Supporting Information

Discovery of Chalcone Derivatives as Antileishmanial Agents: Phenotypic Screening, Exploratory Docking and Enzymatic Assays on Trypanothione Reductase and Related Flavoproteins

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Content

Synthesis

***(E)*-3-(3-(2,4-Bis(prop-2-yn-1-yloxy)phenyl)-3-oxoprop-1-en-1-yl)-1-hexylpyridin-1-ium bromide (2)** [2]. Starting from **Lead 3** (0.23 g, 0.73 mmol), 1-Br-hexane (0.36 g, 0.30 mL, 2.19 mmol) and following the general procedure C, a crude product was obtained that was purified by FCC (gradient elution: first ethyl acetate, then ethyl acetate/methanol 9:1) affording **2** as a solid, mp: 104-105 °C, 0.18 g, 51% yield. ^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ; 9.45 (d, *J* = 1.6 Hz, 2H, H-2'), 9.07 (d, *J* = 6.0 Hz, 1H, H-4'), 8.92 (d, *J* = 8.2 Hz, 1H, H-6'), 8.21 (dd, *J* = 8.2, 6.0 Hz, 1H, H-5'), 7.88 (d, *J* = 16.0 Hz, 1H, α-C=CH), 7.70 (d, *J* = 8.6 Hz, 1H, H-6), 7.60 (d, *J* = 16.0 Hz, 1H, β-C=CH), 6.87 (d, *J* = 2.2 Hz, 1H, H-3), 6.80 (dd, *J* = 8.6, 2.2 Hz, 1H, H-5), 4.97 (d, *J* = 2.4 Hz, 2H, OCH_2), 4.93 (d, *J* = 2.4 Hz, 2H, OCH_2), 4.61-4.58 (m, 2H, CH_2N^+), 3.65 (t, *J* = 2.4 Hz, 1H, C≡CH), 3.62 (t, *J* = 2.4 Hz, 1H, C≡CH), 1.90-2.00 (m, 2H, CH_2), 1.34-1.24 (m, 6H, $(CH_2)_3$), 0.89-0.79 (m, 3H, CH_3). 13C NMR (151 MHz, DMSO-d6) δ (ppm) 188.61, 162.14, 158.37, 144.65, 144.59, 142.65, 135.32, 133.47, 133.08, 132.20, 128.04, 121.60, 107.56, 101.36, 79.22, 79.00, 78.78, 78.58, 61.16, 56.73, 56.02, 30.59, 30.44 25.09, 21.86, 13.87. HRMS (*m/z*): calc for $C_{26}H_{29}BrNO_3$ 482.1331; found 482.1330 $[M + H]^+$.

((*E*)-3-(3-(2,4-Bis(prop-2-yn-1-yloxy)phenyl)-3-oxoprop-1-en-1-yl)-1-dodecylpyridin-1-ium bromide (3) [2]. Starting from **Lead 3** [3] (0.20 g, 0.63 mmol), 1-Br-dodecane (0.20 g, 0.27 mL, 1.9 mmol) in acetonitrile (6.3 mL) and following the General Procedure C, a crude product was obtained that was purified by FCC (gradient elution: ethyl acetate and ethyl acetate/methanol 4:1) affording **3** as a solid, mp: 130-132 °C, 0.09 g, 25% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.45 (s, 1H, H-2'), 9.07 (d, *J* = 6.0 Hz, 1H, H-4'), 8.92 (d, *J* = 8.4 Hz, 1H, H-6'), 8.23-8.19 (m, 1H, H-5'), 7.88 (d, *J* = 16.0 Hz, 1H, α-C=CH), 7.70 (d, *J* = 8.8 Hz, 1H, H-6), 7.59 (d, *J* = 16.0 Hz, 1H, β-C=CH), 6.87 (d, *J* = 2.4 Hz, 1H, H-3), 6.80 (dd, *J* = 2.0, 8.8 Hz, 1H, H-5), 4.97 (d, *J* = 2.4 Hz, 2H, OCH_2), 4.03 (d, *J* = 2.0 Hz, 2H, OCH_2), 4.58 (t, *J* = 7.4, 2H, CH_2N^+), 3.62-3.52 (m, 2H, CH_2), 2.01-1.95 (m, 2H, C≡CH), 1.34-1.18 (m, 18H, $(CH_2)_9$), 0.85 (t, *J* = 6.4 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, CDCl3) δ: 187.73,162.96, 159.10, 144.24, 143.36, 142.78, 136.87, 135.02, 133.65, 131.53, 128.36, 121.57, 107.66, 100.76, 78.02, 77.53, 65.87, 62.57, 57.20, 56.14, 32.03 1, 31.92, 29.67, 29.64, 29.62, 29.58, 29.49, 29.36, 29.33, 29.07, 26.10, 22.69, 14.14. HRMS (m/z): calc for $C_{32}H_{41}BrNO_3$ 566.2270, found 567.2358 $[M + H]^+$.

(*E*)-1-(2,4-bis(prop-2-yn-1-yloxy)phenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one (4) [1]**.** Starting from **27a** (0.15 g, 0.66 mmol), 4-dimethylamino benzaldehyde (0.11 g, 0.72 mmol), EtOH (6.6 mL) piperidine (0.32 mL, 3.3 mmol), AcOH (0.19 mL, 3.3 mmol) and following General Procedure B2, compound **4** was obtained as red crystals, mp: 106-108 °C, 0.075 g, 32% yield. ^{1}H NMR (400 MHz, $CHCl_3$) δ: 8.04 (d, *J*= 8.8 Hz, 2H, H-3' and H-5'), 7.79 (d, *J*= 15.2 Hz, 1H, H-α), 7.56 (d, *J*= 8.8 Hz, 2H, H-2' e H-6'), 7.35 (d, *J*= 15.6 Hz, 1H, H-β), 7.63 (d, *J*= 9.2 Hz, 2H, H-2 e H-6), 6.71 (d, *J*= 8.4 Hz, 2H, H-3 e H-5), 4.78 (d, *J*= 2.8 Hz, 2H, OCH_2), 3.06 (s, 6H, CH_3), 2.57 (t, *J*= 2.4 Hz, 1H, C≡CH). ^{13}C NMR (400 MHz, $CHCl_3$) δ: 191.6, 164.21, 162.11, 150.01, 141.66, 133.47, 133.08, 131.12, 129.34, 129.14, 125.11, 121.36, 112.41, 112.36, 101.34, 79.27, 79.16, 78.78, 78.57, 56.64, 56.27, 41.28, 41.18. HRMS (*m/z*): calc for $C_{23}H_{21}NO_3$ 359.1521, found 360.1590 $[M+H]^+$.

***(E)*-1-(4-(prop-2-yn-1-yloxy)phenyl)-3-(pyridin-3-yl)prop-2-en-1-one (7)** [1]. Starting from **28a** (0.15 g, 0.86 mmol), 3-pyridinecarboxaldehyde (0.10 g, 0.95 mmol), $Ba(OH)_2$ (1.00 g. 5.9 mmol), EtOH (5.0 mL)**,** and following the General Procedure B3, a crude product was obtained that was purified by crystallization from EtOH, to afford analogue **7** as solid, mp: 87–88 °C, 0.10 g, 66.7 % yield. ^{1}H NMR (400 MHz, $CHCl_3$) δ: 8.87 (s, 1H, H-2), 8.64 (dd, *J* = 1.6 and 4.8 Hz, 1H, H-4'), 8.07 (d, *J* = 8.8 Hz, 2H, H-2 and H-6), 7.95 (m, 1H, H-5'), 7.80 (d, *J* = 15.6 Hz, 1H, α-CH=C), 7.61 (d, *J* = 16 Hz, 1H, β-CH=C), 7.37 (dd, *J* = 5.2 and 7.6 Hz, 1H, H-6'), 7.09 (d, *J* = 9.2 Hz, 2H, H-3 and H-5), 4.82 (d, *J* = 2.4 Hz, 2H, OCH_2), 2.58 (d, *J* = 2.0 Hz, 1H, CH).^{13}C NMR (101 MHz, $CDCl_3$) δ: 189.99, 163.25, 149.84, 142.03, 141.74, 132.16, 130.76, 130.45, 129.64, 123.87, 123.45, 114.65, 114.22, 87.91, 76.11, 56.98. HRMS (*m/z*): calc for $C_{17}H_{13}NO_2$ 263.0946, found: 263.1025 [M + 1].

