Synthesis, antiprotozoal activity and cytotoxicity in U-937 macrophages of triclosan–hydrazone hybrids

Sebastian Vergara1 · Miguel Carda2 · Raül Agut2 · Lina M. Yepes3 · Iván D. Vélez3 · Sara M. Robledo3 · Wilson Cardona Galeano1

Received: 23 February 2017 / Accepted: 31 July 2017 © Springer Science+Business Media, LLC 2017

Abstract The synthesis and biological activities (cytotoxicity, leishmanicidal, and trypanocidal) of 11 triclosan–hydrazone hybrids are described herein. The structure of the products was elucidated by spectral data (NMR, IR) and mass spectrometric analyses. The synthesized compounds were evaluated against amastigotes forms of L. (V) panamensis, which is the most prevalent Leishmania species in Colombia, and against Trypanosoma cruzi, which is the major pathogenic species to Chagas disease in humans. In addition, the cytotoxic activity of the synthesized compounds was evaluated against human U-937 macrophages. Hydrazone hybrids were obtained as E-synperiplanar and E-antiperiplanar conformers. Nine of them were active against L. (V) panamensis (5a–5d, 5f–5j) and eight of them against T. cruzi (5a, 5c, 5d, 5f–5j), with EC50 values lower than 40 µM. The compounds 5c, 5e, and 5h exhibit the best selectivity index against both L. (V) panamensis and T. cruzi, with values ranging from 5.90 to 16.55, thus showing potential as starting compounds for the eventual development of drugs against these parasites.

Keywords Leishmaniasis · Chagas disease · Trypanosoma cruzi · Antiprotozoal activity · Cytotoxicity · Triclosan-Hydrazone Hybrids

Introduction

Protozoal diseases are a cause of mortality in various developing countries of tropical and subtropical regions. For leishmaniasis and Chagas-endemic countries these diseases cause significant health problems affecting more than one billion people worldwide (WHO 2002, 2013; Alvar et al. 2012; Nouvellet et al. 2015). This situation is aggravated by increasing treatment failures with available drugs (Bhutta et al. 2014). Chagas disease (American trypanosomiasis) and leishmaniasis are parasitic diseases caused by the parasitic protozoan Trypanosoma cruzi (T. cruzi) and Leishmania species, respectively.

The Leishmaniasis involves a wide spectrum of clinical manifestations in which L. (V) panamensis is one of the most prevalent Leishmania species involved in human cases of cutaneous leishmaniasis in Colombia (Alvar et al. 2012). Chagas disease (also named American trypanosomiasis) is produced by the protozoan parasite T. cruzi that is transmitted to the mammalian host through the bite of triatomine
bugs belonging to *Triatoma*, *Rhodnius*, and *Panstrongylus* genus (Nouvellet et al. 2015). Current treatments for cutaneous leishmaniasis are based on pentavalent antimonials (meglumine antimoniate and sodium stibogluconate). For the treatment of Chagas disease nitroaromatic compounds (benznidazole and nifurtimox) are usually employed. However, these treatments are not without significant side effects, particularly in patients undergoing high-dose and long-term treatments. In addition, the development of drug resistance has significantly increased the health problems associated with these diseases (Chatelain and Ioset 2011; Den Boer et al. 2011; Keenan and Chaplin 2015).

Triclosan is an uncompetitive inhibitor of purified enoyl-acyl carrier protein reductase (ENR), which has demonstrated in vitro inhibitory activity against *Plasmodium falciparum* (Kapoor et al. 2004; McLeod et al. 2001; Surolia and Surolia 2001; Perozzo et al. 2002). A previous study showed that triclosan has in vitro anti-leishmanial activity against axenic amastigotes of *L. panamensis* with an effective concentration (EC\(_{50}\)) of 39 \(\mu\)M.

Further, hydrazones constitute an important type of biologically active compounds (Rollas and Küçükgüzel 2007; Singh and Raghav 2011; Verma et al. 2014) with high ability to elicit anti-leishmanicidal (Bernardino et al. 2006; Rando et al. 2008; Taha et al. 2014) and trypanocidal activity (Carvalho et al. 2012; Porcal et al. 2008; Jorge et al. 2013; Massarico Serafim et al. 2014).

In recent years a promising strategy has emerged based on hybrid molecules, which bear in their structures two distinct pharmacophores having, for example, anti-protozoal, anti-inflammatory, anti-fungal, or anti-cancer activity, thus showing a dual mode of action (Keith et al. 2005; Meunier 2008). These hybrid molecules may display dual activity, but do not necessarily act on the same biological target (Opsenica et al. 2008; Roth et al. 2004; Walsh et al. 2007).

Triclosan–quinoline hybrids with shorter methylene units spacers (1a and 1b) have in vitro activity against intracellular amastigotes of *L. panamensis* with effective concentrations (EC\(_{50}\)) of 13.1 and 4.7 \(\mu\)M, respectively (Arango et al. 2012). Triclosan–chalcone (1c) and triclosan–chromone (1d) hybrids showed no cytotoxicity against U-937 cells but were active against *L. panamensis* amastigotes (LC\(_{50}\) = > 326.7 \(\mu\)M, EC\(_{50}\) = 15.4 and 5.5 \(\mu\)M, respectively) (Otero et al. 2014) (see Fig. 1). Quinoline–hydrazone hybrid 1e showed activity against *L. panamensis* and against *T. cruzi* with EC\(_{50}\) of 2.6 and 4.6 \(\mu\)M, respectively (Coa et al. 2015). Some furoxanyl N-acylhydrazone derivatives were evaluated in vitro against amastigote form of *L. panamensis* and against *T. cruzi*. The compound 1f exhibited excellent profile as anti-*T. cruzi* with an IC\(_{50}\) = 0.91 \(\mu\)M. On the other hand, compounds 1g and 1h showed very good anti-Leishmania activity with IC\(_{50}\) = 1.3 and 1.7 \(\mu\)M, respectively. In addition, compounds 1g and 1h displayed higher selectivity than the reference drug Amph (Hernández et al. 2013). Finally, antileishmanial activity of several phenyl-linked oxadiazole–phenylhydrazone hybrids was evaluated, compound 1i being the most potent antileishmanial agent among this type of hybrids, displaying an IC\(_{50}\) of 0.95 ± 0.01 \(\mu\)M (Taha et al. 2017) (Fig. 1).

In the search for new therapeutic alternatives to treat cutaneous leishmaniasis and Chagas disease a number of triclosan–hydrazone hybrids have been designed and synthesized. Their leishmanicidal and trypanocidal activities, as well as their cytotoxicity in U-937 macrophages, have been evaluated in vitro (Fig. 2).

