Supporting information

Synthesis and biological evaluation against *Leishmania donovani* of novel hybrid molecules containing indazole-based 2-pyrone scaffolds

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EXPERIMENTAL

Melting points were determined using a Büchi-Tottoli apparatus. ¹H and ¹³C NMR spectra were recorded in CDCl₃, DMSO-d₆ and solution (unless otherwise specified) with TMS as an internal reference using a Bruker AC 300 (¹H) or 75MHz (¹³C) instruments. Chemical shifts are given in δ parts per million (ppm) downfield from TMS. Multiplicities of ¹³C NMR resources were assigned by distortionless enhancement by polarization transfer (DEPT) experiments. Low-resolution mass spectra (MS) were recorded on a Perkin-Elmer Sciex API 3000 spectrometer. Column chromatography was carried out on SiO₂ (silica gel 60 Merck 0.063–0.200 mm). Thin-layer chromatography (TLC) was carried out on SiO₂ (silica gel 60, F 254 Merck 0.063–0.200 mm), and the spots were located with UV light. Commercial reagents were used without further purification unless stated.

General procedure for the synthesis of compounds 5a-h, 6a-h, 9a-c and 10a-c.

N-alkyl-6(5)-nitroindazoles (1.0 mmol) were added to a mixture of anhydrous $SnCl_2$ powder (460 mg, 4.0 mmol), and acetic acid (0.572 mL, 10 mmol) in tetrahydrofuran (10 mL), followed by the addition of 2-pyrone (1.0 mmol) in THF (15 mL). The reaction mixture was stirred at 80 °C for 4 to 6 h. After the reaction was completed, the mixture was diluted with ethyl acetate (30 mL), poured into 10% NaHCO₃ (30 mL), and then extracted with ethyl

acetate (50 mL x 3). The combined organic extracts were dried over MgSO₄, filtered, and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate/hexane (3:7) to afford the desired products in good yields. ¹H NMR, ¹³CNMR and DEPT copies of selected compounds are given in the supporting information.

3-(1-(1-Methyl-1H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (5a): Yield: 81%; mp 164-168 °C; ¹H NMR (DMSO-d₆): δ 2.12 (s, 3H, CH₃), 2.55 (s, 3H, CH₃), 4.03 (s, 3H, NCH₃), 5.85 (s, 1H, =CH), 7.08 (dd, 1H, J = 8.4 Hz, J = 1.5 Hz), 7.73 (d, 1H, J = 1.2 Hz), 7.84 (d, 1H, J = 8.4 Hz), 8.10 (s, 1H, H-3), 15.83 (s, 1H, NH); ¹³C NMR (DMSO-d₆): δ 19.8 (CH₃), 20.6 (CH₃), 36.0 (NCH₃), 97.1 (C), 107.2 (CH), 118.9 (CH), 122.4 (CH), 123.0 (C), 133.2 (CH-3), 134.4 (C), 139.9 (C), 162.8 (C), 163.9 (C), 175.7 (CO), 184.4 (CO); EI-MS (m/z) = 298 [M+1]⁺, Anal. Calcd for C₁₆H₁₅N₃O₃; C, 64.64; H, 5.09; N, 14.13. Found: C, 64.71; H, 5.02; N, 14.24.

3-(1-(2-Methyl-2H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (6a): Yield: 75%; 192-194 °C; ¹H NMR (CDCl₃): δ 2.21 (s, 3H, CH₃), 2.66 (s, 3H, CH₃), 4.43 (s, 3H, NCH₃), 5.88 (s, 1H, =CH), 7.08 (dd, 1H, J = 8.4 Hz, J = 1.5 Hz), 7.71 (d, 1H, J = 1.2 Hz), 7.80 (d, 1H, J = 8.4 Hz), 8.21 (s, 1H, H-3), 15.81 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 20.1 (CH₃), 20.7 (CH₃), 46.7 (NCH₃), 97.9 (C), 107.0 (CH), 112.0 (CH), 120.4 (C), 121.8 (CH), 122.8 (CH), 123.7 (C), 125.2 (CH-3), 139.0 (C), 164.0 (C), 175.1 (CO), 184.9 (CO); EI-MS (m/z) = 298 [M+1]⁺, Anal. Calcd for C₁₆H₁₅N₃O₃; C, 64.64; H, 5.09; N, 14.13. Found: C, 64.75; H, 5.05; N, 14.18.