***(E)*-3-(4-nitrophenyl)-1-(4-(prop-2-yn-1-yloxy)phenyl)prop-2-en-1-one (9)** [1]. Starting from **28a** (0.20 g, 1.15 mmol), 4-nitrobenzaldehyde (0.19 g, 1.26 mmol), KOH (0.22 g, 3.96 mmol), EtOH (3.3 mL) and following the General Procedure B1, compound **9** was obtained as a solid, mp: 103–105 °C, 0.06 g, 55 % yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.31 (d, *J* = 8.7 Hz, 2H, H-3' and H-5'), 8.09 (d, *J* = 8.8 Hz, 2H, H-2 and H-5), 7.84 (d, *J* = 15.7 Hz, 1H, α-CH=C), 7.81 (d, *J* = 8.7 Hz, 2H. H-2' and H-6'), 7.67 (d, *J* = 15.7 Hz, 1H, β-CH=C), 7.12 (d, *J* = 8.8 Hz, 2H, H-3 and H-5), 4.82 (d, *J* = 2.4 Hz, 2H, OCH_2), 2.60 (t, *J* = 2.4 Hz, 1H, C≡H). ^{13}C NMR (151 MHz, $CDCl_3$) δ: 189.60, 163.74, 147.64,145.98, 141.07, 130.54, 129.11, 123.87, 121.18, 114.77, 78.11, 76.54,

56.21. HRMS (*m/z):* calc for $C_{18}H_{13}NO_4$ 308.0923, found 309.1002 $[M + H]^+$**(*E*)-3-(4-fluorophenyl)-1-(2-methoxy-4-((3-methylbut-2-en-1-yl)oxy)phenyl)prop-2-en-1-one (12)** [3]**.** Starting from **27b** (0.23 g, 0.98 mmol) and 4-fluorobenzaldheyde (0.14 g, 1.1 mmol), KOH (0.06 g, 6.0 mmol), EtOH (5.0 mL). and following general procedure B1, a crude final product was obtained that was purified by purified by FCC (petroleum ether/ethyl acetate 9:1) and then crystallized from EtOH to obtain **12** as a white solid, mp: 130-132 °C, 0.12 g, 35% yield,. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 8.8 Hz, 1H, H-6), 7.65 (d, *J* = 16.0 Hz, 1H, α-CH=C), 7.57-7.60 (m, 2H, H-3' and H-5'), 1.78 (s, 3H, CH_3), 7.46 (d, *J* = 16.0 Hz, 1H, β-CH=C), 7.06-7.11 (m, 2H, H-2' and H-6'), 6.58 (dd, *J* = 1.6 Hz and 8.0 Hz, 1H, H-5), 6.53 (d, *J* = 1.6 Hz, 1H, H-3), 5.49-5.53 (m, 1H, CH), 4.59 (d, *J* = 6.4 Hz, 2H, OCH_2), 3.91 (s, 3H, OCH_3), 1.83 (s, 3H,CH3), 1.78 (s, 3H,CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 193.4, 168.2, 162.7, 145.9, 138.4, 131.2, 130.0 (d, J = 8.5 Hz), 124.6, 119.3 (d, *J* = 22 Hz), 118.9, 107.4, 103.8, 65.1, 55.7, 25.8, 18.2. ESI-MS (*m/z*): calcd for $C_{21}H_{21}FO_3$ 340.1475, found 341.1558 $[M + H]^+$.

(*E*)-1-(4-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(4-nitrophenyl)prop-2-en-1-one (13) [1]**.** Starting from **26b** (0.45 g, 1.22 mmol), 4-nitrobenzaldehyde (0.24 g, 1.6 mmol), $Ba(OH)_2$ (1.64 g, 9.6 mmol), EtOH (12 mL) and following general procedure B3, a crude product was obtained that was purified by crystallisation from EtOH, yielding **13** as a solid, mp: 100-102 °C, 0.26 g, 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.29 (d, *J* = 8.4 Hz, 2H, H-3' and H-5'), 8.07 (d, *J* = 9.2 Hz, 2H, H-2 and H-6), 7.90 (d, *J* = 15.6 Hz, 1H, α-CH=C), 7.79 (d, *J* = 8.0 Hz, 2H, H-2' and H-6'), 7.66 (d, *J* = 15.6 Hz, 1H, β-CH=C), 7.00 (d, *J* = 8.4 Hz, 2H, H-3 and H-5), 5.51 (t, *J* = 6.8 Hz, 1H, CH), 4.62 (d, *J* = 7.2Hz, 2H, OCH_2), 1.85 (s, 3H, CH_3), 1.79 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) 189.32, 163.21, 145.36, 138.11, 129.95, 129.45, 123.87, 121.08, 119.08, 114.63, 64.08, 24.11, 18.52. HRMS (*m/z*): calc for $C_{20}H_{19}NO_4$ 337.1314, found 338.1389 $[M+H]^+$.

(*E*)-1-(4-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(pyridin-3-yl)prop-2-en-1-one (14) [1]**.** Starting from **26b** (0.25 g, 1.22 mmol), 3-pyridinecarboxaldehyde (0.14 g, 1.34 mmol), $Ba(OH)_2$ (1.25 g, 7.32 mmol), EtOH (12 mL) and following the General Procedure B3, a crude product was obtained that was purified by crystallization from EtOH, to obtain **14** as solid, mp: 87-88 °C, 0.17 g, 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.87 (d, *J*= 2.0 Hz, 1H, H-2'), 8.63 (dd, *J*= 1.6 and 4.8 Hz, 1H, H-4'), 8.05 (d, *J*= 8.8 Hz, 2H, H-2 and H-6), 7.95 (dd, *J*= 1.6 and 7.6 Hz, 1H, H-4'), 7.79 (d, *J*= 15.6 Hz, 1H, α-CH=C), 7.62 (d, *J*= 15.6 Hz, 1H, β-CH=C), 7.37 (dd, *J* = 2.4 and 8.2

Hz, 1H, H-5'), 7.01 (d, *J* = 8.8 Hz, 2H, H-3 and H-5), 5.11 (t, *J* = 1.2 Hz, 1H, CH), 4.62 (d, *J* = 6.8 Hz, 2H, OCH_2), 1.83 (s, 3H, CH_3), 1.78 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 195.3 167.04, 163.54, 162.47, 162.18, 142.85, 131.64, 130.56, 130.31, 130.21, 128.64, 123.44, 118.21, 113.11, 105.74, 101.33, 72.15, 60.02, 55.87, 54.21. HRMS (*m/z*): calc for $C_{19}H_{19}NO_2$ 293.1416 found 294.1490 $[M+H]^+$.

(E)-1-(4-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(pyridin-4-yl)prop-2-en-1-one (15) [1]**.** Starting from **26b** (0.25 g, 1.22 mmol), 4-pyridinecarboxaldehyde (0.14 g, 1.34 mmol), $Ba(OH)_2$ (1.25 g, 7.32 mmol), EtOH (12 mL) and following the General Procedure B3, a crude product was obtained that was purified by crystallization from EtOH, to obtain **15** as solid mp: 91-93 °C, 0.41 g, 75% yield. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.69 (d, *J* = 4.2 Hz, 2H, H-3' and H-5'), 8.04 (d, *J*= 8.4 Hz, 2H, H-2 and H-6), 7.66 (s, 2H, H-α and H-β), 7.48 (d, *J*= 4.2 Hz, 2H, H-2' and H-6'), 7.01 (d, *J*= 8.8 Hz, 2H, H-3 and H-5), 5.51 (t, *J*= 6.8 Hz, 1H, CH), 4.62 (d, *J*= 7.2 Hz, 2H, OCH_2), 1.83 (s, 3H, CH_3), 1.78 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 191.66, 164.77, 149.16, 144.00, 138.08, 130.51, 127.88,123.74, 119.65, 114.22, 64.21, 21.41, 18.32, 18.57. HRMS (*m/z*): calc for $C_{19}H_{19}NO_2$ 293.1416 found 294.1488 $[M+H]^+$.

(*E*)-3-(4-fluorophenyl)-1-(4-(2-hydroxyethoxy)phenyl)prop-2-en-1-one (18) [1]**.** Starting from **26c** (0.18 g, 1.0 mmol) and 4-fluorobenzaldehyde (0.14 g, 1.1 mmol), KOH (0.06 g, 6.0 mmol), and following the general procedure B1, a solid was formed and collected by *vacuum* filtration and purified by FCC (Petroleum Ether/EtOAc 4:1), to give **18** as solid (0.19 g), 67% yield. ^1H NMR (acetone-d_6) δ: 8.12 (d, *J* = 6.8 Hz, 2H, H-2 and H-6), 7.83-7.88 (m, 2H, H-2' and H-6'), 7.81 (d, *J* = 15.6 Hz, 1H, α-CH=), 7.72 (d, *J* = 15.6 Hz, 1H, β-CH=), 7.20 (t, *J* = 8.8 Hz, 2H, H-3' and H-5'), 7.05 (d, *J* = 6.8 Hz, 2H, H-3 and H-5), 4.02-4.04 (br, 1H, OH), 4.18 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.92-3.96 (m, 2H, CH_2OH). ^{13}C NMR δ (acetone-d_6) δ: 168. 01, 160.21, 145.16, 131.45, 131.15, 130.97, 130.22, 129.65, 120.54, 116.80, 114.12, 69.9, 60.9. HRMS: calcd for $C_{17}H_{15}FO_3$ 287.1083, found 288.1154 [$[M+H]^+$.

***E*)-2-(4-fluorobenzylidene)-6-(prop-2-yn-1-yloxy)-3,4-dihydronaphthalen-1(2*H*)-one (21)** [4]**.** Starting from **33** (0.20 g, 1.0 mmol), 4-F-benzaldehyde (0.17 g, 1.1 mmol), KOH (0.34 g. 5.1 mmol), EtOH (5.1 mL) and following general procedure B1, a crude product was obtained that was purified by FCC (toluene), affording **21** as solid mp >250 °C, 0.153 g, 50%, yield. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, *J* = 8.8 Hz, 1H, H-7), 7.82 (s, 1H, =CH), 7.68 (dd, *J* = 6.0 Hz and 8.8 Hz, 2H, H-2' and H-6'), 7.34-7.41 (m, 2H, H-3' and H-5'), 7.13 (dd, *J* = 8.4 Hz and 2.4 Hz

1H, H-5), 7.05 (d, J = 2.4 Hz, 1H, H-5), 5.02 (d, J = 2.0 Hz, 2H, OCH_2), 3.21-3.27 (m, 2H, CH_2), 3.08 (t, J = 6.4 Hz, 2H, CH_2), 2.17 (t, J = 2.2 Hz, 1H,C≡CH). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 187.65, 137.98, 129.33, 130.76, 130.48, 130.43, 136.46, 131.28, 162.25, 166.02, 126.55, 115.74, 115.98, 113.54, 112.77, 56.09, 28.26, 27.72, 23.11, 16.07. HRMS (*m/z*): calc for $C_{20}H_{15}FO_2$ 306.1056. found 307.1155 $[M+H]^+$.