**Materials and methods**

**Chemical synthesis**

**General remarks**

Microwave reactions were carried out in a CEM Discover microwave reactor in sealed vessels (monowave, maximum power 300 W, temperature control by IR sensor, fixed

---

**Fig. 1** Compounds with antiprotozoal activity
temperature). $^1$H and $^{13}$C NMR spectra were recorded on a Varian instrument operating at 500 and 125 MHz, respectively. The signals of the deuterated solvent (CDCl$_3$) were used as reference (the singlet at $\delta = 7.27$ ppm for $^1$H NMR and the triplet centered at $\delta = 77.00$ ppm for $^{13}$C NMR). Carbon atom types (C, CH, CH$_2$, CH$_3$) were determined by using the DEPT or APT pulse sequence. Signals were assigned using two-dimensional heteronuclear correlations (COSY and HSQC). High-resolution mass spectra were recorded using electrospray ionization-mass spectrometry (ESI-MS). A QTOF Premier instrument with an orthogonal Z-spray (ESI-MS). A QTOF Premier instrument with an orthogonal Z-spray–electrospray interface (Waters, Manchester, UK) was used for operating in the W-mode. The drying and cone voltage was set to 10 V. For accurate mass measurements, a 2 mg/L standard solution of leucine enkephalin was introduced via the Z-spray electrically heated under microwave irradiation to reflux for a period of 5 min. Then, the reaction mixture was poured on ice and the resulting precipitate was filtered out, affording the title compound 3 in 83% yield (3.90 g, 0.011 mmol). Synthetic procedure for hydrazones

A triclosan–carbohydrazide 3 (0.5 g, 1.38 mmol) solution in methanol (2 mL) was sonicated for 2 min and then
benzaldehyde 4 (1.38 mmol) and acetic acid (0.1 mL) were added dropwise to the reaction mixture. Upon completion of the reaction (5–10 min), the product was filtered, sequentially washed with water (20 mL) and ethyl ether (5 mL), dried in vacuo and recrystallized from ethanol, affording the corresponding hydrzones in yields ranging from 42 to 93%.

(E)-2-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)-N’-(2-hydroxybenzylidene)acetohydrazide (5a) Yield 91% (1.256 mmol, 585 mg); white solid, m.p. 178–180 °C; IR (cm⁻¹): νmax max 3450 (Ar–OH), 3100 (N–H), 1680 (C=O), 1494 (C=N), 1409 (C=C₆H₅), 1259 ((C=O)–N), 829 (C=H₆), 754 (C=C). ¹H-NMR (DMF-d₇): δ 4.78, 5.23 (–1:1, –OCH₂ₛ, s), 6.82–6.94 (H₃, H₅, H₆, m), 7.01–7.10 (H₄, H₆, m), 7.19–7.31 (H₆, H₆, m), 7.34 (H₅, dd, δ = 8.9, 2.0 Hz), 7.18–7.38 (H₃, m), 8.28, 8.43 (–1:1, N=C–H, s), 9.98, 10.92 (OH), 11.50, 11.71 (–1:1, NH). ¹H (Acetone-d₆): δ 5.23, 4.79 (–2:1 –OCH₂ₛ, s), 8.28, 8.42 (–2:1, N=C–H, s), 11.53, 11.75 (–2:1, NH, s). ¹³C-NMR (DMF-d₇): δ 66.10, 67.34 (–1:1 –OCH₂ₛ, s), 115.58, 116.07 (C₆), 116.54, 116.80 (C₆), 119.08, 119.38 (C₇), 121.68, 122.38 (C₈), 122.62, 122.80 (C₈), 123.68, 129.94 (C₉), 127.06, 127.26 (C₉), 127.28, 127.61 (C₉), 128.74, 129.90 (C₉), 129.55, 129.80 (C₉), 129.93, 130.09 (C₁₀), 130.11, 130.22 (C₁₀), 131.65, 131.99 (C₁₁), 142.07, 142.66 (C₁₂), 148.08, 148.25 (C₁₂), 150.63, 151.10 (N=CH), 152.13, 152.35 (C₁₂), 156.83, 157.73 (C₁₂), 168.42 (C=O). EIMS: m/z 465.0176 [M + H]⁺, calcd for C₂₁H₁₅Cl₁₃N₂O₅: 481.0126.

(E)-2-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)-N’-(3,4-dihydroxybenzylidene)acetohydrazide (5d) Yield 64% (0.883 mmol, 425 mg); yellow solid, m.p. 222–226 °C; IR (cm⁻¹): νmax max 3448 (Ar–OH), 3344 (N–H), 1705 (C=O), 1496 (C=N), 1471 (C=C₆H₅), 1251 ((C=O)–N), 866 (C=H₆), 770 (C=C). ¹H-NMR (DMF-d₇): δ 4.75, 5.18 (–1:1, –OCH₂ₛ, s), 6.28–6.32 (H₃, H₅, m), 6.35, 6.33 (H₃, H₅, dd, δ = 2.2 Hz), 6.87, 6.89 (H₃, H₅, d, δ = 8.9 Hz), 7.01–7.10 (H₆, H₆, m), 7.20 (H₆, H₆, d, δ = 2.0 Hz), 7.27, 7.35 (H₆, H₆, m), 7.67, 7.70 (H₆, d, δ = 2.4 Hz), 8.15, 8.36 (–2:1, N=C–H, s), 11.07, 9.94 (OH), 11.31, 11.51 (–1:1, NH). ¹H (Acetone-d₆): δ 5.23, 5.31 (–2:1 –OCH₂ₛ, s), 8.16, 8.25 (–1:2, N=C–H, s), 11.39, 10.38 (–1:2, NH, s). ¹³C-NMR (DMF-d₇): δ 66.89, 67.77 (–OCH₂ₛ, s), 102.28, 102.52 (C₉), 110.30, 111.41 (C₁₀), 115.07, 115.56 (C₁₀), 118.62, 118.89 (C₁₀), 121.16, 121.87 (C₁₀), 122.12, 122.30 (C₁₀), 123.19, 123.45 (C₁₀), 126.79, 127.13 (C₁₀), 128.26, 128.41 (C₁₀), 129.31, 129.43 (C₁₀), 129.59, 129.62 (C₁₀), 129.73, 129.76 (C₁₀), 130.98, 131.00 (C₁₀), 142.17, 142.62 (C₁₀), 148.78 (C₁₀), 150.16, 150.64 (N=CH), 152.64, 151.87 (C₁₁), 158.01, 159.26 (C₁₂), 160.45, 160.82 (C₁₂), 162.87, 167.45 (C=O). EIMS: m/z 481.0125 [M + H]⁺, calcd for C₂₁H₁₅Cl₁₃N₂O₅: 481.0130.

(E)-2-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)-N’-(2,5-dihydroxybenzylidene)acetohydrazide (5e) Yield 90% (1.242 mmol, 597 mg); yellow solid, m.p. 220–224 °C; IR
Yield 63% (0.870 mmol, 430 mg); white solid, m.p. 148 °C.