3-(1-(1-Ethyl-1H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (5b): Yield: 86%; mp 177-179 °C; ¹H NMR (CDCl₃): δ 1.54 (t, 3H, CH₃, J= 7.2 Hz), 2.21 (s, 3H, CH₃), 2.68 (s, 3H, CH₃), 4.46 (q, 2H, NCH₂, J= 7.2 Hz), 6.07 (s, 1H, =CH), 6.96 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz), 7.27 (d, 1H, J = 1.2 Hz), 7.81 (d, 1H, J = 8.4 Hz), 8.07 (s, 1H, H-3), 15.57 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 14.9 (CH₃), 20.1 (CH₃), 21.0 (CH₃), 44.1 (NCH₂), 98.2 (C), 106.1 (CH), 106.5 (CH), 118.7 (CH), 122.9 (CH), 123.4 (C), 132.7 (CH-3), 134.4 (C), 138.7 (C), 160.1 (C), 164.0 (C), 175.8 (CO), 184.0 (CO); EI-MS (m/z) = 312 [M+1]⁺, Anal. Calcd for C₁₇H₁₇N₃O₃; C, 65.58; H, 5.50; N, 13.50. Found: C, 65.67; H, 5.42; N, 13.64.

3-(1-(2-Ethyl-2H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (6b): Yield: 72%; mp 159-161 °C; ¹H NMR (CDCl₃): δ 1.75 (t, 3H, CH₃, J= 7.2 Hz), 2.19 (s, 3H, CH₃), 2.66 (s, 3H, CH₃), 4.72 (q, 2H, NCH₂, J= 7.2 Hz), 5.91 (s, 1H, =CH), 7.03 (dd, 1H, J = 9.0 Hz, J = 1.2 Hz), 7.65 (d, 1H, J = 1.2 Hz), 7.83 (d, 1H, J = 9.0 Hz), 8.23 (s, 1H, H-3), 15.88 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 15.6 (CH₃), 20.0 (CH₃), 20.7 (CH₃), 49.0 (NCH₂), 98.2 (C), 106.8 (CH), 111.4 (CH), 120.2 (C), 121.8 (CH), 122.6 (CH), 123.4 (C), 125.6 (CH-3), 138.7 (C), 164.2 (C), 175.6 (CO), 184.5 (CO); EI-MS (m/z) = 312 $[M+1]^+$, Anal. Calcd for $C_{17}H_{17}N_3O_3$; C, 65.58; H, 5.50; N, 13.50. Found: C, 65.71; H, 5.38; N, 13.58.

3-(1-(1-Allyl-1H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (5c): Yield: 91%; mp 118-120 °C; ¹H NMR (CDCl₃): δ 2.21 (s, 3H, CH₃), 2.68 (s, 3H, CH₃), 5.04–5.06 (m, 2H, NCH₂), 5.13–5.28 (m, 2H, =CH₂), 5.97–6.06 (m, 1H, =CH), 6.13 (s, 1H, =CH), 6.97 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz), 7.27 (d, 1H, J = 1.2 Hz), 7.82 (d, 1H, J = 8.4 Hz), 8.07 (s, 1H, H-3), 15.48 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 20.1 (CH₃), 21.0 (CH₃), 52.1 (NCH₂), 98.2 (C), 106.5 (CH), 110.5 (CH), 118.5 (=CH₂), 118.8 (CH), 122.7 (CH), 123.7 (C), 132.1 (CH), 133.3 (CH), 134.3 (C), 139.2 (C), 163.9 (C), 164.7 (C), 176.0 (CO), 184.0 (CO); EI-MS (m/z) = 324 [M+1]⁺, Anal. Calcd for C₁₈H₁₇N₃O₃; C, 66.86; H, 5.30; N, 13.00. Found: C, 66.74; H, 5.42; N, 13.08.

3-(1-(2-Allyl-2H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (6c): Yield: 82%; mp 96-98 °C; ¹H NMR (CDCl₃): δ 2.16 (s, 3H, CH₃), 2.64 (s, 3H, CH₃), 5.10–5.13 (m, 2H, NCH₂), 5.35–5.42 (m, 2H, =CH₂), 5.83 (s, 1H, =CH), 6.09–6.18 (m, 1H, =CH), 6.90 (dd, 1H, J = 8.4 Hz, J = 1.5 Hz), 7.54 (d, 1H, J = 1.0 Hz), 7.74 (d, 1H, J = 8.4 Hz), 8.10 (s, 1H, H-3), 15.71 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 19.9 (CH₃), 20.6 (CH₃), 56.3 (NCH₂), 97.4 (C), 107.2 (CH), 113.5 (CH), 120.5 (CH), 120.7 (=CH₂), 122.1 (CH), 122.7 (C), 123.9 (CH), 131.3 (CH), 147.3 (C), 163.4 (C), 163.8 (C), 175.6 (CO), 184.6 (CO); EI-MS (m/z) = 324 [M+1]⁺, Anal. Calcd for C₁₈H₁₇N₃O₃; C, 66.86; H, 5.30; N, 13.00. Found: C, 66.72; H, 5.38; N, 13.15.