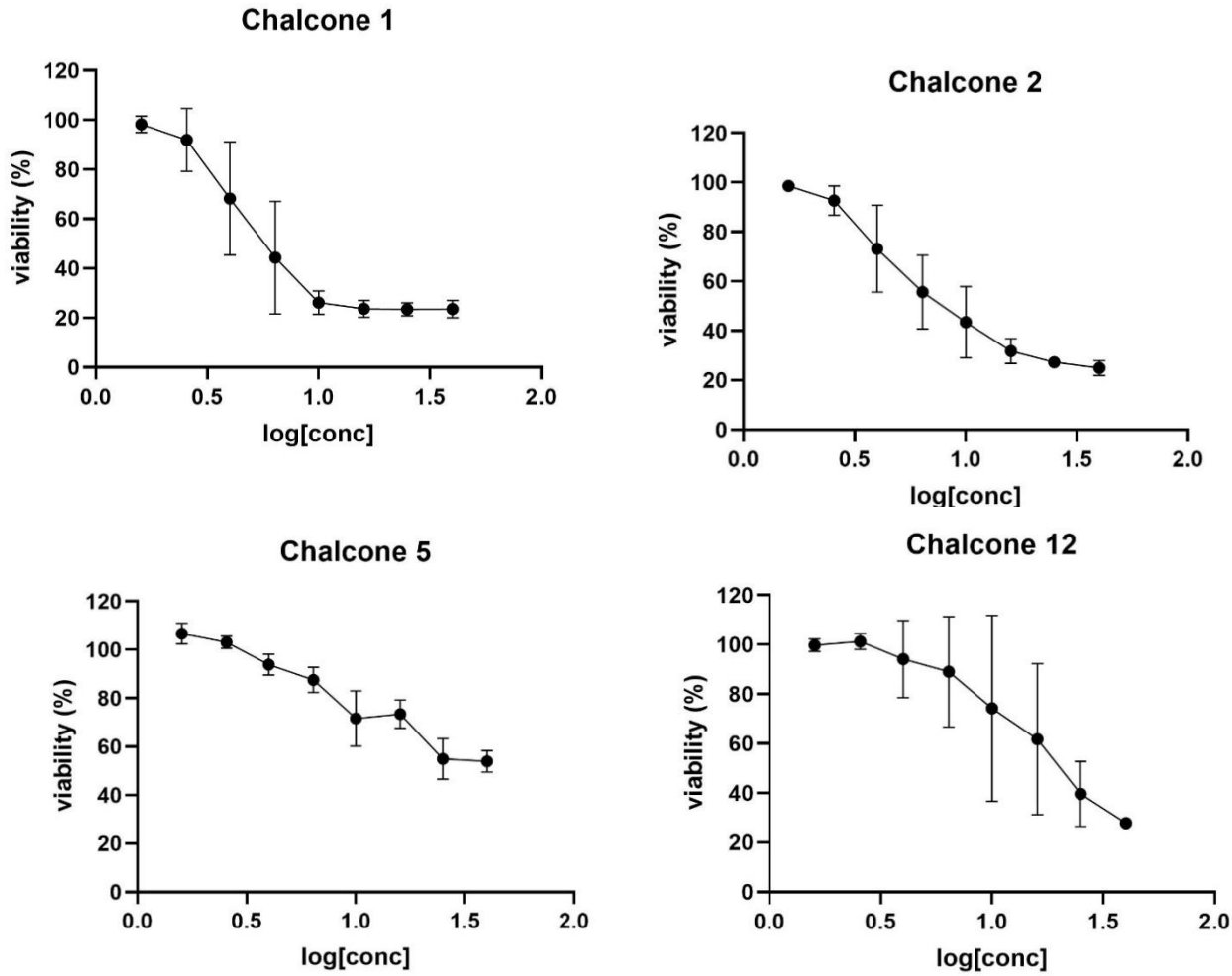

Figure S1. Dose-response curves for the determination of the inhibitory activity (IC_{50}) of the chalcones 1, **2**, **5**, and **12 against *L.infantum* promastigotes**. Four experimental replicates were carried out for each chalcone analogue.

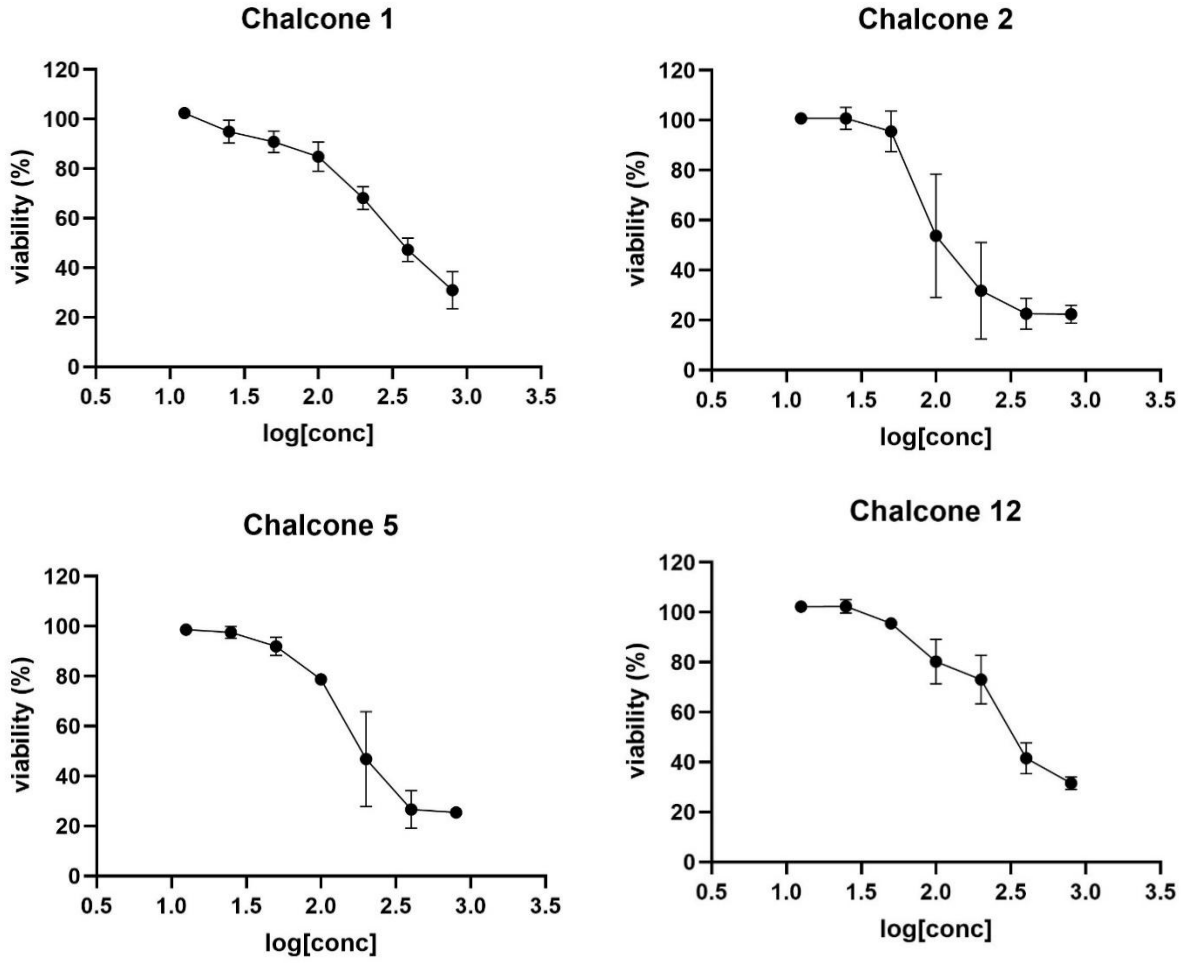

Figure S2. Dose-response curves for the determination of the cytotoxicity (CC_{50}) of the chalcones 1, **2**, **5**, and **12 in U937-derived human macrophages.** Two experimental replicates were carried out for each chalcone analogue.

