Synthesis and Leishmanicidal Activity of Molecular Hybrids 1,2,3-Triazole-Chalcones

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Abstract: Leishmaniasis is a largely neglected infection caused by Leishmania spp. parasites. The first-line treatment, antimoniate meglumine, has a large number of adverse effects, high cost and is prone to resistance development, hence the necessity of new alternatives. We report the synthesis of (2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-arylprop-2-en-1-one derivatives and the in vitro evaluation of their leishmanicidal activities. A series of 16 compounds were synthesized in three steps starting from p-anisidine with moderate to good yields and their biological activities were evaluated in vitro against Leishmania mexicana promastigotes and RAW 264.7 cells. The synthesis of 1,2,3-triazol and chalcone moieties were made using a cycloaddition with acetylacetone followed by a Claisen-Schmidt condensation using different substituted benzaldehydes. Nine compounds were active against L. mexicana with IC50 between 1.0 and 29.2 µM.  

Keywords: molecular hybrids; 1,2,3-triazole; chalcone; Leishmania

1. Introduction  

Leishmaniasis is a tropical disease that affects humans and some animals and is transmitted by the bite of a sandfly infected with a protozoan parasite of the genus Leishmania [1,2]. It is known a neglected disease since it affects millions of people, causing more than 50,000 deaths each year [3,4]. This disease affects, especially, low-income people and is associated with malnutrition, population displacement, poor housing, and a weak immune system [5]. It has a worldwide distribution, since it is present in more than 98 countries, especially in Central and South America, southern Europe, North, and East Africa, the Middle East, and India [6].  

Following the initial stage of infection, the disease may evolve to different clinical forms, classified as cutaneous, mucocutaneous, and visceral, the most important and deadly disease [2]. Actually, there is not an effective cure for leishmaniasis, and current treatments are not sufficiently effective, highly toxic, very expensive, and long lasting, which contributes to a high dropout rate [4]. First-line treatments for leishmaniasis recommended by the WHO [7], generally include medications derived from pentavalent antimony, including antimoniate meglumine (Glucantime) and sodium stibogluconate (Pentostam), and also amphotericin B (Amph B), pentamide, and paromomycin.  

Heterocycles are common structural units in marketed drugs and in medicinal chemistry targets in the drug discovery process. Nitrogen-containing rings especially play an important role in drug development because of their wide variety of therapeutic and pharmacological properties [8]. 1,2,3-triazoles and their derivatives have attracted great
interest due to their wide range of biological activities, such as antimicrobials, analgesics, anti-inflammatory, local anesthetics, anticonvulsants, antineoplastic, antimalarial, antileishmania [9], antiviral, and anticancer among others [10]. Likewise, chalcones exhibit interesting biological properties, such as, antibacterial, antifungal, anti-inflammatory, antitumor, antioxidative, antiprotozoal (trypanocidal and leishmanicidal), antiviral, antimalaria, antioxidant, among others [11].

Molecular hybridization is a strategy used in medical chemistry that aims to combine two or more bioactive molecules, or parts of them, into a new single structure, so that they will maintain and improve the pharmacological characteristics of the original compounds [12]. In view of the above facts, we synthesized some molecular hybrids 1,2,3-triazole-chalcones and evaluated their in vitro cytotoxicity against promastigotes of Leishmania mexicana and RAW 264.7 cells.

2. Methods
2.1. General

All solvents and reagents were from Aldrich and used without further purification. All melting points are uncorrected and were determined on a Büchi Melting Point M-560 apparatus. FTIR spectra were recorded by a VARIAN 660-IR/FT-IR Spectrometer (4000–400 cm$^{-1}$). The $^1$H-NMR and $^{19}$F-NMR spectra of the compounds were determined by coupling on the Oxford Instruments nuclear magnetic resonance spectrometer at 60 MHz, chemical shifts ($\delta$) were given in parts per million (ppm), relative to tetramethylsilane (TMS, $\delta$ = 0 ppm) for protons and trifluoroacetic acid (TFA, $\delta$ = −75.39 ppm) for fluorine. Reactions were monitored by TLC on silica gel using ethyl acetate/hexane mixtures as a solvent and compounds visualized by UV lamp. The reported yields are for the purified material and are not optimized.