3-{1-[1-(4-Methyl-benzyl)-1H-indazol-6-ylamino]-ethylidene}-6-methylpyran-2,4-dione

(5d): Yield: 88%; mp 98–100 °C; ¹H NMR (CDCl₃): δ 2.23 (s, 3H, CH₃), 2.31 (s, 3H, CH₃), 2.59 (s, 3H, CH₃), 5.58 (s, 2H, NCH₂), 5.79 (s, 1H, =CH), 6.94 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz), 7.11-7.15 (m, 5H), 7.82 (d, 1H, J = 8.4 Hz), 8.11 (s, 1H, H-3), 15.37 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 20.1 (CH₃), 21.1 (2CH₃), 53.3 (NCH₂), 98.2 (C), 106.0 (CH), 106.6 (CH), 118.7 (CH), 122.9 (CH), 123.8 (C), 127.3 (2CH), 129.5 (C), 129.7 (2CH), 132.7 (C), 133.2 (CH), 134.2 (C), 138.1 (C), 139.2 (C), 163.4 (C), 175.1 (CO), 184.1 (CO); EI-MS (m/z) = 388 [M+1]⁺, Anal. Calcd for C₂₃H₂₁N₃O₃; C, 71.30; H, 5.46; N, 10.85. Found: C, 71.18; H, 5.57; N, 10.74.

3-{1-[2-(4-Methyl-benzyl)-2H-indazol-6-ylamino]-ethylidene}-6-methylpyran-2,4-dione (6d): Yield: 78%; mp 154-156 °C; ¹H NMR (CDCl₃): δ 2.16 (s, 3H, CH₃), 2.34 (s, 3H, CH₃), 2.64 (s, 3H, CH₃), 5.59 (s, 2H, NCH₂), 5.79 (s, 1H, =CH), 6.85 (dd, 1H, J = 8.4 Hz, J = 1.0 Hz), 7.17 (d, 1H, J = 7.8 Hz), 7.23 (d, 1H, J = 7.8 Hz), 7.52 (d, 1H, J = 1.0 Hz), 7.68 (d, 1H, J = 8.4 Hz), 7.95 (s, 1H, H-3), 15.72 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 19.9 (CH₃), 20.5 (CH₃), 21.2 (CH₃), 57.5 (NCH₂), 97.5 (C), 107.2 (CH), 113.2 (CH), 120.6 (C), 120.7 (CH), 122.2 (CH), 124.3 (CH), 128.5 (2CH), 129.8 (2CH), 131.4 (C), 135.1 (C), 138.9 (C), 163.5 (C), 163.7 (C), 175.5 (CO), 184.6 (CO); EI-MS (m/z) = 388 [M+1]⁺, Anal. Calcd for $C_{23}H_{21}N_3O_3$; C, 71.30; H, 5.46; N, 10.85. Found: C, 71.21; H, 5.61; N, 10.78.

3-(1-(3-Chloro-1-methyl-1H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-

dione (5e): Yield: 78%; mp 185-187 °C; ¹H NMR (CDCl₃): δ 2.15 (s, 3H, CH₃), 2.58 (s, 3H, CH₃), 4.08 (s, 3H, NCH₃), 5.91 (s, 1H, =CH), 7.05 (d, 1H, J = 8.4 Hz), 7.20 (d, 1H, J = 1.0 Hz), 7.81 (d, 1H, J = 8.4 Hz), 15.65 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 20.0 (CH₃), 20.7 (CH₃), 47.6 (NCH₃), 97.8 (C), 106.8 (CH), 107.1 (CH), 119.0 (CH), 121.0 (C), 121.4 (CH), 134.1 (C), 136.0 (C), 140.8 (C), 163.7 (C), 164.9 (C), 175.8 (CO), 184.6 (CO); EI-MS (m/z) = 332 (³⁵Cl) [M+1]⁺, 334 (³⁷Cl) [M+3]⁺ Anal. Calcd for C₁₆H₁₄ClN₃O₃; C, 57.93; H, 4.25; N, 12.67. Found: C, 57.82; H, 4.36; N, 12.54.