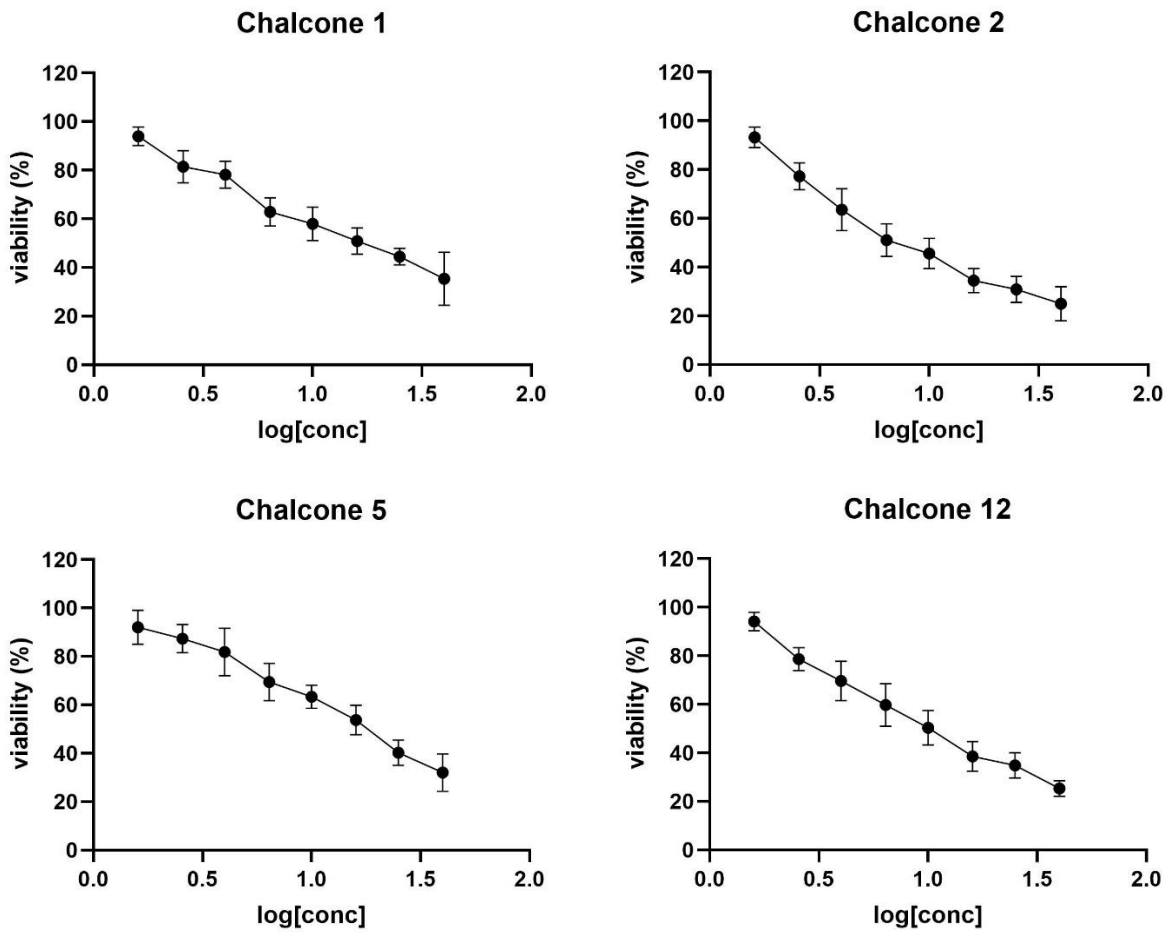

Figure S3. Dose-response curves for the determination of the inhibitory activity (IC_{50}) of the chalcones 1, **2**, **5**, and **12 against *L.infantum* amastigotes.** Three experimental replicates were carried out for each chalcone analogue.

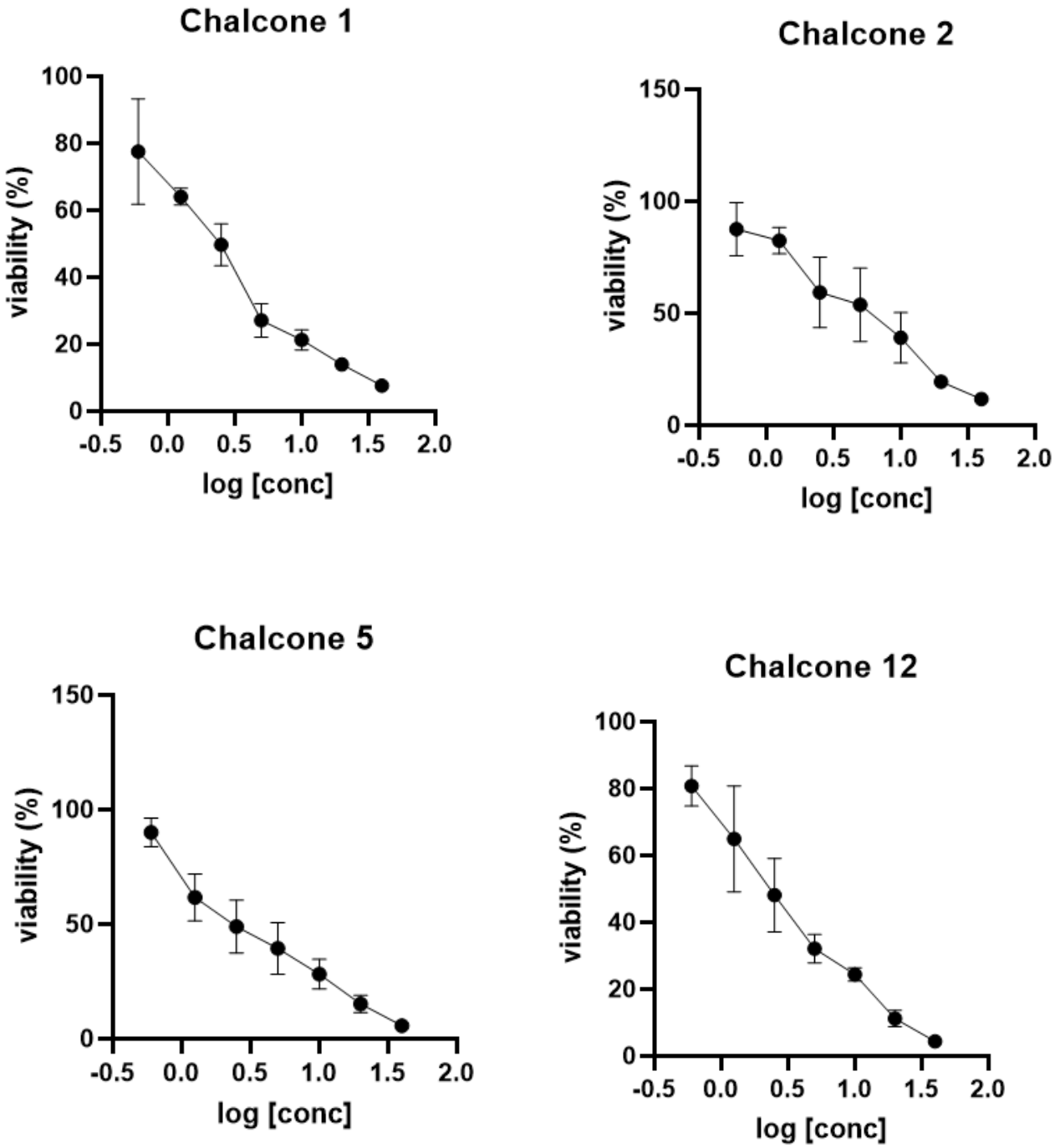

Figure S4. Dose-response curves for the determination of the inhibitory activity (IC_{50}) of the chalcones 1, **2**, **5**, and **12 against *L.donovani* amastigotes.** Two experimental replicates were carried out for each chalcone analogue.

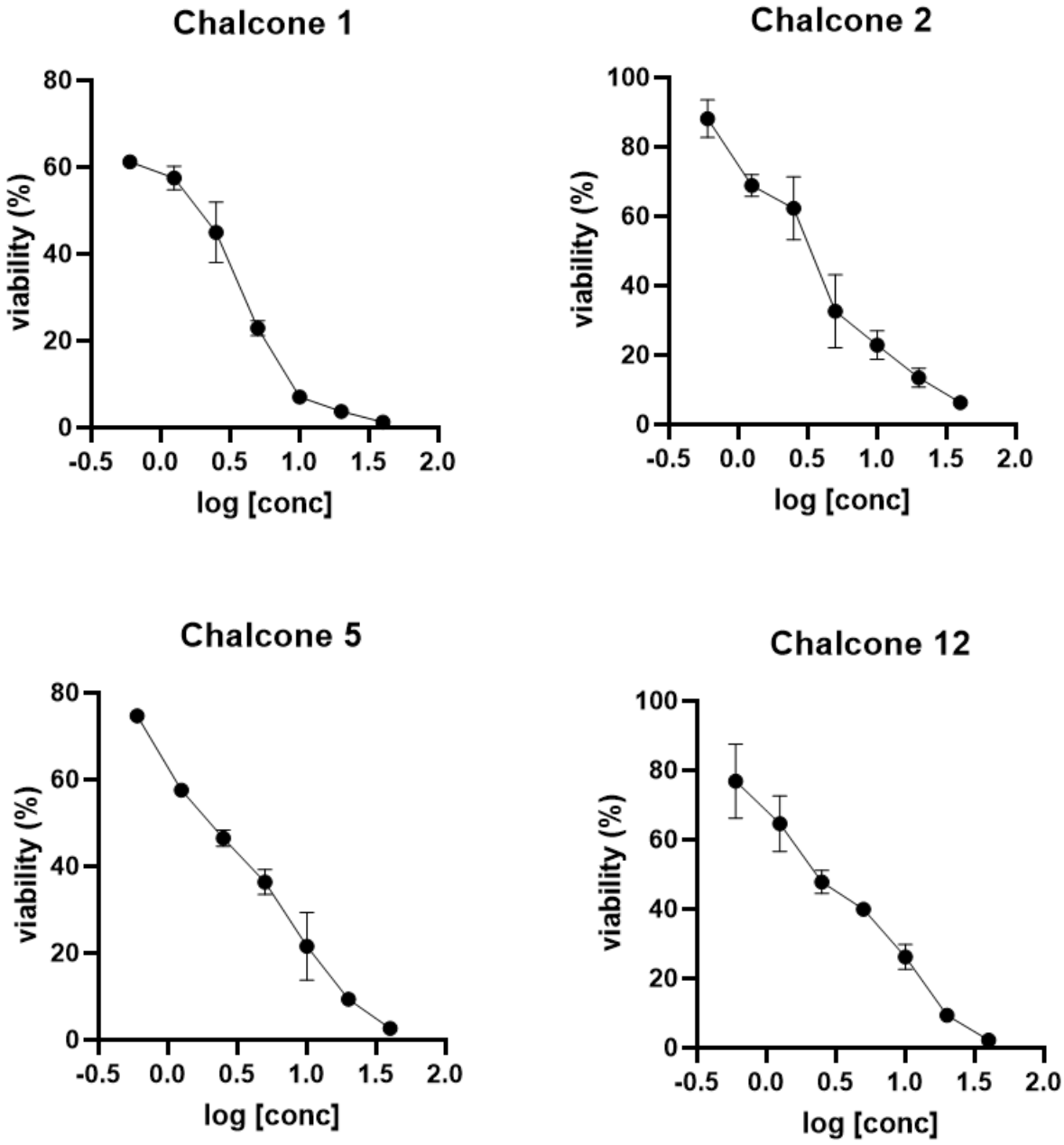

Figure S5. Dose-response curves for the determination of the inhibitory activity (IC_{50}) of the chalcones 1, 2, 5, and 12 against *L.tropica* amastigotes. Two experimental replicates were carried out for each chalcone analogue.