(E)-2-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)-N’-(4-hydroxy-3-methoxybenzylidene) acetohydrazide (5f)
Yield 63% (0.870 mmol, 430 mg); white solid, m.p. 148–150 °C; IR (cm⁻¹): νmax max 3341 (Ar–OH), 3167 (N–H), 1667 (C=O), 1492 (C=N), 1471 (C=Caryl), 1255 ((C=O)–N), 910 (C=H-ar), 785 (C–Cl).¹H-NMR (DM SO-d6): δ 4.77, 5.22 (1:1, –OCH2 s), 6.67–6.74 (H3, H4, m), 6.89 (H5, d, J = 8.8 Hz), 6.94–7.11 (H6, H5, m), 7.21, 7.29 (H6, d, J = 2.0 Hz), 7.30, 7.34 (H5, dd, d = 8.9, 2.2 Hz), 7.68, 7.69 (H3, d, J = 2.2 Hz), 8.22, 8.35 (1:1, N=C–H, s), 8.86, 8.94, 9.29, 10.07 (OH), 11.46, 11.59 (1:1, NH).¹H (Acetone-d6): δ 3.58, 3.84 (~1:2, –OCH2 s), 8.21, 8.28 (~1:2, N=C–H, s), 11.54, 10.62 (~1:2, NH, s).¹3C-NMR (DM SO-d6): δ 65.60, 66.86 (~OCH2 s), 111.39, 113.35 (Cp), 115.00, 115.54 (C6), 116.91, 117.00 (C7, C5), 118.64, 118.77 (C7), 118.88, 119.09 (C3), 120.12 (C7), 121.19, 121.85 (C8), 122.12, 122.28 (C4), 123.21, 123.44 (C4), 126.81, 127.12 (C6), 128.26, 128.40 (C4), 129.32, 129.44 (C3), 129.61, 129.72 (C4), 142.51, 142.40 (C6), 146.79, 147.10 (N=C), 149.36, 149.84 (C8), 149.87, 150.04 (C1), 150.15, 150.61 (C1), 151.64, 151.86 (C7, C5), 162.23, 167.83 (C=O). EIMS: m/z 495.0281 [M + H]+, calcd for C22H17Cl3N2O5: 495.0278.

(E)-2-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)-N’-(3,4-dihydroxy-5-methoxybenzylidene) acetohydrazide (5g)
Yield 78% (1.076 mmol, 550 mg); yellow solid, m.p. 106–110 °C; IR (cm⁻¹): νmax max 3466 (Ar–OH), 3280 (N–H), 1659 (C=O), 1495 (C=N), 1475 (C=Caryl), 1269 ((C=O)–N), 806 (C=Haryl), 716 (C–Cl).¹H-NMR (DM SO-d6): δ 3.31–3.79 (~OCH3 s), 4.73, 5.22 (~1:1, ~OCH2 s), 6.76–6.82 (H3, m), 6.89 (H3, d, J = 8.8 Hz), 7.02, 7.04 (H2, H5, d, J = 2 Hz), 7.07 (H4, d, J = 9.5 Hz), 7.19, 7.27 (H4, d, J = 2.0 Hz), 7.39, 7.54 (H5, dd, d = 8.9, 2.5 Hz), 7.79. EIMS: m/z 497.0074 [M + H]+, calcd for C23H18Cl3N2O6: 497.0075.

¹H (Acetone-d6): δ 3.42–6.53 (H3, H5, m), 6.88, 6.89 (H5, d, J = 8.9 Hz), 6.98–7.11 (H4, H6, m), 7.21, 7.28 (H6, d, J = 2.0 Hz), 7.30, 7.34 (H4, dd, d = 8.8, 2.4 Hz), 7.42, 7.57 (H6, d, J = 8.6 Hz), 7.68, 7.70 (H3, d, J = 2.4 Hz), 8.19, 8.33 (~1:1, N=C–H, s), 10.15 (OH), 11.25, 11.41 (~1:1, NH).¹H (Acetone-d6): δ 4.82, 5.25 (~1:1, ~OCH2 s), 8.30, 8.20 (~3:1, N=C–H, s), 10.58, 10.64 (~3:1, NH, s).¹3C-NMR (DM SO-d6): δ 55.37, 55.62 (~OCH3 s), 66.00, 67.70 (~OCH2), 101.30, 101.55 (C5), 106.80, 106.99 (C5), 112.07, 113.36 (C1), 114.46, 115.95 (C6), 119.04, 119.32 (C3), 121.66, 122.34 (C1), 122.68, 122.87 (C4), 123.64, 123.89 (C7), 127.26, 127.59 (C2), 128.59, 128.79 (C7), 128.95, 129.22 (C8), 129.84, 129.96 (C3), 130.13, 130.25 (C7), 142.56, 143.02 (N=C=H), 147.88, 148.86 (C1), 150.67, 151.14 (C2), 152.17, 152.37 (C5), 154.81, 155.69 (C4), 162.36, 162.66 (C4), 163.56, 168.14 (C=O). EIMS: m/z 495.0281 [M + H]+, calcd for C23H17Cl3N2O5: 495.0278.
Yield 93% (1.283 mmol, 674 mg); white solid, m.p. 210 °C; IR (cm\(^{-1}\)) 3383 (Ar-OH), 1485 (C=O). EIMS: m/z 511.0230 [M + H]+, calcd for C\(_{23}H_{19}Cl_3N_2O_6\): 511.0152.

(E)-2-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)-N′-(4-hydroxy-3,5-dimethoxybenzylidene)acetohydrazide (5j) Yield 93% (1.283 mmol, 674 mg); white solid, m.p. 166–170 °C; IR (cm\(^{-1}\)) \(\nu_{\max}\) max 3383 (Ar-OH), 3224 (N-H), 1662 (C=O, cm\(^{-1}\)), 1494 (C=N), 1269 (C=O–N), 803 (C–H\(_2\)), 712 (C–Cl). \(^1\)H-NMR (DMSO-d\(_6\)): \(\delta\) 3.79, 3.80 (–OCH\(_3\), s), 4.75, 5.26 (–1:1, –OCH\(_2\), s), 6.86, 6.88 (H\(_1\), d, \(J = 8.9\) Hz), 6.96 (H\(_6\), d, \(J = 8.6\) Hz), 7.01, 7.04 (H\(_2\)–H\(_4\), H\(_5\)–H\(_6\), d, \(J = 2.0\) Hz), 7.07 (H\(_2\), d, \(J = 8.9\) Hz), 7.19, 7.27 (H\(_6\), d, \(J = 8.9, 2.5\) Hz), 7.29, 7.34 (H\(_5\), dd, \(J = 8.9, 2.5\) Hz), 7.69, 7.71 (H\(_4\), d, \(J = 2.5\) Hz), 7.85, 8.03 (–1:1, N=C–H, s), 8.87, 8.43 (OH). \(\nu_{\max}\) max 3383 (Ar-OH), 1485 (C=O). EIMS: m/z 511.0230 [M + H]+, calcd for C\(_{23}H_{19}Cl_3N_2O_6\): 511.0152.