3-(1-(3-Chloro-2-methyl-2H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4dione (6e): Yield: 76%; mp 107-109 °C; ¹H NMR (CDCl₃): δ 2.16 (s, 3H, CH₃), 2.63 (s, 3H, CH₃), 4.17 (s, 3H, NCH₃), 5.77 (s, 1H, =CH), 6.88 (dd, 1H, J = 8.7 Hz, J = 1.5 Hz), 7.42 (d, 1H, J = 1.5 Hz), 7.62 (d, 1H, J = 8.7 Hz), 15.88 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 19.9 (CH₃), 20.5 (CH₃), 37.8 (NCH₃), 97.5 (C), 107.2 (C), 114.2 (CH), 111.8 (C), 118.1 (C), 120.1 (CH), 120.6 (CH), 135.0 (C), 147.1 (C), 163.4 (C), 175.5 (CO), 184.8 (CO); EI-MS (m/z) = 332 (³⁵Cl) [M+1]⁺, 334 (³⁷Cl) [M+3]⁺ Anal. Calcd for C₁₆H₁₄ClN₃O₃; C, 57.93; H, 4.25; N, 12.67. Found: C, 57.78; H, 4.34; N, 12.52.

3-(1-(3-Chloro-1-ethyl-1H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione

(**5f**) : Yield: 77%; mp 67-69 °C; ¹H NMR (CDCl₃): δ 1.51 (t, 3H, CH₃, J= 7.2 Hz), 2.26 (s, 3H, CH₃), 2.58 (s, 3H, CH₃), 4.40 (q, 2H, NCH₂, J= 7.2 Hz), 5.81 (s, 1H, =CH), 6.90 (dd, 1H, J = 8.7 Hz, J = 1.5 Hz), 7.45 (d, 1H, J = 1.5 Hz), 7.66 (d, 1H, J = 8.7 Hz), 15.82 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 14.8 (CH₃), 20.2 (CH₃), 21.0 (CH₃), 44.5 (NCH₂), 97.8 (C), 107.4 (C), 114.1 (CH), 111.9 (C), 118.5 (C), 120.3 (CH), 120.8 (CH), 135.1 (C), 146.9 (C), 163.8 (C), 175.6 (CO), 184.2 (CO); EI-MS (m/z) = 346 (³⁵Cl) [M+1]⁺, 348 (³⁷Cl) [M+3]⁺ Anal. Calcd for C₁₆H₁₄ClN₃O₃; C, 59.05; H, 4.66; N, 12.15. Found: C, 59.18; H, 4.54; N, 12.22.

3-(1-(3-Chloro-2-ethyl-2H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (**6f**) : Yield: 80%; mp 62-64 °C; ¹H NMR (CDCl₃): δ 1.45 (t, 3H, CH₃, J= 7.2 Hz), 2.20 (s, 3H, CH₃), 2.64 (s, 3H, CH₃), 4.72 (q, 2H, NCH₂, J= 7.2 Hz), 5.81 (s, 1H, =CH), 6.91 (dd, 1H, J = 8.4 Hz, J = 1.5 Hz), 7.45 (d, 1H, J = 1.5 Hz), 7.68 (d, 1H, J = 8.4 Hz), 15.78 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 15.7 (CH₃), 20.0 (CH₃), 20.6 (CH₃), 48.8 (NCH₂), 97.8 (C), 107.1 (C), 113.9 (CH), 112.0 (C), 118.2 (C), 120.4 (CH), 120.8 (CH), 134.9 (C), 146.8 (C), 163.5 (C), 175.7 (CO), 184.5 (CO); EI-MS (m/z) = 346 (35 Cl) [M+1]⁺, 348 (37 Cl) [M+3]⁺ Anal. Calcd for C₁₆H₁₄ClN₃O₃; C, 59.05; H, 4.66; N, 12.15. Found: C, 59.24; H, 4.50; N, 12.28.