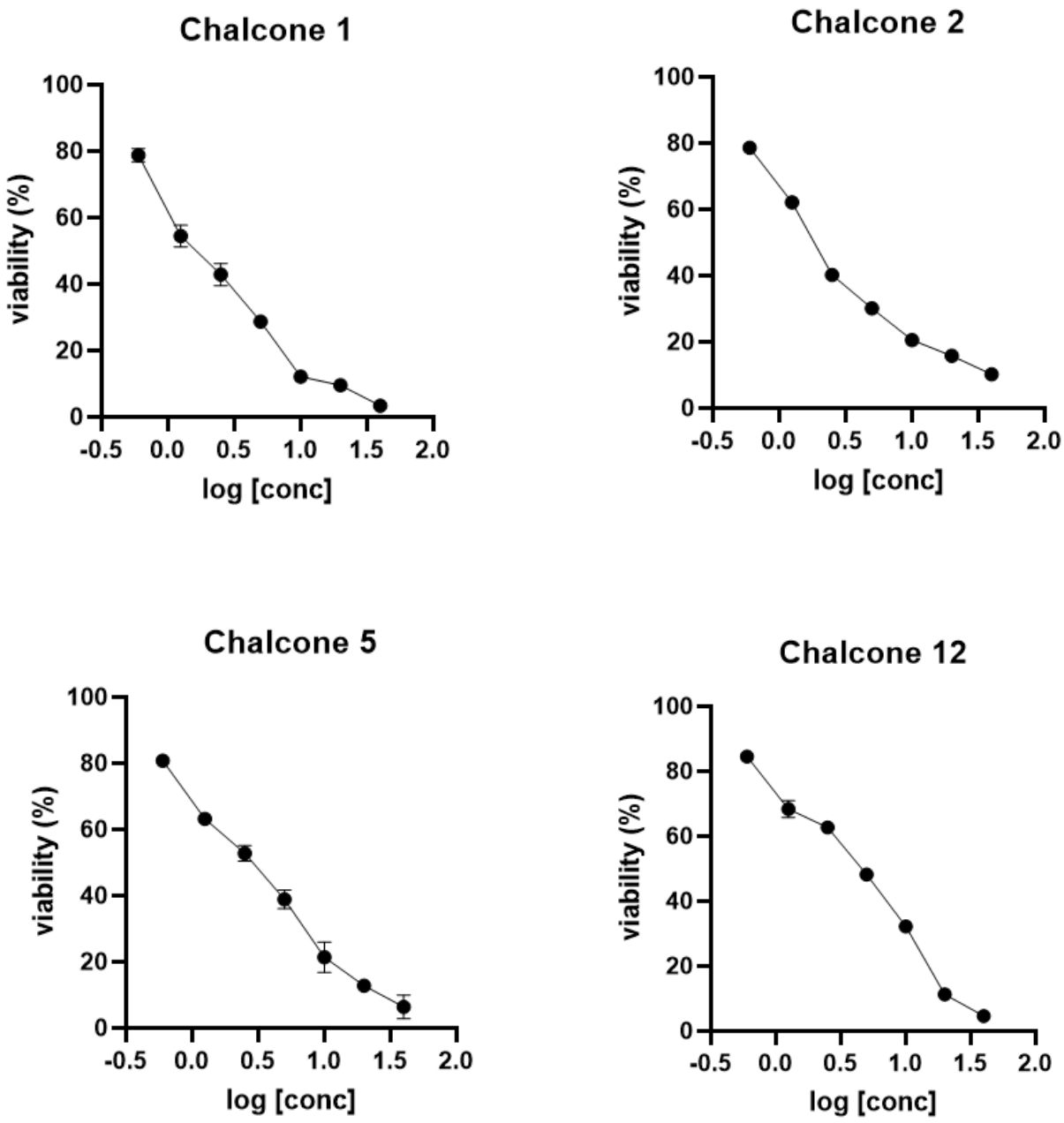

Figure S6. Dose-response curves for the determination of the inhibitory activity (IC_{50}) of the chalcones 1, 2, 5, and 12 against *L.major* amastigotes. Two experimental replicates were carried out for each chalcone analogue**.**

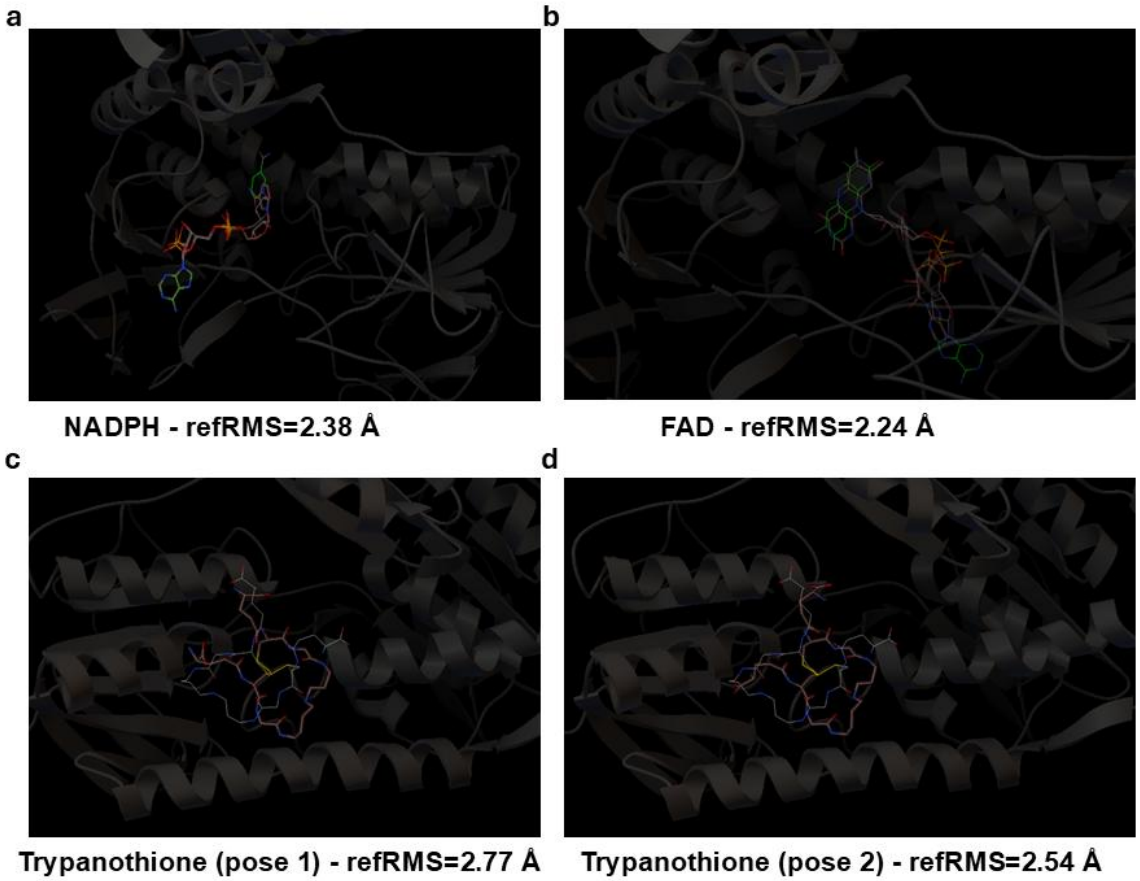

Figure S7. Redocking. Best-ranked docked poses of natural cofactors in *Leishmania infantum* trypanothione reductase (TR). (**a**) NADPH, (**b**) FAD, and (**c**) GCG are shown in their best-scoring docked conformations superimposed onto the corresponding crystallographic binding sites. (**d**) Second-best docked pose of trypanothione, displaying a slightly lower RMSD relative to the crystallographic reference compared with the pose shown in panel c. Docking poses were obtained using AutoDock4 and evaluated based on binding free energy and RMSD values calculated with respect to the experimental structures. Protein residues are shown as cartoon representation, while ligands are displayed as sticks.