2.2. Synthesis of 1-Azido-4-methoxybenzene (1)

$p$-Anisidine (1.007 g, 8.175 mmol) was ground with potassium bisulfate (3.339 g, 24.52 mmol) and 0.3 mL of distilled water in a mortar for 5 min. After that, sodium nitrite (1.410 g, 20.43 mmol) was added and continued grinding for additional 10 min, controlling that the paste does not dry out. Sodium azide (1.328 g, 20.43 mmol) was added, and ground for additional 10 min. Finally, the product was washed with cold water, filtered and dried under vacuum, obtaining 992.1 mg of a brown solid 1 with a yield of 81.4%. The reaction was monitored by TLC using ethyl acetate/hexane 1:4 as eluent. Mp: 31.8–33.0 °C (lit. [13], 34 °C); $^1$H NMR (60 MHz, CDCl$_3$): $\delta$ 3.79 (s, 3H, OCH$_3$), 6.92 (m, 4H, Ar); $^{13}$C-NMR (15 MHz, CDCl$_3$): $\delta$ 55.7, 115.4, 120.2, 132.6, 157.3.

2.3. Synthesis of 1-[1-(4-Methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-ethanone (2)

Potassium carbonate (955.9 mg, 6.914 mmol) was added to a solution of 1 (1.031 g, 6.914 mmol) and acetylacetone (0.792 mL, 7.605 mmol), in DMSO (6.876 mL). The reaction mixture was stirred at room temperature, and the progress of the reaction was monitored by TLC. After 4 h the product was precipitated by slowly pouring the solution over an ice-water mixture. Finally, the mixture was allowed to stand at 4 °C overnight. The precipitate formed was filtered, washed with cold water, and dried under vacuum. The triazole 2 was obtained as a yellowish powder with a 90.2% yield (1,442 g). Mp: 122.1–122.5 °C, (lit. [14], 120 °C); $^1$H NMR (60 MHz, CDCl$_3$): $\delta$ 2.55 (s, 3H, CH$_3$), 2.75 (s, 3H, COCH$_3$), 3.89 (s, 3H, OCH$_3$), 7.04 (d, $J = 9.1$ Hz, 2H, Ar), 7.36 (d, $J = 9.1$ Hz, 2H, Ar).

2.4. General Procedure for the Synthesis of (2E)-1-[1-(4-Methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-arylprop-2-en-1-one Derivatives (4a–o)

To a well-stirred solution of triazole 2 (115.0 mg, 0.4973 mmol) in 3.5 mL of ethanol at room temperature was slowly added 0.6 mmol of benzaldehydes 3a–o. The solution was placed in an ice bath, and with constant stirring, 0.5 mL of an aqueous solution of potassium hydroxide (35.71 mg, 0.6365 mmol) was added. The reaction mixture was stirred for 2 h at this temperature and then allowed to reach room temperature. The progress of the
reaction was followed by TLC in EtOAc/Hexane 1:1 until the reaction was complete (5–24 h), and then kept in a refrigerator overnight. The precipitated solid was filtered off, washed with cold water and dried under vacuum. All products were recrystallized from ethanol.

(2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-phenylprop-2-en-1-one (4a): White crystals; yield: 95.7%; mp: 160.6–161.4 °C (Lit. [15], 153–155 °C); 1H RMN (60 MHz, CDCl3): δ 2.64 (s, 3H, CH3), 3.90 (s, 3H, OCH3), 7.06 (d, J = 9.1 Hz, 2H, Ar), 7.30–7.58 (m, 3H, Ar), 7.40 (d, J = 8.9 Hz, 2H, Ar), 7.58–7.91 (m, 2H, Ar), 7.85 (d, J = 16.1 Hz, 1H, CH=CH), 8.17 (d, J = 16.0 Hz, 1H, CH=CH); FTIR (cm⁻¹): 3106, 1665, 1604, 1257, 837.

(2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-(2-fluorophenyl)prop-2-en-1-one (4b): White crystals; yield: 73.6%; mp: 113.9–115.5 °C; 1H RMN (60 MHz, CDCl3): δ 2.64 (s, 3H, CH3), 3.90 (s, 3H, OCH3), 7.06 (d, J = 9.1 Hz, 2H, Ar), 7.29–7.57 (m, 5H, Ar), 7.58–8.03 (m, 1H, Ar), 8.13 (s, 2H, CH=CH); 19F RMN (56.17 MHz, CDCl3): δ -115.60 (m, 1F).