3-(1-(1-Allyl-3-chloro-1H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione

(**5g**):. Yield: 81%; mp 146-148 °C; ¹H NMR (CDCl₃): δ 2.23 (s, 3H, CH₃), 2.66 (s, 3H, CH₃), 4.95–4.98 (m, 2H, NCH₂), 5.18–5.30 (m, 2H, =CH₂), 5.82–6.02 (m, 1H, =CH), 6.14 (s, 1H, =CH), 7.02 (d, 1H, J = 8.4 Hz), 7.24 (d, 1H, J = 1.0 Hz), 7.77 (d, 1H, J = 8.4 Hz), 15.54 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 20.1 (CH₃), 21.1 (CH₃), 52.5 (NCH₂), 97.1 (C), 106.3 (CH), 106.9 (CH), 118.9 (=CH₂), 119.3 (CH), 121.0 (C), 121.6 (CH), 131.7 (CH), 133.5 (C), 135.3 (C), 140.5 (C), 163.8 (C), 165.0 (C), 176.2 (CO), 184.0 (CO); EI-MS (m/z) = 358 (³⁵Cl) [M+1]⁺, 360 (³⁷Cl) [M+3]⁺ Anal. Calcd for C₁₈H₁₆ClN₃O₃; C, 60.43; H, 4.51; N, 11.74. Found: C, 60.32; H, 4.64; N, 11.63.

3-(1-(2-Allyl-3-chloro-2H-indazol-6-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione

(**6g**): Yield: 75%; mp 70-72 °C; ¹H NMR (CDCl₃): δ 2.18 (s, 3H, CH₃), 2.67 (s, 3H, CH₃), 5.08–5.12 (m, 2H, NCH₂), 5.32–5.40 (m, 2H, =CH₂), 5.86 (s, 1H, =CH), 6.10–6.17 (m, 1H, =CH), 6.92 (dd, 1H, J = 8.4 Hz, J = 1.5 Hz), 6.91 (dd, 1H, J = 8.4 Hz, J = 1.5 Hz), 7.45 (d, 1H, J = 1.5 Hz), 7.61 (d, 1H, J = 8.4 Hz), 15.77 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 20.0 (CH₃), 20.6 (CH₃), 56.4 (NCH₂), 97.6 (C), 106.8 (C), 113.6 (CH), 112.0 (C), 118.2 (C), 120.4 (CH), 120.7 (=CH₂), 121.0 (CH), 131.5 (CH), 135.0 (C), 146.2 (C), 164.0 (C), 175.4 (CO), 184.8 (CO); EI-MS (m/z) = 358 (³⁵Cl) [M+1]⁺, 360 (³⁷Cl) [M+3]⁺ Anal. Calcd for C₁₈H₁₆ClN₃O₃; C, 60.43; H, 4.51; N, 11.74. Found: C, 60.28; H, 4.60; N, 11.61.

3-(1-(1-Methyl-1H-indazol-5-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione 9a: Yield: 92%; mp 173-175 °C; ¹H NMR (CDCl₃): δ 2.23 (s, 3H, CH₃), 2.65 (s, 3H, CH₃), 4.13 (s, 3H, NCH₃), 6.24 (s, 1H, =CH), 7.20 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz), 7.53 (d, 1H, J = 8.4 Hz), 7.57 (d, 1H, J = 1.2 Hz), 8.05 (s, 1H, H-3), 15.22 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 20.1 (CH₃), 21.0 (CH₃), 35.9 (NCH₃), 98.2 (C), 106.2 (CH), 110.5 (CH), 118.1 (CH), 123.8 (C), 124.1 (CH), 128.8 (C), 133.0 (CH-3), 138.9 (C), 164.1 (C), 164.9 (C), 176.3 (CO), 183.6 (CO); EI-MS (m/z) = 298 [M+1]⁺, Anal. Calcd for C₁₆H₁₅N₃O₃; C, 64.64; H, 5.09; N, 14.13. Found: C, 64.75; H, 5.18; N, 14.20.

3-(1-(2-Methyl-2H-indazol-5-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (10a): Yield: 84%; mp 142-144 °C; ¹H NMR (CDCl₃): δ 2.16 (s, 3H, CH₃), 2.61 (s, 3H, CH₃), 4.29 (s, 3H, NCH₃), 5.81 (s, 1H, =CH), 7.09 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz), 7.48 (d, 1H, J = 1.8 Hz), 7.77 (d, 1H, J = 8.4 Hz), 8.02 (s, 1H, H-3), 15.59 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 19.9 (CH₃), 20.5 (CH₃), 40.6 (NCH₃), 97.3 (C), 107.3 (CH), 116.8 (CH), 118.6 (CH), 121.4 (C), 124.9 (CH), 125.5 (CH-3), 130.5 (C), 146.8 (C), 163.4 (C), 163.8 (C), 175.7 (CO), 184.6 (CO); EI-MS (m/z) = 298 [M+1]⁺, Anal. Calcd for $C_{16}H_{15}N_3O_3$; C, 64.64; H, 5.09; N, 14.13. Found: C, 64.72; H, 5.21; N, 14.24.