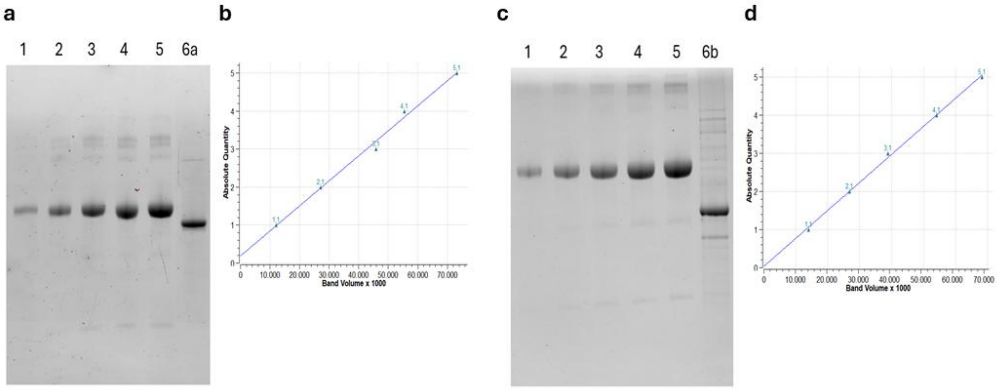

Figure S8. SDS-PAGE analysis for *Li*TR and *Ct*NDH2 quantification. (**a**), (**c**): 1-2-3-4-5 µg of BSA (lanes 1-5); purified *Li*TR 1 µl (lane 6a); purified CtNDH 2-5 µl (lane 6b). (**b**), (**d**): Standard curves calculation through ImageLab software.

Spectra copies of compounds 1, **2**, **5**, and **12.**

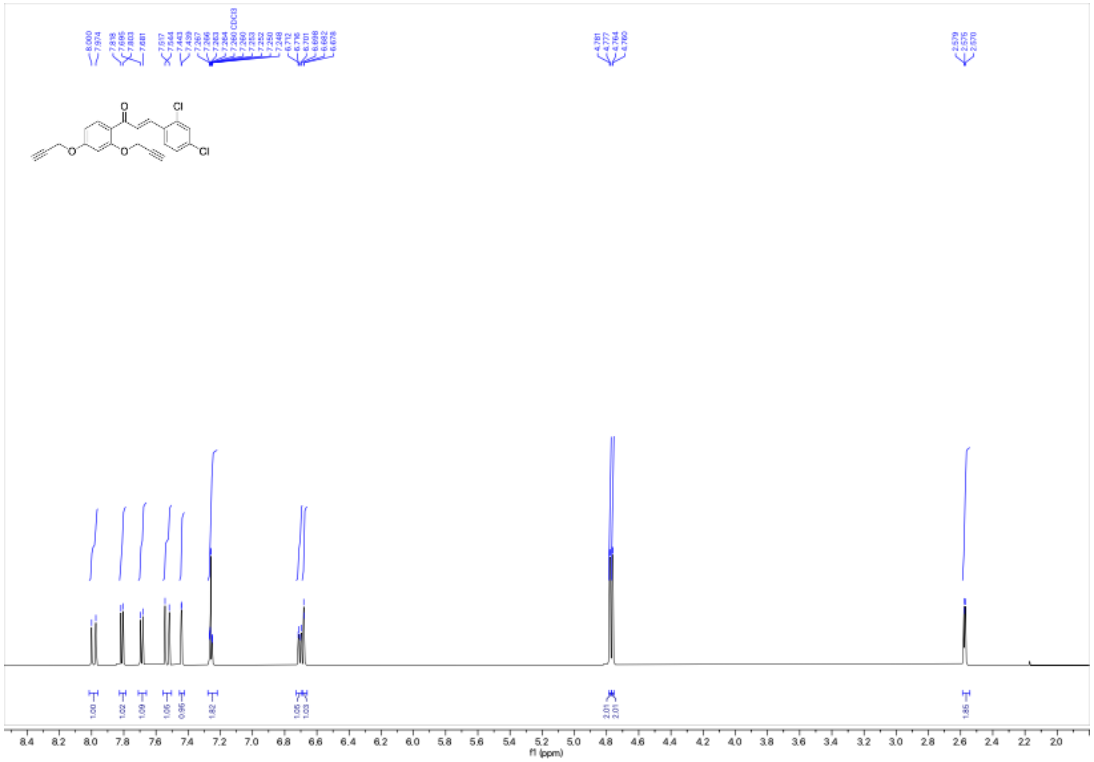

Figure S9. ^{1}H NMR spectra (600 MHz, $CDCl_3$) of compound **1**.

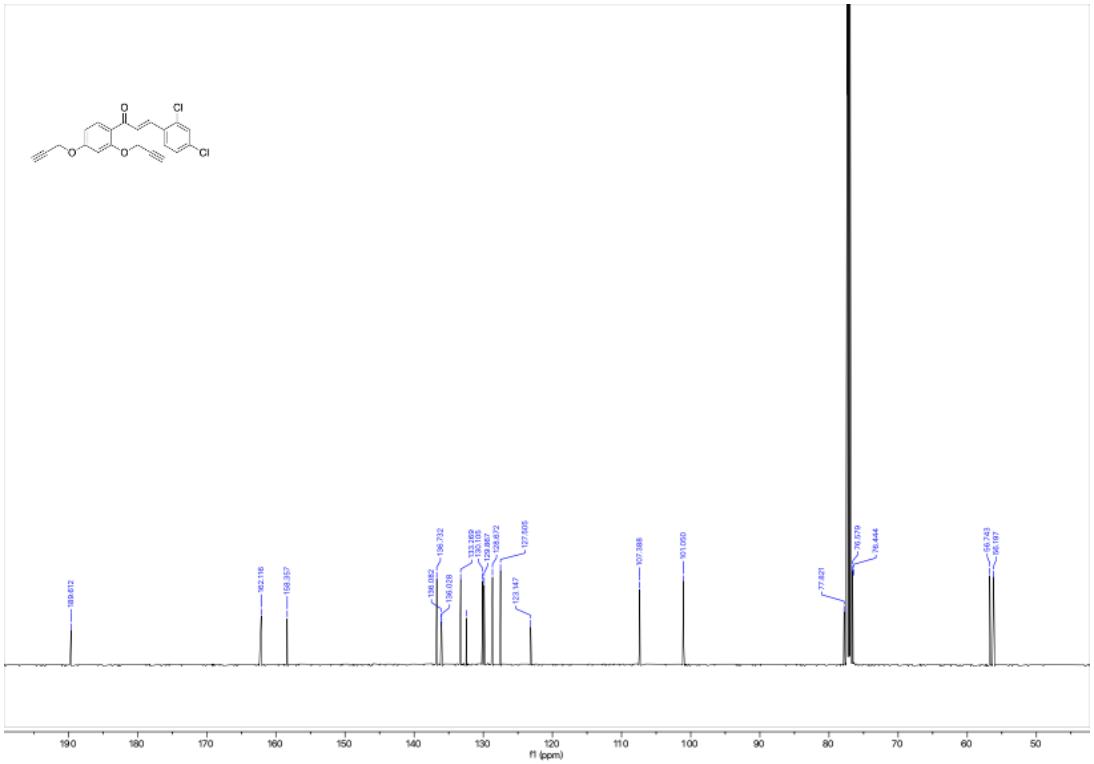

Figure S10. ^{13}C NMR spectra (600 MHz, $CDCl_3$) of compound **1**.

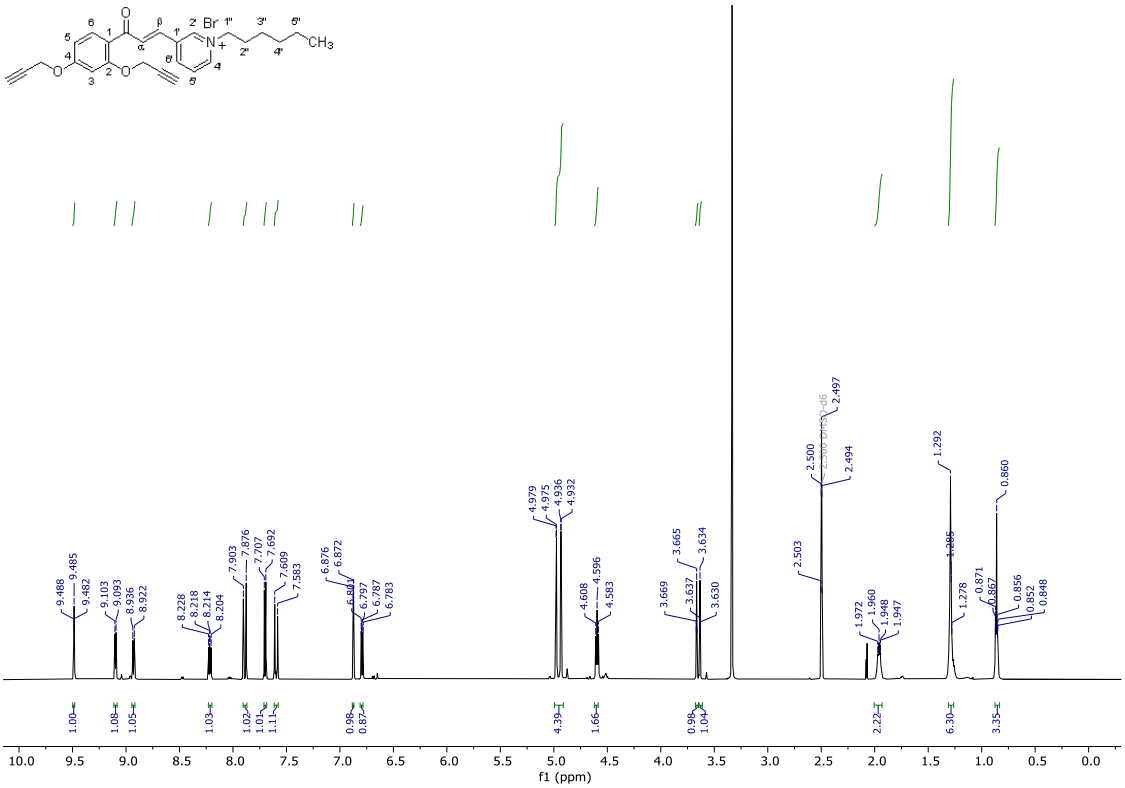

Figure S11. ^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **2**.