(2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-(3-fluorophenyl)prop-2-en-1-one (4c): White crystals; yield: 76.7%; mp: 127.9–128.5 °C; 1H RMN (60 MHz, CDCl3): δ 2.64 (s, 3H, CH3), 3.90 (s, 3H, OCH3), 6.92–7.22 (m, 1H, Ar), 7.06 (d, J = 9.1 Hz, 2H, Ar), 7.28–7.59 (m, 3H, Ar), 7.40 (d, J = 8.9 Hz, 2H, Ar), 7.80 (d, J = 15.9 Hz, 1H, CH=CH), 8.14 (d, J = 16.0 Hz, 1H, CH=CH); 19F RMN (56.17 MHz, CDCl3): δ -111.88 (m, 1F).

(2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-(4-fluorophenyl)prop-2-en-1-one (4d): White crystals; yield: 98.0%; mp: 162.5–163.8 °C; 1H RMN (60 MHz, CDCl3): δ 2.64 (s, 3H, CH3), 3.90 (s, 3H, OCH3), 7.06 (d, J = 9.1 Hz, 2H, Ar), 7.10 (t, J = 8.8 Hz, 2H, Ar), 7.40 (d, J = 9.1 Hz, 2H, Ar), 7.72 (dd, J = 8.8, 5.5 Hz, 2H, Ar), 7.80 (d, J = 16.1 Hz, 1H, CH=CH), 8.10 (d, J = 16.0 Hz, 1H, CH=CH); 19F RMN (56.17 MHz, CDCl3): δ -108.92 (m, 1F).

(2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-(4-bromophenyl)prop-2-en-1-one (4e): White crystals; yield: 96.4%; mp: 159.1–160.2 °C; 1H RMN (60 MHz, CDCl3): δ 2.63 (s, 3H, CH3), 3.90 (s, 3H, OCH3), 7.05 (d, J = 9.1 Hz, 2H, Ar), 7.40 (d, J = 9.1 Hz, 2H, Ar), 7.41–7.72 (m, 4H, Ar), 7.78 (d, J = 16.2 Hz, 1H, CH=CH), 8.13 (d, J = 16.3 Hz, 1H, CH=CH); FTIR (cm⁻¹): 1663, 1600, 1562, 1008.

(2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-[2-(trifluoromethyl)phenyl]prop-2-en-1-one (4f): White crystals; yield: 97.1%; mp: 137.6–144.6 °C; 1H RMN (60 MHz, CDCl3): δ 2.65 (s, 3H, CH3), 3.90 (s, 3H, OCH3), 7.06 (d, J = 9.2 Hz, 2H, Ar), 7.40 (d, J = 9.1 Hz, 2H, Ar), 7.51–7.85 (m, 3H, Ar), 7.85–8.35 (m, 3H, Ar and CH=CH); 19F RMN (56.17 MHz, CDCl3): δ -58.75 (s, CF3).

(2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-[3-(trifluoromethyl)phenyl]prop-2-en-1-one (4g): White crystals; yield: 77.5%; mp: 140.5–142.9 °C; 1H RMN (60 MHz, CDCl3): δ 2.65 (s, 3H, CH3), 3.90 (s, 3H, OCH3), 7.06 (d, J = 9.0 Hz, 2H, Ar), 7.40 (d, J = 9.0 Hz, 2H, Ar), 7.51–7.76 (m, 2H, Ar), 7.79–8.18 (m, 4H, Ar and CH=CH); 19F RMN (56.17 MHz, CDCl3): δ -62.85 (s, CF3); FTIR (cm⁻¹): 1670, 1608, 1343, 1264, 1199, 1111, 989, 633.