3-(1-(1-Ethyl-1H-indazol-5-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (9b): Yield: 87%; mp 156-158 °C; ¹H NMR (CDCl₃): δ 1.54 (t, 3H, CH₃, J= 7.2 Hz), 2.22 (s, 3H, CH₃), 2.65 (s, 3H, CH₃), 4.48 (q, 2H, NCH₂, J= 7.2 Hz), 6.21 (s, 1H, =CH), 7.19 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz), 7.51 (d, 1H, J = 8.4 Hz), 7.58 (d, 1H, J = 1.5 Hz), 8.06 (s, 1H, H-3), 15.25 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 14.9 (CH₃), 20.1 (CH₃), 20.9 (CH₃), 44.2 (NCH₂), 96.8 (C), 106.3 (CH), 110.4 (CH), 118.2 (CH), 123.9 (C), 124.1 (CH), 128.8 (C), 133.0 (CH-3), 138.0 (C), 164.1 (C), 164.8 (C), 176.2 (CO), 183.7 (CO); EI-MS (m/z) = 312 [M+1]⁺, Anal. Calcd for C₁₇H₁₇N₃O₃; C, 65.58; H, 5.50; N, 13.50. Found: C, 65.75; H, 5.41; N, 13.64.

3-(1-(2-Ethyl-2H-indazol-5-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (10b): Yield: 76%; mp 113-115 °C; ¹H NMR (CDCl₃): δ 1.69 (t, 3H, CH₃, J= 7.2 Hz), 2.17 (s, 3H, CH₃), 2.62 (s, 3H, CH₃), 4.59 (q, 2H, NCH₂, J= 7.2 Hz), 5.83 (s, 1H, =CH), 7.13 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz), 7.51 (d, 1H, J = 1.0 Hz), 7.82 (d, 1H, J = 8.4 Hz), 8.10 (s, 1H, H-3), 15.61 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 15.7 (CH₃), 19.9 (CH₃), 20.5 (CH₃), 48.9 (NCH₂), 97.3 (C), 107.2 (CH), 117.0 (CH), 118.3 (CH), 121.0 (C), 124.0 (CH), 125.5 (CH-3), 130.7 (C), 145.7 (C), 163.5 (C), 163.8 (C), 175.7 (CO), 184.6 (CO); EI-MS (m/z) = 312 [M+1]⁺, Anal. Calcd for C₁₇H₁₇N₃O₃; C, 65.58; H, 5.50; N, 13.50. Found: C, 65.72; H, 5.38; N, 13.60.

3-(1-(1-Allyl-1H-indazol-5-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (**9c**): Yield: 90%; mp 90-92 °C; ¹H NMR (CDCl₃): δ 2.22 (s, 3H, CH₃), 2.65 (s, 3H, CH₃), 5.05–5.08 (m, 2H, NCH₂), 5.15–5.26 (m, 2H, =CH₂), 5.97–6.08 (m, 1H, =CH), 6.19 (s, 1H, =CH), 7.17 (dd, 1H, J = 8.4 Hz, J = 1.8 Hz), 7.50 (d, 1H, J = 9.0 Hz), 7.58 (d, 1H, J = 1.8 Hz), 8.07 (s, 1H, H-3), 15.27 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 20.1 (CH₃), 20.8 (CH₃), 52.1 (NCH₂), 96.9 (C), 106.5 (CH), 110.7 (CH), 118.1 (CH), 118.4 (=CH₂), 124.1 (CH), 124.3 (C), 128.9 (C), 132.2 (CH), 133.5 (CH), 138.5 (C), 164.1 (C), 164.7 (C), 176.2 (CO), 183.8 (CO); EI-MS (m/z) = 324 [M+1]⁺, Anal. Calcd for C₁₈H₁₇N₃O₃; C, 66.86; H, 5.30; N, 13.00. Found: C, 66.70; H, 5.37; N, 13.16.