(E)-2-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)-N′-(4-hydroxy-3,5-dimethoxybenzylidene)acetohydrazide (5k) Yield 42% (0.580 mmol, 288 mg); light yellow solid, m.p. 210–214 °C; IR (cm\(^{-1}\)) \(\nu_{\max}\) max 3425 (Ar-OH), 3310 (N-H), 1687 (C=O), 1495 (C=N), 1473 (C=C\(_\alpha\)), 1259 (C=O–N), 827 (C–H\(_2\)), 702 (C–Cl). \(^1\)H-NMR (DMSO-d\(_6\)): \(\delta\) 4.74, 5.14 (–1:1, –OCH\(_2\), s), 5.83 (H\(_3\), H\(_5\), m), 6.87 (H\(_2\), H\(_6\), d, \(J = 8.9\) Hz), 6.99–7.09 (H\(_4\), m), 7.25–7.30 (H\(_6\), m), 7.34 (H\(_5\), dd, \(J = 8.9, 2.5\) Hz), 7.69, 7.68 (H\(_3\), d, \(J = 2.5\) Hz), 8.35, 8.55 (–1:1, N=C–H, s), 10.92, 10.25, 9.85 (OH), 11.38, 11.58 (–1:1, NH). \(^1\)H Acetone-d\(_6\)): \(\delta\) 5.22, 4.80 (–1:4, –OCH\(_2\), s), 8.54, 8.63 (–1:4, N=C–H, s), 10.24, 10.37 (–1:4, NH, s). \(^13\)C-NMR (DMSO-d\(_6\)): \(\delta\) 56.48, 67.24 (–OCH\(_2\)), 94.80 (C\(_3\)), 99.14, 99.25 (CH), 116.01, 116.08 (C\(_9\), 119.05, 119.35 (C\(_3\)), 121.69, 122.35 (C\(_9\)), 122.63, 122.79 (C\(_9\)), 123.91, 123.64 (C\(_2\)), 127.26, 127.61 (C\(_5\)), 127.87, 128.49 (C\(_2\)), 129.82, 129.97 (C\(_3\)), 130.11, 130.26 (C\(_3\)), 142.52, 143.05 (N=–C\(_\alpha\)), 144.59, 147.26 (C\(_2\)), 150.07, 151.03 (C\(_1\)), 152.13, 152.35 (C\(_1\)), 156.79, 160.13 (C\(_2\), C\(_\alpha\)), 162.21, 163.09 (C\(_\alpha\)), 165.53, 167.12 (C=O). EIMS: m/z 497.0074 [M + H]+, calcd for C\(_{23}H_{19}Cl_3N_2O_6\): 497.0074.

Biological activity assays

The compounds were subjected to in vitro evaluation as regards their cytotoxicity, leishmanicidal, and trypanocidal activity against U-937 human cells and intracellular amastigotes of L. (V) panamensis and T. cruzi, respectively.

In vitro cytotoxicity

The cytotoxic activity of the compounds was assessed based on the viability of the human promonocytic cell line U-937 (ATCC CRL-1593.2TM) evaluated by the MIT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay following the methodology described elsewhere (Pulido et al. 2012). Briefly, cells grown in tissue flasks were harvested and washed with phosphate buffered saline (PBS) by centrifuging. Cells were counted and adjusted at 1 × 10\(^6\) cells/mL of RPMI-1640 supplemented with complete 10% fetal bovine serum (FBS) and 1% antibiotics (100 U/mL penicillin and 0.1 mg/mL streptomycin). One hundred µL was dispensed into each well of a 96-well cell-culture plate and then 100 µL of RPMI-1640 and the corresponding concentrations of the compounds were added, starting at 200 µg/mL in duplicate. Plates were incubated at 37 °C, 5% CO\(_2\) for 72 h in the presence of compounds. The effect of compounds was determined by measuring the activity of the mitochondrial dehydrogenase by adding 10 µL/well of MITT solution (0.5 mg/mL) and incubated at 37 °C for 3 h. The reaction was stopped by adding 100 µL/well of 50% isopropanol solution with 10% sodium dodecyl sulfate and 30 min incubation. The cell viability was determined based on the quantity of formazan produced according to the intensity of color (absorbance) registered as optical densities (O.D.) obtained at 570 nm in a spectrophotometer (VarioskanTM Flash Multimode Reader—Thermo Scientific, USA). Cells cultured in the absence of compounds were used as control of viability (100% viability), while amphotericin B (AmB) was used as non-cytotoxic and cytotoxic drug control, respectively. Assays were conducted in two independent runs with three replicates per each concentration tested.

In vitro anti-leishmanial activity

The activity of the compounds was evaluated on intracellular amastigotes of L. (V) panamensis transfected with the
green fluorescent protein gene (MHOM/CO/87/UA140-EGFP) (Taylor et al. 2011). The effect of each compound was determined according to the inhibition of the infection evidenced by the decrease of the infected cells and parasite inside the cells. Briefly, U-937 human cells at a concentration of \(3 \times 10^5\) cells/mL in RPMI 1640 and 0.1 \(\mu\)g/mL of phorbol-12-myristate-13-acetate (PMA) were dispensed into each well of a 24-well cell culture plate and then infected with 5 days old promastigotes in a 15:1 parasites per cell ratio. Plates were incubated at 34 \(^\circ\)C, 5% CO\(_2\) for 3 h and cells were washed two times with PBS to eliminate internalized parasites. One mL of fresh RPMI 1640 supplemented with 10% FBS and 1% antibiotics was added into each well, cells were incubated again to guarantee multiplication of intracellular parasites. After 24 h of infection, culture medium was replaced by fresh culture medium containing each compound at four-fold dilutions (100–25–6.25 and 1.56 \(\mu\)g/mL) and plates were incubated at 37 \(^\circ\)C, 5% CO\(_2\). After 72 h, inhibition of the infection was determined. Shortly, cells were removed from the bottom plate with a trypsin/EDTA (250 mg) solution, recovered cells were centrifuged at 1100 rpm for 10 min at 4 °C, the supernatant was discarded and cells were washed with 1 mL of cold PBS by centrifuging at 1100 rpm for 10 min at 4 °C. The supernatant was discarded and cells were suspended in 500 \(\mu\)L of PBS and analyzed by flow cytometry (FC 500MPL, Cytomics, Brea, CA, USA). All determinations for each compounds and standard drugs were carried out in triplicate, in two independent experiments (Pulido et al. 2012). Activity of the tested compounds was carried out in parallel with infection progress in culture medium alone and in culture medium with AmB as antileishmanial drugs.