(2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-[4-(trifluoromethyl)phenyl]prop-2-en-1-one (4h): White crystals; yield: 76.8%; mp: 145.2–147.6 °C; 1H RMN (60 MHz, CDCl3): δ 2.64 (s, 3H, CH3), 3.90 (s, 3H, OCH3), 7.06 (d, J = 9.1 Hz, 2H, Ar), 7.40 (d, J = 9.1 Hz, 2H, Ar), 7.65 (d, J = 9.1 Hz, 2H, Ar), 7.84 (d, J = 9.1 Hz, 2H, Ar), 7.86 (d, J = 16.0 Hz, 1H, CH=CH), 8.21 (d, J = 16.0 Hz, 1H, CH=CH); 19F RMN (56.17 MHz, CDCl3): δ -63.06 (s, 3F, CF3); FTIR (cm⁻¹): 1667, 1612, 1275, 1113, 996, 746.

(2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-[2-nitrophenyl]prop-2-en-1-one (4i): White crystals; yield: 33.5%; mp: 193.6–195.5 °C; 1H RMN (60 MHz, CDCl3): δ 2.65 (s, 3H, CH3), 3.90 (s, 3H, OCH3), 7.06 (d, J = 9.1 Hz, 2H, Ar), 7.40 (d, J = 9.2 Hz, 2H, Ar), 7.51–8.17 (m, 4H, Ar), 7.97 (d, J = 15.8 Hz, 1H, CH=CH), 8.38 (d, J = 15.8 Hz, 1H, CH=CH); FTIR (cm⁻¹): 1670, 1610, 1469, 1284, 996.

(2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-(3-nitrophenyl)prop-2-en-1-one (4j): Yellowish crystals; yield: 75.2%; mp: 177.5–178.1 °C; 1H RMN (60 MHz,
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(2E)-1-[1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-(4-nitrophenyl)-prop-2-en-1-one (4k): White crystals; yield: 96.7%; mp: 231.7–236.9 °C; ¹H RMN (60 MHz, CDCl₃): δ 2.65 (s, 3H, CH₃), 3.90 (s, 3H, OCH₃), 7.06 (d, J = 9.0 Hz, 2H, Ar), 7.41 (d, J = 9.0 Hz, 2H, Ar), 7.66 (d, J = 8.0 Hz, 1H, Ar), 7.91–8.76 (m, 5H, Ar and CH=CH); FTIR (cm⁻¹): 1668, 1607, 1275, 1258, 994.

2.6. Evaluation of Leishmanicidal Activity

Leishmanicidal activities of all compounds were evaluated by measuring promastigotes' mitochondrial activity using MTT colorimetric assay as described previously [17]. In this study, promastigotes from Leishmania mexicana were used. Promastigotes were cultured at 25 °C in Schneider’s Drosophila Medium (Gibco) supplemented with 10% fetal bovine serum (Eurobio). The medium was renewed every third day. Parasite density was determined by counting the cells using a Neubauer chamber.
Into each well of a 96-well cell-culture plate were dispensed $1 \times 10^6$ parasites/well. A stock solution of the compounds was prepared in DMSO (Sigma), and serial dilutions were added to the parasite suspension, leading to concentrations ranging from 100 to 0.01 µM, keeping the solvent concentration at 0.5%. The final volume was 200 µL for each well and triplicate conditions were carried out. Amphotericin B (Gibco) treatment (1 µM) untreated parasites and DMSO 0.5% were used as positive and negative controls, respectively. After exposure to the compounds for 48 h in culture medium, 20 µL of a solution of 5 mg/mL MTT dissolved in PBS was added to each well. The plate was incubated at 25 °C for 2 h in darkness. Later, the plate was centrifuged at 4400 rpm for 10 min and the culture medium was then aspirated. Total of 50 µL of DMSO was added into each well to solubilize the formazan crystals, the plate was shaken for 5 min, and then it was measured by recording changes in absorbance at 570 nm using a microplate reader Cytation 5 (BioTek, Winooski, VT, USA) spectrophotometer. Data were analyzed with the statistical software GraphPad Prism 7.02 (GraphPad Software, Corp.).