3-(1-(2-Allyl-2H-indazol-5-ylamino)ethylidene)-6-methyl-3H-pyran-2,4-dione (10c): Yield: 81%; mp 78-80 °C; ¹H NMR (CDCl₃): δ 2.18 (s, 3H, CH₃), 2.63 (s, 3H, CH₃), 5.16– 5.19 (m, 2H, NCH₂), 5.40–5.46 (m, 2H, =CH₂), 5.92 (s, 1H, =CH), 6.05–6.20 (m, 1H, =CH), 7.15 (dd, 1H, J = 8.4 Hz, J = 1.5 Hz), 7.53 (d, 1H, J = 1.0 Hz), 7.68 (d, 1H, J = 8.4 Hz), 8.12 (s, 1H, H-3), 15.56 (s, 1H, NH); ¹³C NMR (CDCl₃): δ 19.9 (CH₃), 20.6 (CH₃), 56.2 (NCH₂), 97.6 (C), 106.9 (CH), 117.2 (CH), 118.3 (CH), 121.4 (=CH₂), 124.8 (CH), 121.7 (C), 128.8 (CH), 130.7 (CH), 132.4 (C), 163.8 (C), 163.9 (C), 175.8 (CO), 184.4 (CO); EI-MS (m/z) = 324 [M+1]⁺, Anal. Calcd for C₁₈H₁₇N₃O₃; C, 66.86; H, 5.30; N, 13.00. Found: C, 66.77; H, 5.43; N, 13.21.

Biology

Parasite and cell cultures. Promastigotes of the *Leishmania donovani* (MHOM/ET/67/HU3/LV9) were cultured in the dark at 26°C with 5% CO₂ in M199 complete medium containing M199 medium supplemented with 100 μ M adenosine, 0.5 mg/L hemin, 40 mM Hepes pH 7.4 and 10 % heat inactivated foetal bovine serum (HIFBS). Cultures of axenic amastigotes of *L. donovani* were obtained from late log promastigotes diluted at 1 x 10⁶/mL in M199 complete medium acidified at pH 5.5 and cultured at 37°C with 5% CO₂.

Macrophage. The macrophages RAW 264.7 were cultured at 37°C with 5% CO₂ in DMEM complete medium containing Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 100 U/mL penicillin-streptomycin, and 10% HIFBS.

Evaluation of compounds cytotoxicity. Cytotoxicity was evaluated on RAW 264.7 macrophages. Cells were plated in 96-well microplates at a density of 2 x 10^4 cells per well. After an incubation of 24 h at 37°C with 5% CO₂, the medium was removed in each well, and 100 µl of DMEM complete medium containing two fold serial dilutions of the compounds was added to each well. After 48 h of incubation at 37°C with 5% CO₂, 10 µl of resazurin (450 µM) was added to each well, and further incubated in the dark for 4 h at 37°C with 5% CO₂. Cell viability was then monitored as described above. The cytotoxicity of the compounds was expressed as CC50 (Cytotoxic Concentration 50%: concentration inhibiting the macrophages growth by 50%).

In vitro antileishmanial evaluation of compounds on axenic and intramacrophage amastigotes. The evaluations of activity on axenic and intramacrophage amastigotes of *L. donovani* were adapted from the protocols previously described [19]. Briefly, for the evaluation on axenic amastigotes, two fold serial dilutions of the compounds were performed in 100 μ l of complete medium (see above) in 96-well microplates. Axenic amastigotes were then added to each well at a density of 10⁶/ml in a 200 μ l final volume. After 72 h of incubation at 37°C with 5 % CO₂, 20 μ l of resazurin (450 μ M) was added to each well and further incubated in the dark for 24 h at 37°C with 5% CO₂. In living cells, resazurin is reduced in resorufin and this conversion is monitored by measuring OD570nm (resorufin) and OD600nm (resazurin; Lab systems Multiskan MS). The activity of the compounds was expressed as IC₅₀ in μ M. Amphotericin B (AmB) was used as the reference drug.

Concerning the evaluation on intramacrophage amastigotes, RAW 264.7 macrophages were plated in 96-well microplates at a density of 2 x 104 cells per well and incubated for 24 h at

 37° C with 5% CO2. Axenic amastigotes were differenciated as described above, centrifuged at 2,000 g for 10 min, resuspended in DMEM complete medium, and added to each well to reach a 16 :1 parasite to macrophage ratio. After 24 h of infection at 37° C with 5% CO₂, extracellular parasites were removed, and DMEM complete medium (100 µl) containing two fold serial dilutions of the compounds from a maximal concentration of 100 µM was added to each well. After 48 h of treatment, the medium was removed and replaced by Direct PCR Lysis Reagent (100 µl; Euromedex) before 3 freeze-thaw cycles at room temperature, addition of 50 µg/ml proteinase K, and a final incubation at 55°C overnight to allow cell lysis. 10 µl of each cell extract was then added to 40 µl of Direct PCR Lysis reagent containing Sybr Green I (0.05%; Invitrogen). DNA fluorescence was monitored using Mastercycler® realplex (Eppendorf). The activity of the compounds was expressed as IC₅₀ in µM. Amphotericin B (AmB) was used as the reference drug.