**In vitro anti-trypanosomal activity**

Compounds were tested on intracellular amastigotes of *T. cruzi*, Tulahuen strain transfected with \(\beta\)-galactosidase gene (donated by Dr. F. S. Buckner, University of Washington) (Buckner et al. 1996). The activity was determined according to the ability of the compounds to reduce the infection of U-937 cells by *T. cruzi*. In this case, 100 \(\mu\)L of U-937 human cells at a concentration of 2.5 \(\times\) \(10^5\) cells/mL in RPMI-1640, 10% SFB, and 0.1 \(\mu\)g/mL of PMA were placed in each well of 96-well plates and then infected with phase growth epimastigotes in 5:1 (parasites per cell) ratio and incubated at 34 \(^\circ\)C, 5% CO\(_2\). After 24 h of incubation four-fold dilutions of each compound (100–25–6.25 and 1.56 mg/mL) were added to infected cells. After 72 h of incubation, the effect of all compounds on viability of intracellular amastigotes was determined by measuring the \(\beta\)-galactosidase activity by spectrophotometry adding 100 \(\mu\)M CPRG and 0.1% nonidet P-40 to each well. After 3 h of incubation, plates were read at 570 nm in a spectrophotometer (Varioskan™ Flash Multimode Reader—Thermo Scientific, USA) and intensity of color (absorbance) was registered as O.D. Infected cells exposed to benznidazol (BNZ) were used as control for anti-trypanosomal activity while infected cells incubated in culture medium alone were used as control for infection. Non-specific absorbance was corrected by subtracting the O.D. from the blank. Determinations were done by triplicate in at least two independent experiments (Buckner et al. 1996; Insuasty et al. 2015).

**Statistical analysis**

Cytotoxicity was determined according to viability and mortality percentages obtained for each isolated experiment (compounds, amphotericin B, Benznidazole, and culture medium alone). The results were expressed as 50 lethal concentrations (LC\(_{50}\)) that corresponds to the concentration necessary to eliminate 50% of cells and calculated by Probit analysis (Finney 1978). Percentage of viability was calculated by Eq. 1, where the O.D. of control corresponds to 100% of viability. In turn, mortality percentage corresponds to 100%-%viability:

\[
\% \text{ Viability} = \frac{(\text{O.D Exposed cells})}{(\text{O.D unexposed cells})} \times 100
\]

The degree of toxicity was graded according to the LC\(_{50}\) value using the following scale: high cytotoxicity: LC\(_{50}\) < 200 \(\mu\)M; moderate cytotoxicity: LC\(_{50}\) > 200–<400 \(\mu\)M and potential non-cytotoxicity: LC\(_{50}\) > 400 \(\mu\)M.

Anti-leishmanial activity was determined according to the percentage of infection (amount of parasites in infected cells) obtained for each experimental condition by flow cytometry. The percentage of infected cells was determined as the number of positive events by double fluorescence (green for parasites and red for cells) using dotplot analysis. On the other hand, the amount of parasites in the infected cells was determined by analysis of mean fluorescence intensity (MFI) in fluorescent parasites (Pulido et al. 2012). The parasite inhibition was calculated by Eq. 2, where the MFI of control corresponds to 100% of parasites. In turn, inhibition percentage corresponds to 100%-%Parasites. Results of anti-leishmanial activity were expressed as EC\(_{50}\) determined by the Probit method (Finney 1978):

\[
\% \text{ Parasites} = \frac{(\text{MFI Exposed parasites})}{(\text{MFI unexposed parasites})} \times 100
\]
The parasite inhibition was calculated by Eq. 3, where the O.D. of unexposed parasites corresponds to 100% of parasites. In turn, percentage of inhibition corresponds to 100%–% Parasites. Results of anti-trypanosomonal activity were also expressed as EC_{50} determined by the Probit method (Finney 1978):

\[
\% \text{ Parasite} = \frac{(O.D \text{ Exposed parasites})}{(O.D \text{ Unexposed parasites})} \times 100
\]

The anti-leishmanial and anti-trypanosomonal activities were graded according to the EC_{50} value using the following scale: high activity: EC_{50} < 40 μM, moderate activity: EC_{50} > 40–<80 μM; and potential non-activity: EC_{50} > 80 μM.

The selectivity index (SI), was calculated by dividing the cytotoxic activity and the anti-leishmanial or anti-trypanosomonal activity using the following formula: SI = LC_{50}/EC_{50}.

**Results and discussion**

**Chemistry**

Microwave-assisted Williamson etherification of triclosan 1 with ethyl bromoacetate gave rise to ester 2 in 75% yield (Otero et al. 2014). Nucleophilic reaction of hydrazine hydrate on compound 2 gave rise to acylhydrazide 3 in 83% yield. Coupling compound 3 with a number of aldehydes in alcoholic medium provided hydrazones 5a–6h in 42–93% yields (Coa et al. 2015). This synthetic strategy involves ultrasound-assisted or microwave-assisted reactions which allows to achieve the compounds with shorter reaction times than the conventional heating methods. In addition, the products were obtained in very good to excellent yields and without appreciable by-product formation (Scheme 1).

The structures and stereochemistry of all hydrazones have been established by a combined study of IR, ESI-MS, ^1^H-NMR, ^1^C-NMR, COSY, and NOESY spectra. IR spectra exhibited characteristic absorption peaks corresponding to Ar–OH (3325–3468 cm⁻¹), N–H (3100–3290 cm⁻¹), C=O (1658–1705 cm⁻¹), C=N (1492–1598 cm⁻¹), C=C_{Ar} (1409–1480 cm⁻¹), (C=O–N (1251–1274 cm⁻¹), C–H_{Ar} (802–910 cm⁻¹), and C–Cl (702–780 cm⁻¹). ESI-MS spectra showed characteristic [M + 1]^+ peaks corresponding to their molecular weights. The assignments of all the signals to individual H-atoms or C-atoms have been performed on the basis of typical δ-values and J-constants. The ^1^H-NMR and ^1^C-NMR spectra of these compounds dissolved in DMSO-d_{6} showed double signals. For example, for compounds 5a we have the following ^1^H-NMR signals: C(=O)–NH– (11.50, 11.71 ppm), –N=CH (8.28, 8.43 ppm), and Ar–O–CH_{2}– (4.78, 5.23 ppm). ^1^C-NMR spectra showed signals at 150.63 and 151.10 ppm due to the presence of CH=N, signals at 163.83 and 168.42 ppm corresponding to C=O group and signals to 66.10 and 67.34 due to Ar=O–CH_{2}–, which indicated that these molecules are present in two stereoisomeric forms (e.g., geometric or conformational isomers) (see Fig. 3).

