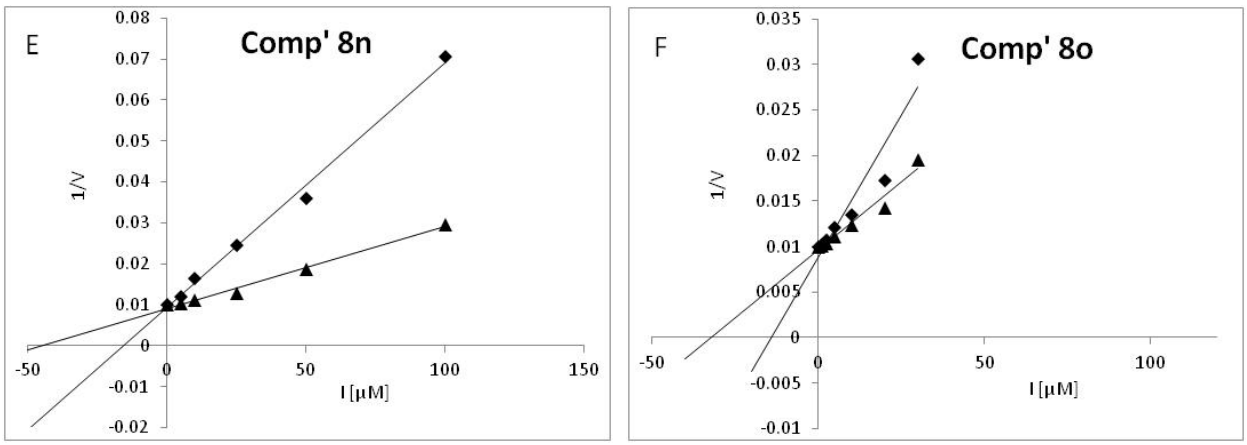


Figure 4: A Plot of reciprocal of velocity as function of concentration of inhibitor(s) at two substrate concentrations [(♦) 50 μM and (▲) 100 μM] to determine type of inhibition and *Ki* values. **A**: Dixon plot for compound **8j**; **B:** Dixon plot for compound **8k**; **C:** Dixon plot for compound **8l**; **D:** Dixon plot for compound **8m**; **E:** Dixon plot for compound **8n**; **F:** Dixon plot for compound **8o**. Points are experimental and lines are theoretical for linear competitive inhibition.

Experimental Procedures for *in vitro* Antileishmanial activity

***In vitro* antipromastigote assay:** The antileishmanial activity of these compounds on the extracellular promastigote form of *L. donovani* was assessed as described earlier.[18] The late log phase of promastigotes (expressing firefly luciferase gene) were seeded with complete M-199 medium at 5 x 10^5/mL/100μL/well in 96-well plates and incubated with tested compounds in a 24ºC incubator for 96 h. Miltefosine was used as a standard drug. After 96 h of incubation, 50 μL of promastigote suspension was pipette out from each well in to another 96-well plate and mixed with an equal volume of Steady Glo® reagent (Promega) and luminescence was measured by using a luminometer. The values were expressed as relative luminescence unit (RLU). The inhibition of parasitic multiplication is determined by comparison of the luciferase activity of compound treated parasites with that of untreated control.

***In vitro* antiamastigote assay**: For assessing the activity of compounds against the amastigote form of parasite, mouse macrophage cell line (J-774A.1) infected with WHO reference strain of

L. donovani promastigotes (expressing luciferase firefly reporter gene) was used. Cells (4 x 10^3/100µL/well) were seeded in a 96-well plate in RPMI-1640 medium containing 10% foetal calf serum and incubated at 37°c in a CO_2 incubator. After 24 h, the medium was replaced with fresh medium containing stationary phase promastigotes (4 x 10^5 /100µL/well). Promastigotes were phagocytized by the macrophages and inside the phagolysosomes, they were transformed into amastigotes (non-motile form). Each well of the plate was washed with plain RPMI medium after 24 h of incubation to remove the un-internalized promastigotes. The test compounds were added up to 7 points in complete medium starting from 40 µM conc. and the plates were incubated at 37°C in a CO_2 incubator for 72 h. After incubation, the drug containing medium was aspirated and 50µL PBS was added in each well and mixed with an equal volume of Steady Glo reagent®. After gentle shaking for 1-2 minute, the reading was taken in a luminometer. The values are expressed as relative luminescence units (RLU). 50% inhibitory concentration (IC_{50}) of the parasite growth was determined by comparison of luciferase activity of drug treated with that of untreated controls.[18]

Cytotoxicity assay: The cell viability was determined by following the method of Mosmann with slight modifications. Exponentially growing mammalian kidney fibroblast cells (Vero cell line) (1 x 10^5cells/100µL/well) were incubated with test compounds for 72 h. The test compounds were added at three fold dilutions up to 7 points in complete medium starting from 400 µM concentration, and were incubated at 37ºC in a CO_2 incubator. After incubation, 25 µL of MTT reagent (5mg/mL) in PBS was added to each well and incubated at 37°C for 2 h. At the end of the incubation period, the supernatant were removed by tilting plate completely without disturbing cell layer and 150 µL of pure DMSO was added to each well. After 15 min of shaking the readings were recorded as absorbance at 544 nm on a micro plate reader. The cytotoxic effect

expressed as 50% lethal dose (CC_{50} values) was estimated as described by Huber and Koella. The selectivity index (SI) for each compound was calculated as ratio between, cytotoxicity (CC_{50}) and activity (IC_{50}) against *Leishmania* amastigotes.[19,20]

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