Selected ¹H NMR, ¹³C NMR and DEPT spectra of compounds **5a-d**, **5g**, **6b-e**, **9a-c**, **10a** and **10b**.

Figure S1. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 5a	P9-P10
Figure S2. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 5b	P10-P11
Figure S3. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 6b	P12-P13
Figure S4. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 5c	P13-P14
Figure S5. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 6c	P15-P16
Figure S6. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 5d	P16-P17
Figure S7. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 6d	P18-P19
Figure S8. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 6e	P19-P20
Figure S9. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 5g	P21-P22
Figure S10. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 9a	P22-P23
Figure S11. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 10a	P24-P25
Figure S12. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 9b	P25-P26
Figure S13. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 10b	P27-P28
Figure S14. ¹ H NMR, ¹³ C NMR and DEPT spectra of compound 9c	P28-P29



Figure S1. ¹H NMR (300 MHz, DMSO-d₆) spectra of compound 5a



Figure S1. ¹³C NMR (75 MHz, DMSO-d₆) spectra of compound 5a



Figure S1. ¹³C NMR (75 MHz, DMSO-d₆, DEPT) spectra of compound 5a



Figure S2. ¹H NMR (300 MHz, CDCl₃) spectra of compound 5b



Figure S2. ¹³C NMR (75 MHz, CDCl₃) spectra of compound 5b



Figure S2. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 5b



Figure S3. ¹H NMR (300 MHz, CDCl₃) spectra of compound 6b



Figure S3. ¹³C NMR (75 MHz, CDCl₃) spectra of compound 6b



Figure S3. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 6b



Figure S4. ¹H NMR (300 MHz, CDCl₃) spectra of compound 5c



Figure S4. ¹³C NMR (75 MHz, CDCl₃) spectra of compound 5c



Figure S4. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 5c



Figure S5. ¹H NMR (300 MHz, CDCl₃) spectra of compound 6c



Figure S5. ¹³C NMR (75 MHz, CDCl₃) spectra of compound 6c



Figure S5. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 6c



Figure S6. ¹H NMR (300 MHz, CDCl₃) spectra of compound 5d



Figure S6. ¹³C NMR (75 MHz, CDCl₃) spectra of compound 5d



Figure S6. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 5d



Figure S7. ¹H NMR (300 MHz, CDCl₃) spectra of compound 6d



Figure S7. ¹³C NMR (75 MHz, CDCl₃) spectra of compound 6d



Figure S7. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 6d



Figure S8. ¹H NMR (300 MHz, CDCl₃) spectra of compound 6e



Figure S8. ¹³C NMR (75 MHz, CDCl₃) spectra of compound 6e



Figure S8. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 6e



Figure S9: ¹H NMR (300 MHz, CDCl₃) spectra of compound 5g



Figure S9: ¹³C NMR (75 MHz, CDCl₃) spectra of compound 5g



Figure S9. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 5g



Figure S10: ¹H NMR (300 MHz, CDCl₃) spectra of compound 9a



Figure S10: ¹³C NMR (75 MHz, CDCl₃) spectra of compound 9a



Figure S10. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 9a



Figure S11: ¹H NMR (300 MHz, CDCl₃) spectra of compound 10a



Figure S11: ¹³C NMR (75 MHz, CDCl₃) spectra of compound 10a



Figure S11. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 10a



Figure S12: ¹H NMR (300 MHz, CDCl₃) spectra of compound 9b



Figure S12: ¹³C NMR (75 MHz, CDCl₃) spectra of compound 9b



Figure S12. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 9b



Figure S13: ¹H NMR (300 MHz, CDCl₃) spectra of compound 10b



Figure S13: ¹³C NMR (75 MHz, CDCl₃) spectra of compound 10b



Figure S13. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 10b



Figure S14: ¹H NMR (300 MHz, CDCl₃) spectra of compound 9c



Figure S14: ¹³C NMR (75 MHz, CDCl₃) spectra of compound 9c



Figure S14. ¹³C NMR (75 MHz, CDCl₃, DEPT) spectra of compound 9c