Integration values of these signals showed a ~1:1 ratio between the two stereoisomers when compounds were dissolved in DMSO-d_{6}. However, a ~2:1 ratio was obtained when ^1^H-NMR spectra of hydrazones were taken in acetone-d_{6}. This result shows the presence of an equilibrium system that is dependent on the polarity of the solvent (Rahman et al. 2005). NOESY experiment showed coupling between C(=O)–NH and –N=CH, and between –N=CH and Ar–O–CH_{2}– hydrogens. These couplings can be explained for the presence of E-antiperiplanar isomer, which is depicted in Fig. 3 (Basilio et al. 2013). Therefore, the other constituent of the isomeric mixture must be the E-syneriplanar isomer (see Fig. 3).

**Biological activities**

The effect of hydrazones on cell growth and viability was assessed in human macrophages (U-937 cells) (Pulido et al. 2015), which are the host cells for L. (V) panamensis and T. cruzi parasites. On the other hand, the antiparasite activity of these compounds was tested on intracellular amastigotes of L. (V) panamensis (Taylor et al. 2011) and T. cruzi (Buckner et al. 1996; Insuasty et al. 2015) according to the ability of these compounds to reduce the amount of parasite inside infected macrophages. Results are summarized in Table 1.

All synthetic hydrazones, with the exception of 5c, 5e, and 5h, were highly cytotoxic to U-937 cells showing LC_{50} < 200.0 μM (Table 1). Compounds 5c, 5e, and 5h showed no cytotoxicity (LC_{50} > 400 μM). In turn, amphotericin B and benznidazole showed high and moderate and no cytotoxicity, respectively.

The anti-leishmanial and anti-trypanosomonal activities were measured by determining the effective concentration 50 (EC_{50}) that corresponds to the concentration of drug that gives the half-maximal reduction of the amount of intracellular parasites (Table 1). Dose–response relationship showed that compounds 5a–5d, 5f–5j, and triclosan were active against intracellular amastigotes of L. (V) panamensis with EC_{50} < 40 μM. The most active hybrid compounds were 5a, 5d, 5g, 5f, 5j, and 5i with an EC_{50} of >1.29, 1.64, 2.36, 6.88, 9.30, and 11.92 μM, respectively, followed by 5b, 5c, triclosan, and 5h with EC_{50} of 24.70, 25.08, 38.61, and 39.24 μM, respectively. Compounds 5k and 5e showed moderate activity with a EC_{50} > 40 μM. As expected, the anti-leishmanial drug amphotericin B showed activity with low EC_{50} values.
On the other hand, compounds 5d, 5a, 5f, 5g, and 5i were highly active against intracellular amastigotes of T. cruzi with EC\textsubscript{50} of 2.28, 2.36, >5.04, 2.95, and 11.31 \( \mu \)M, respectively, followed by compounds 5j, 5c, 5h, and triclosan with an EC\textsubscript{50} of 25.11, 31.31, 37.11, and 48.97 \( \mu \)M, respectively. In this case, benznidazole showed activity with an EC\textsubscript{50} of 40.3 \( \mu \)M.

In general, the anti-leishmanial and anti-trypanosomal activity of the hydrazones were higher than their cytotoxicity. Thus, the SI values calculated for these compounds were >1. Compounds 5c, 5e, and 5h showed the best SI with values from 5.90 to 16.55 (Table 1). Amphotericin B has very high SI values. Benznidazole exhibited a SI of 17.0. Although several hybrid compounds showed better activity than benznidazole, the SI of these compounds is affected by their cytotoxicity. These results suggest that biological activity of the triclosan–hydrazone hybrids is selective, being more active against T. cruzi parasites than U-937 cells.

On a structure–activity relationship basis, it is worth noting the synergistic effect of the parent subunits in the hybrids in comparison with the unlinked cases. For example, triclosan is by itself less potent than their hybrids 5a, 5d, 5f, 5g, 5i, and 5j. The presence of hydroxy or methoxy groups in positions 2 and 4 on the benzylidene moiety increases both the activity and cytotoxicity (5d and 5g vs. 5b, 5c, and 5e), which could be explained by a better molecular recognition ability towards target bioreceptors.

There is not a clear relationship between the antiprotozoal activity and the methylation of the hydroxy groups, since in some cases methylation decreases the activity (5d vs. 5g) while in other cases an increase in activity is measured (5c vs. 5f and 5i vs. 5j). Furthermore, increasing the number of hydroxy groups decreases the activity (5a vs. 5b and 5h; 5a vs. 5d and 5k). The biological activity of these compounds could be explained by their action as iron chelators (Walcourt et al. 2004; Coa et al. 2015) or/and as alkylating...
agents (Michael acceptor) (Cardona et al. 2014). Studies in vitro have shown that chelating agents are able to inhibit parasite growth and proliferation by deprivation of iron, which is an essential nutrient for cell growth and division (Richardson et al. 1995). An electrophilic-conjugated system could be generated from o-hydroxybenzylidene-N-acylhydrazone framework due to the ability of this system to be converted into an electrophilic quinone methide intermediate through a pericyclic rearrangement (Ifa et al. 2000). The generation of such a system would allow conjugate addition of nucleophilic amino acid residues such as those found in Leishmania cysteine proteases (Mottram et al. 2004).

Conclusions

The synthesis, cytotoxicity, and activity against *L. (V) panamensis* and *T. cruzi* amastigotes of 11 triclosan–hydrazone hybrids are reported. Hydrazones were obtained as two *E*-synperiplanar and *E*-antiperiplanar conformers. Nine of them were active against *L. panamensis* (*5a–5d, 5f–5j*) and eight of them were active against *T. cruzi* (*5a, 5c, 5d, 5f–5j*), with EC50 values lower than 40 μM. Compounds *5c, 5e*, and *5h* showed the best SI against both *L. panamensis* and *T. cruzi*, with values between 5.90 and 16.55. These results suggest that these compounds have potential as templates for drug development against protozoal diseases. The presence of hydroxy or methoxy groups in positions 2 and 4 on the benzylidene moiety increases both activity and cytotoxicity. There is no clear relationship between the antiprotozoal activity and the methylation pattern of the hydroxy groups, since in some cases methylation decreases the activity (*5d* vs. *5g*), while in other cases the activity is increased (*5c* vs. *5f* and *5i* vs. *5j*). The mechanism of action of these compounds needs to be addressed and will be the objective of further studies.