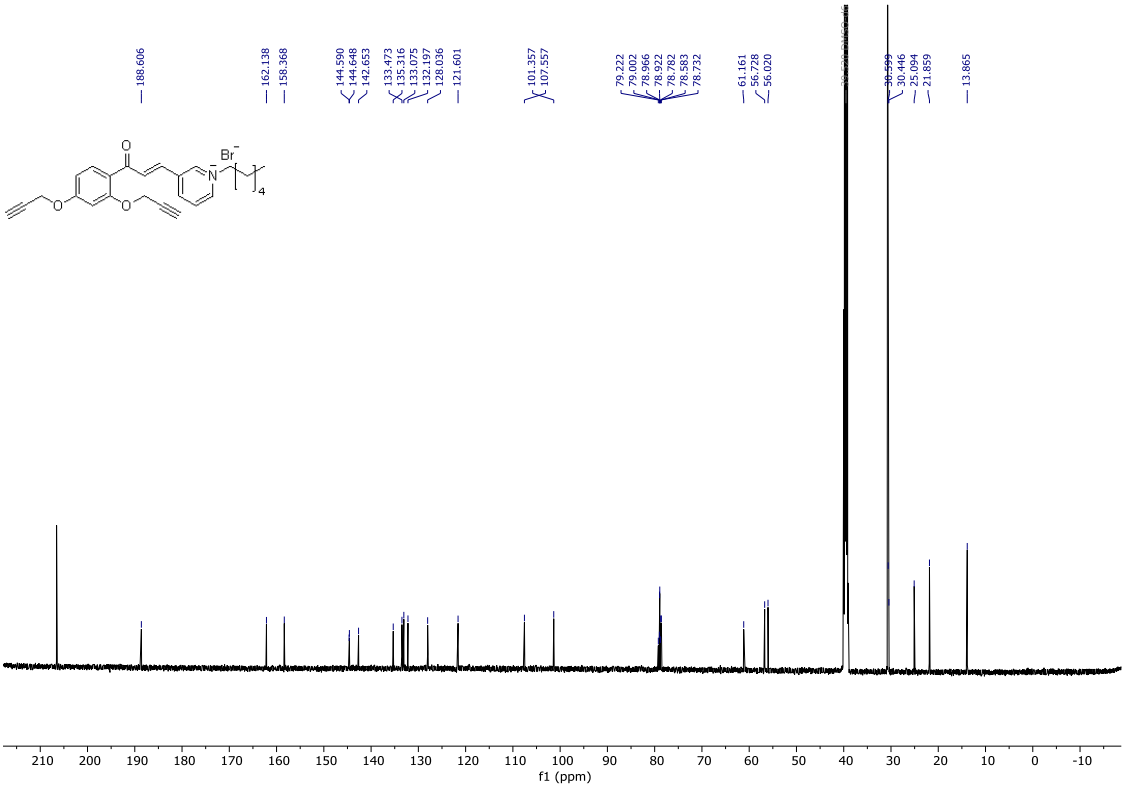

Figure S12. ^{13}C NMR (151 MHz, DMSO-d_6) of compound **2**.

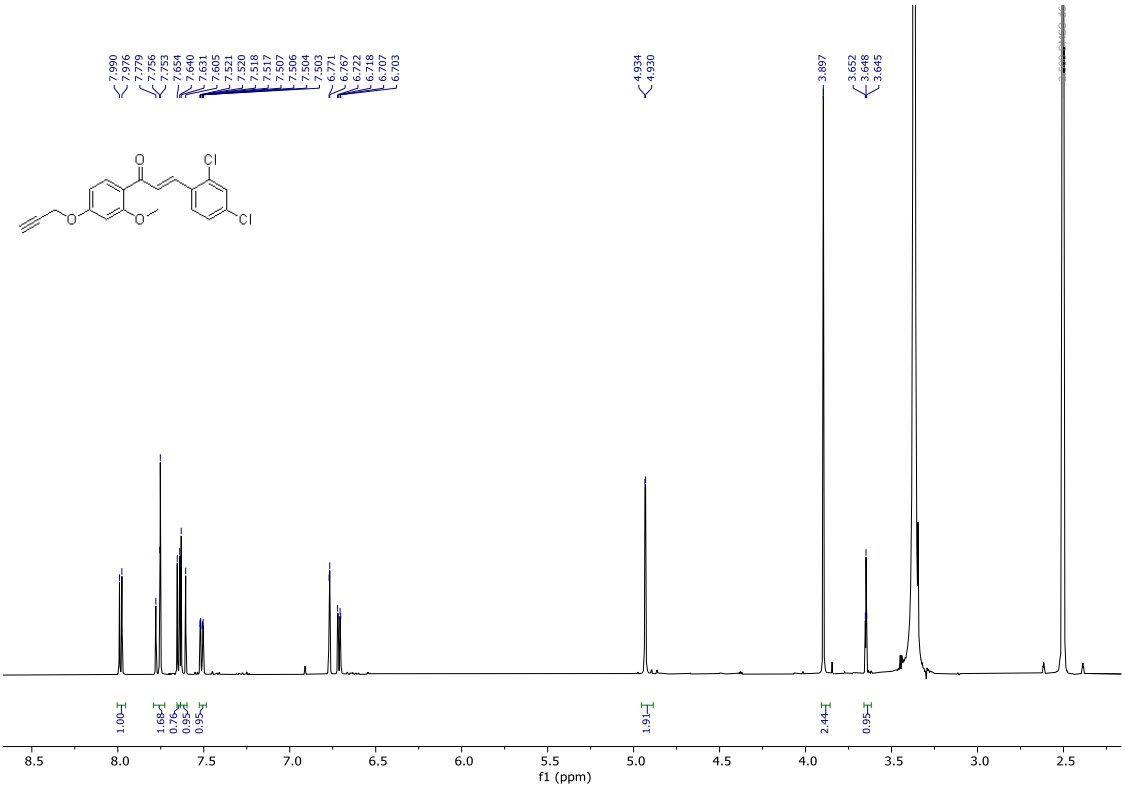

Figure S13. ^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **5**.

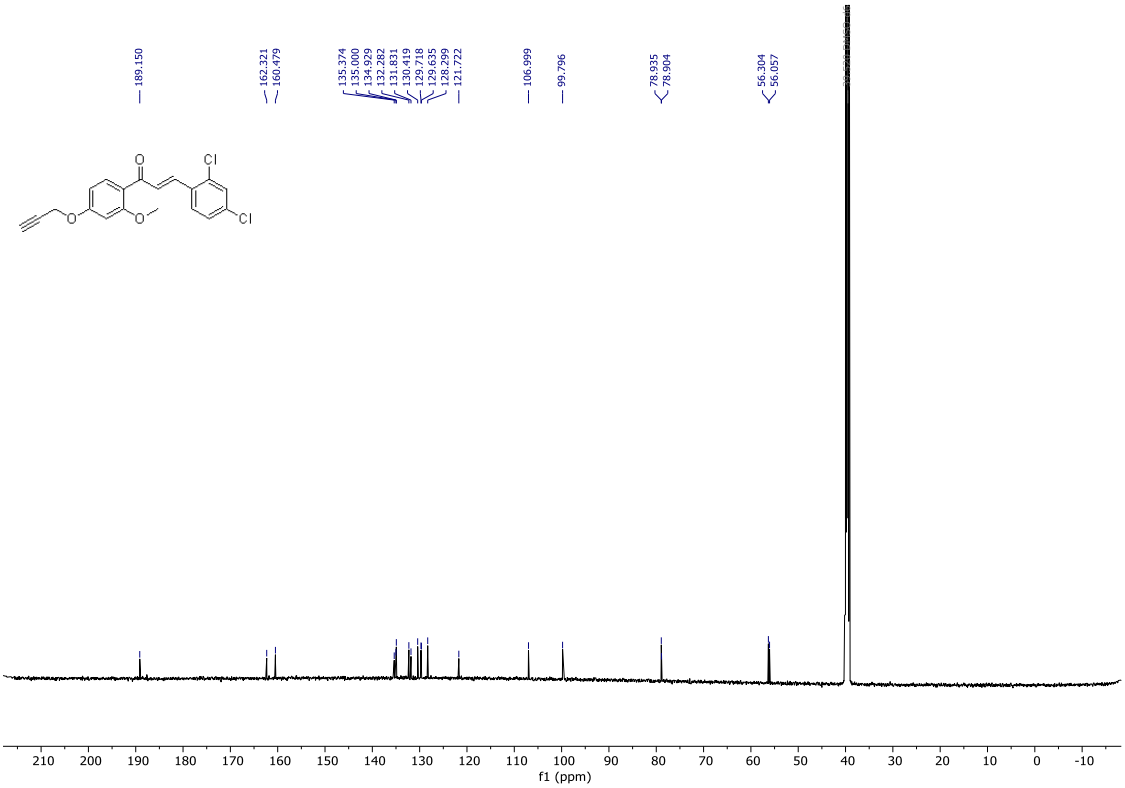

Figure S14. ^{13}C NMR (151 MHz, DMSO-d_6) of compound **5.**

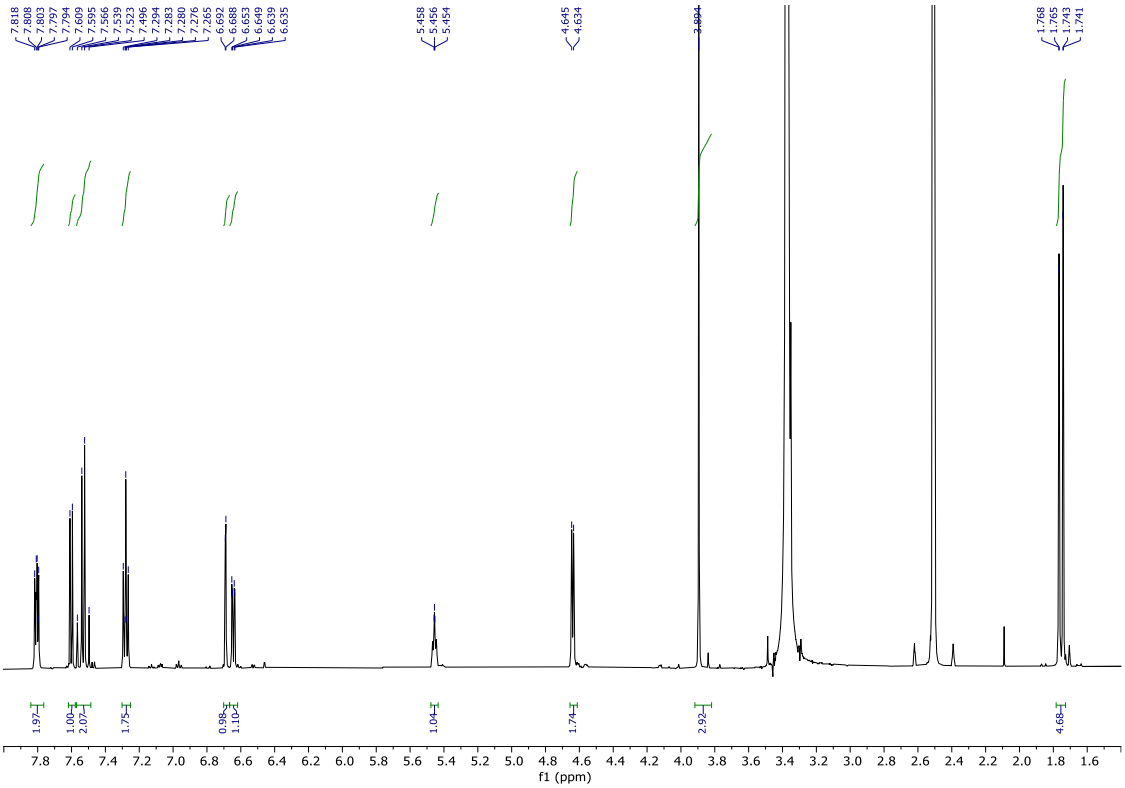

Figure S15. ^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **12**.

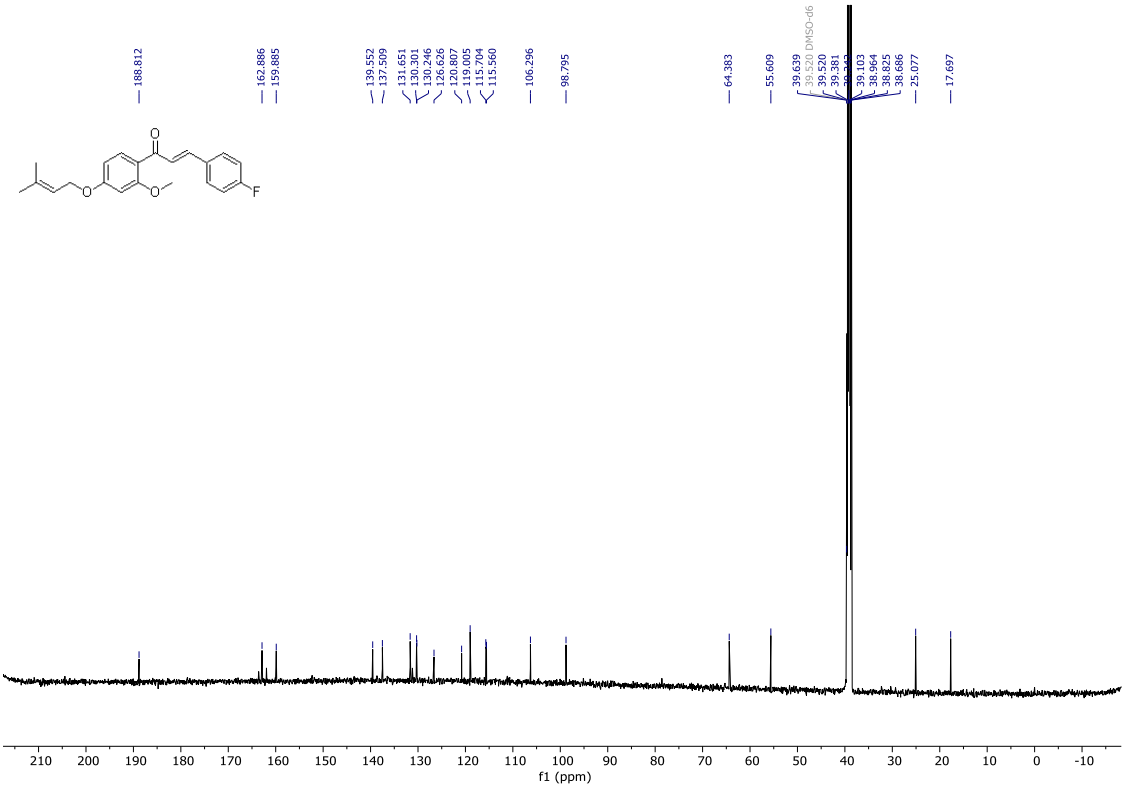

Figure S16. ^{13}C NMR (151 MHz, DMSO-d_6) of compound **12.**

Table S1: Results of the docking of chalcones on TR crystallized structure (4apn.pdb)

PDB ID	Cofactor binding Site	Binding energy calculated for the corresponding cofactor (kcal/mol)	Ki calculated for the corresponding cofactor	Inhibitor (chalcone number)	Binding energy of the chalcone inhibitors (kcal/mol)	Ki of the chalcone inhibitors
4apn	FAD	-8.92	289.54nM	1	-10.85	11.06nM
				2	-11.54	3.5nM
				5	-9.76	69.94nM
				12	-10.24	31.05nM
	NADPH	-7.67	2.4mM	1	-9.28	156.96nM
				2	-8.64	462.55nM
				5	-8.46	633.48nM
				12	-7.81	1.89µM
	UQ5c	-6.42	19.61mM	1	-7.33	4.24µM
				2	-6.95	8.03µM
				5	-6.97	7.79µM
				12	-6.96	7.90µM
	GCGc	-0.65	334.9mM	1	-5.52	89.51µM
				2	-4.48	518.94µM
				5	-5.36	118.08µM
				12	-5.15	168.91µM

PDB ID, protein databank accession number

[c] GCG corresponds to trypanothione, whereas UQ5 is an ubiquinone derivative.

Table S2. Results of docking on the *L.infantum* DLD (XP_001468025.1) 3D comparative model**.**

Modelled sequence	Cofactor binding Site	Binding energy calculated for the corresponding cofactor (kcal/mol)	Ki calculated for the corresponding cofactor	Inhibitor (chalcone number)	Binding energy of the chalcone inhibitors (kcal/mol)	Ki of the chalcone inhibitors
XP001468025.1 (DLD)	FAD	-7.52	3.05μM	1	-10.89	40.15nM
				2	-10.02	44.99nM
				5	-9.31	148.62nM
				12	-9.83	61.98nM
	NADH	-7.77	2.01μM	1	-8.49	599.01nM
				2	-6.9	8.71µM
				5	-7.95	1.49µM
				12	-7.51	3.13µM
	UQ5c	-4.55	463.67μM	1	-6.22	27.61µM
				2	-5.27	136.32µM
				5	-5.74	61.58µM
				12	-5.46	99.67µM

c UQ5 is a ubiquinone derivative.

Table S3. Results of the docking of the four selected chalcones on *L.infantum* NDH2 (XP_001469921.1) 3D comparative model**.**

Modelled sequence	**Cofactor binding Site**	**Binding energy calculated for the correspondin g cofactor (kcal/mol)**	**Ki calculated for the corresponding cofactor**	**cmpd**	**Binding energy of the chalcone inhibitors (kcal/mol)**	**Ki of the chalcone inhibitors**
XP001469921.1 (NDH2)	FAD	-9.03	151.39 nM	1	-9.35	140.94 nM
				2	-7.62	2.62 μM
				5	-8.47	614.24 nM
				12	-8.37	737.97 nM
	NADH	-7.83	1.82 μM	1	-8.06	1.23 μM
				2	-6.76	11.12 μM
				5	-6.91	8.60 μM
				12	-6.88	9.02 μM
	UQ5 c	-7.09	6.36 μM	1	-7.31	4.38 μM
				2	-7.25	4.83 μM
				5	-7.35	4.12 μM
				12	-7.69	2.31μM

c UQ5 is a ubiquinone derivative.

Table S4. Results of the docking of the four selected chalcones on deCoAR (XP_001468165.1) 3D comparative model**.**

Modelled sequence	**Cofactor binding Site**	**Binding energy calculated for the corresponding cofactor (kcal/mol)**	**Ki calculated for the corresponding cofactor**	**Inhibitor (chalcone number)**	**Binding energy of the chalcone inhibitors (kcal/mol)**	**Ki of the chalcone inhibitors**
XP001468165.1 (deCoAR)	FAD	-10.48	20.76nM	1	-8.2	975.36nM
				2	-7.94	1.50µM
				5	-8.69	427.02nM
				12	-8.69	425.71nM
	NADPH	-9.58	95.53nM	1	-6.54	16.19µM
				2	-6.01	39.56µM
				5	-7.11	6.10µM
				12	-7.06	6.66µM

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