---

Table 1 In vitro cytotoxicity and antiprotozoal activity of triclosan–hydrazone hybrids

| Compound | Cytotoxicity | Anti-leishmanial activity | Anti-trypanosomal activity |
|----------|-------------|---------------------------|----------------------------|
|          | LC50 (Mean ± SEM) [μM] | EC50 (Mean ± SEM) [μM] | SI | EC50 (Mean ± SEM) [μM] | SI |
| 5a       | 2.79 ± 0.65 | >1.29                     | <2 | 2.36 ± 0.13 | 1.18 |
| 5b       | 91.96 ± 9.38 | 24.70 ± 1.54 | 3.72 | 47.81 ± 4.24 | 1.92 |
| 5c       | >415.19 | 25.08 ± 0.44 | >16.55 | 31.31 ± 1.27 | >13.26 |
| 5d       | 2.08 ± 0.13 | 1.64 ± 0.27 | 1.31 | 2.28 ± 0.21 | 0.95 |
| 5e       | >415.19 | 70.33 ± 12.48 | 5.90 | 58.48 ± 6.85 | 7.10 |
| 5f       | 10.89 ± 0.81 | 6.88 ± 0.36 | 1.58 | >5.04 | <2.16 |
| 5g       | 5.45 ± 0.20 | 2.36 ± 0.12 | 2.28 | 2.95 ± 0.49 | 1.83 |
| 5h       | >401.84 | 39.24 ± 2.18 | >10.24 | 37.11 ± 6.43 | >10.83 |
| 5i       | 36.15 ± 5.10 | 11.92 ± 0.82 | 3.03 | 11.31 ± 0.27 | 3.20 |
| 5j       | 23.78 ± 5.15 | 9.30 ± 0.52 | 2.55 | 25.11 ± 4.31 | 0.95 |
| 5k       | 62.08 ± 1.61 | 45.61 ± 6.19 | 1.36 | 42.39 ± 6.73 | 1.46 |
| Triclosan | 193.41 ± 32.81 | 38.61 ± 2.38 | 5.01 | 48.97 ± 4.21 | 3.95 |
| Amphotericin B | 45.60 ± 2.16 | 0.054 ± 0.011 | 842 | NA | NA |
| Benzimidazole | 687.8 ± 16.14 | NA | NA | 40.3 ± 6.92 | 17.0 |

Data represent mean value ± standard deviation

LC50, lethal concentration 50 in μM, EC50, effective concentration 50 in μM, SI, selectivity index = LC50/EC50, NA, not applicable.
Acknowledgements The authors thank COLCIENCIAS (contract no. 0333-2013, code: 111556933423) for financial support.

Compliance with ethical standards

Conflict of interests The authors declare that they have no competing interests.

References

Alvar J, Vélez ID, Bern C, Herrera M, Desjeux P, Cano J, Jamin J, den Boer M (2012) Leishmaniasis worldwide and global estimates of its incidence. PLOS One 7:e35671

Arango V, Domínguez JJ, Cardona W, Robledo SM, Muñoz DL, Figadere B, Saéz J (2012) Synthesis and leishmanicidal activity of quinoline-tricosan and quinoline-eugenol hybrids. Med Chem Res 21:3445–3454

Basilio A, Miguez E, Kümmelper AE, Rumianek VM, Manssour CA, Barreiro EJ (2013) Characterization of amide bond conformers for a novel heterocyclic template of N-acrylhydrazine derivatives. Molecules 18:111683–11704

Bernardino AM, Gomes AO, Charret KS, Freitas AC, Machado GM, Canto-Cavalheiro MM, Leon LL, Amaral VF (2006) Synthesis and leishmanicidal activities of 1-(4-X-phenyl)-1″-[(4-Y-phenyl)methylene]-1H-pyrazole-4-carboxyhydrazides. Eur J Med Chem 41:80–87

Bhutta ZA, Sommerfeld J, Lassi ZS, Salam RA, Das JK (2014) Tackling the existing burden of infection diseases in the developing world: existing gaps and the way forward. Infect Dis Poverty 3:1–6

Buckner FS, Verlinde CL, La Flamme AC, Van Voorhis WC (1996) δ-substituted α,β-unsaturated cyclic lactones with antileishmanial activity. Mol Simul 40:477–484

Carvalho S, Fetiota L, Soares M, Costa T, Henriques MG, Salomão K, de Castro SL, Kaiser M, Brun R, Wardell JL, Wardell S, Trossini G, Andricopulo AD, da Silva EF, Fraga C (2012) Design and synthesis of new (E)-cinnamic N-acrylhydrazones as potent antipyramsonama cruzi using parasites expressing beta-galactosidase. Antimicrob Agents Chemother 40:2592–2597

Cardona W, Guerra D, Restrepo A (2014) Reactivity of δ-substituted α,β-unsaturated cyclic lactones with antileishmanial activity. Mol Simul 40:477–484

Carvalho S, Fetiota L, Soares M, Costa T, Henriques MG, Salomão K, de Castro SL, Kaiser M, Brun R, Wardell JL, Wardell S, Trossini G, Andricopulo AD, da Silva EF, Fraga C (2012) Design and synthesis of new (E)-cinnamic N-acrylhydrazones as potent antipyramsonama cruzi using parasites expressing beta-galactosidase. Antimicrob Agents Chemother 40:2592–2597

Chatelain E, Ioset JR (2011) Drug discovery and development for neglected diseases. Nature Reviews Drug Discovery 10:746–753

Cao IC, Castrillón W, Cardona W, Carda M, Osipina V, Muñoz JA, Vélez ID, Robledo SM (2015) Synthesis, leishmanicidal, trypanocidal and cytotoxic activity of quinoline-hydrazone hybrids. Eur J Med Chem 101:746–753

Den Boer M, Argaw D, Jannin J, Alvar J (2011) Leishmaniasis impact and treatment access. Clin Microbiol Infect 17:1471–1477

Finney JD (1978) Probit analysis: statistical treatment of the sigmoid dose–response curve, 3rd edn. Cambridge University Press, Cambridge, UK, p 550

Hernández P, Rojas R, Gilman RH, Sauvain M, Lima LM, Barreiro EJ, González M, Cerecetto H (2013) Hybrid furaxanyl N-acrylhydrazine derivatives as hits for the development of neglected diseases drug candidates. Eur J Med Chem 59:64–74

Ifa DR, Rodrigues CR, Alencastro RB, Fraga CAM, Barreiro EJ (2000) A possible molecular mechanism for the inhibition of cysteine proteases by salycilaldehyde N-acrylhydrazones and related compounds. J Mol Struct Theochem 505:11–17

Insuaity B, Ramírez J, Becerra D, Echeverry C, Quiroga J, Abonia R, Robledo SM, Velez ID, Upegui Y, Muñoz JA, Osipina V, Nogueras M, Cobo J (2015) An efficient synthesis of a new caffeine-based chalcones, pyrazolines and pyrazololo[3-4-b][1-4] diazepines as potential antimalarial, antitrypanosomal and antileishmanial agents. Eur J Med Chem 93:401–413

Jorge SD, Plateau-Berl F, Mesquita Pasqualto KF, Ishii M, Ferreira AK, Berra CM, Bosch RV, Maria DA, Tavares LC (2013) Ligand-based design, synthesis, and experimental evaluation of novel benzofuroxan derivatives as anti-Trypanosoma cruzi agents. Eur J Med Chem 64:200–214

Kapoor M, Reddy C, Krishnasasav MY, Surolia N, Surolia A (2004) Slow-tight-binding inhibition of enolyl-acyl carrier protein reductase from Plasmodium falciparum by triclosan. Biochem J 381:719–724

Keenan M, Chaplin JH (2015) A new era for chagas disease drug discovery? Prog Med Chem 54:185–230

Keith CT, Borisy A, Stockwell BR (2005) Multicomponent therapeutics for networked systems. Nat Rev Drug Discov 4:71–78

Massarico Serafim RA, Gonçalves JE, de Souza FP, de Melo Loureiro AP, Raffortis S, Krogh R, Andricopulo AD, Dias LC, Ferreira EI (2014) Design, synthesis and biological evaluation of hybrid bioisoster derivatives of N-acrylhydrazine and furoxan groups with potential and selective anti-Trypanosoma cruzi activity. Eur J Med Chem 82:418–425

McLeod R, Muench SP, Rafferty JB, Kyle DE, Mui EJ, Kirsits MJ, Mack DG, Roberts CW, Samuel BU, Lyons RE, Dorris M, Milhous WK, Rice DW (2004) Triclosan inhibits the growth of Plasmodium falciparum and Toxoplasma gondii by inhibition of apicomplexan Fab I. Int J Parasitol 34:109–113

Meunier B (2008) Hybrid molecules with a dual mode of action: dream or reality? Acc Chem Res 41:69–77

Mottram JC, Coombs GH, Alexander J (2004) Cysteine peptidases as virulence factors of Leishmania.Curr Opin Microbiol 7:375–381

Nouvellet P, Cucunubá ZM, Gourbière S (2015) Ecology, evolution and control of Chagas disease: a century of neglected modelling and promising future. Adv Parasitol 87:135–191

Opsenica I, Opsenica D, Lanteri CA, Anova L, Milhous WK, Smith KS, Solaja BA (2008) New chimeric antimalarials with 4-aminoquinoline moiety linked to a tetraoxane skeleton. J Med Chem 51:6216–6219

Otero E, Vergara S, Robledo SM, Cardona W, Carda M, Vélez ID, Rojas C, Otulvaro F (2014) Synthesis, leishmanicidal and cytotoxic activity of triclosan-chalcone, triclosan-chromone and triclosan-coumarin hybrids. Molecules 19:13251–13266

Porcal W, Hernández P, Boiain L, Boiain M, Ferreira A, Chidichimo A, Cazzulo J, Olea-Azar C, González M, Ceretccito H (2008) New trypanocidal hybrid compounds from the association of hydrazine moieties and benzofuroxan heterocycle. Bioorg Med Chem 16:6995–7004

Perozzo R, Kuo M, Sidhu AbS, Valiyaveettil JT, Bittman R, Jacobs Jr WR, Fidock DA, Sacchettini JC (2002) Structural elucidation of the specificity of the antibacterial agent triclosan for malarial enoyl acyl carrier protein reductase. J Biol Chem 277:13106–13114

Pulido SA, Muñoz DL, Restrepo AM, Mesa CV, Alzate JF, Velez ID, Robledo SM (2012) Improvement of the green fluorescent protein reporter system in Leishmania spp. for the in vitro and in vivo screening of antileishmanial drugs. Acta Trop 122:36–45

Rahman M, Mukhtar S, Ansari WH, Lemiere G (2005) Synthesis, and promising future. Adv Parasitol 87:135–191

Rahman M, Mukhtar S, Ansari WH, Lemiere G (2005) Synthesis, stereochemistry and biological activity of some novel long alkyl chain substituted thiazolidine-4-ones and thiazan-4-one from 10- undecenoic acid hydrazide. J Med Chem 40:173–184

Rando D, Avery M, Tekwani B, Khan S, Ferreira E (2008) Antileishmanial activity screening of 5-nitro-2-heterocyclic benzylidine hydrazides. Bioorg Med Chem 16:6724–6731
Richardson DR, Tran EH, Ponka P (1995) The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents. Blood 86:4295–4306
Rollas S, Küçükgüzel ŞG (2007) Biological activities of hydrazone derivatives. Molecules 12:1910–1939
Roth BL, Sheffler DJ, Kroeze WK (2004) Magic shotguns versus magic bullets: Selectively non-selective drugs for mood disorders and schizophrenia. Nat Rev Drug Discov 3:353–359
Singh M, Raghav N (2011) Biological activities of hydrazones: a review. Int J Pharm Pharm Sci 3:26–32
Surolia N, Surolia A (2001) Triclosan offers protection against blood stages of malaria by inhibiting enoyl-ACP reductase. Nat Med 7:167–173
Taha M, Ismail NH, Ali M, Khan KM, Jamil W, Kashif SM, Asraf M (2014) Synthesis of indole-2- hydrazones in search of potential leishmanicidal agents. Med Chem Res 23:5282–5293
Taha M, Ismail NH, Imran S, Anouar EH, Selvaraj M, Jamil W, Ali M, Kashif SM, Rahim F, Khan KM, Adenan MI (2017) Synthesis and molecular modelling studies of phenyl linked oxadiazole-phenylhydrazone hybrids as potent antileishmanial agents. Eur J Med Chem 126:1021–1033
Taylor VM, Cedeño DL, Muñoz DL, Jones MA, Lash TD, Young AM, Constantin MH, Esposito N, Vélez ID, Robledo SM (2011) In vitro and in vivo studies of the utility of dimethyl and diethyl carbaporphyrin ketal in treatment of cutaneous leishmaniasis. Antimicrob Agents Chemother 55:4755–4764
Verma G, Marela A, Shauquzzaman M, Akhtar M, Rahmat Ali M, Mumtaz Alam M (2014) A review exploring biological activities of hydrazones. J Pharm Bioallied Sci 6:69–80
Walcourt A, Loyevsky M, Lovejoy DB, Gordeuk VR, Richardson DR (2004) Novel aroylhydrazone and thiosemicarbazone iron chelators with anti-malarial activity against chloroquine-resistant and -sensitive parasites. Int J Biochem Cell Biol 36:401–407
Walsh JJ, Coughlan D, Heneghan N, Gaynor C, Bell A (2007) A novel artemisinin-quinine hybrid with potent antimalarial activity. Bioorg Med Chem Lett 17:3599–3602
WHO (2013) Why are Some Tropical Diseases Called 'Neglected'? http://www.who.int/features/qa/58/en/. Accessed 12 Apr 2013.
WHO (2002) Control of Chagas disease: second report of the WHO expert committee, vol 905. World Health Organization, Geneva, p 109. ISBN 9241209054. http://www.who.int/iris/handle/10665/42443