2.7. Evaluation of the Cell Viability

Raw 264.7 (ATCC® TIB-71™) was maintained in Dulbecco’s modified Eagle medium (DMEM) (Gibco, Invitrogen) supplemented with 10% fetal bovine serum (FBS) (Eurobio) and 100 IU/mL penicillin + 100 µg/mL streptomycin (Gibco), at 37 °C in an atmosphere containing 5% CO2. The medium was renewed once a week. The viability was determined using MTT dye assay as described before for leishmanicidal activity assessment, with some variations. Here, $5 \times 10^4$ cells/well in a final volume of 100 µL were deposited into a 96-wells plate, in triplicate. Saponin (2.4 mg/mL) and untreated cells were used as positive control and negative control, respectively. Compounds were dissolved in DMSO to obtain different serial concentrations (100–0.01 µM). After 48-h exposure to the compounds, 10 µL/well of MTT (5 mg/mL MTT in PBS), and the plate was incubated at 37 °C for 2 h. in darkness. Cells were pelleted by centrifugation at 4400 rpm for 10 min and the media was removed. Total of 100 µL/well of DMSO were added and the absorbance at 570 nm was recorded.

3. Results and Discussion

3.1. Synthesis

The products were synthesized according to Scheme 1. The synthesis of the azide 1 was performed using the procedure reported by Gorlushko et al. [18], starting for p-anisidine. The diazotization was accomplished using sodium hydrogen sulfate as an ecologically safe acidic reagent, and the three-step reaction was carried out in a mortar. When the diazotization was complete, sodium azide was added to the paste-like diazonium salt. Finally, the aqueous paste containing azide 1 was treated with water, and the pure product was isolated by simple filtration as a brown solid in 81.4% yield.

$$\text{OMe}$$ $\text{MeO}$ $\text{N}_3$

$p$-anisidine $1$

$$\text{MeO}$$ $\text{MeO}$ $\text{N} = \text{N} = \text{N}$

2

$$\text{OMe}$$ $\text{OMe}$ $\text{MeO}$ $\text{R}$

4a-p

4a= H 4b= 2-CF3 4c= 3-CF3 4d= 4-CF3 4e= 4-Br 4f= 3-OCH3-4-F 4g= 3-OPh-4-F 4h= 2-OH 4i= 2-CF3 4j= 3-NO2 4k= 4-NO2 4l= 4-OCF3 4m= 4-SCH3

Scheme 1. General reaction for the synthesis of 1,2,3-triazole-chalcones 4a-p. Reagents and conditions: (a) i. KHSO4, ii. NaN3, r.t., (b) acetylacetone, K2CO3, DMSO, r.t., (c) benzaldehyde 3a-p, KOH, EtOH, 0 °C, 2h → rt.
With the azide 1 in hand, the next step was the preparation of the triazol 2 via enolate–azide cycloaddition as previously reported [19]. The product was obtained as a yellow powder in a 90.2% yield. Finally, the hybrids 1,2,3-triazole-chalcones 4 were synthesized by the Claisen–Schmidt reaction [15]. Initially, the reaction of 2 with 3a afforded pure product 4a in 56.5% yield, but after the reaction was optimized, the yield was increased to 96%. With the optimized conditions the hybrids 4a-p (Table 1) were obtained from the respective benzaldehyde 3a-p in moderate to excellent yield (74–99%), except 4i, which presented a yield of 33.5% even after 26 h of reaction. The analysis by TLC of the reaction mixture showed, in addition to 4i and starting materials, one additional spot. Thereby, using this synthetic route the hybrids 1,2,3-triazole-chalcones 4 were synthesized between 54% and 73% overall yields from 1, except for 4i which was obtained in 24% overall yield.

Table 1. Synthesis of hybrids 1,2,3-triazole-chalcones 4a-p by reaction of 2 with benzaldehydes 3a-p.

| Compound | R       | Time (h) | Appearance/Color | M.p. (°C) | Yield (%) |
|----------|---------|----------|------------------|-----------|-----------|
| 4a       | H       | 8        | Crystals/White   | 160.6–161.4 | 95.7      |
| 4b       | 2-F     | 5        | Crystals/White   | 113.9–115.5 | 73.6      |
| 4c       | 3-F     | 5        | Crystals/White   | 127.9–128.5 | 76.7      |
| 4d       | 4-F     | 5        | Crystals/White   | 162.5–163.8 | 98.0      |
| 4e       | 4-Br    | 24       | Crystals/White   | 159.1–160.2 | 96.4      |
| 4f       | 2-CF₃   | 24       | Crystals/White   | 137.6–144.6 | 97.1      |
| 4g       | 3-CF₃   | 6        | Crystals/White   | 140.5–142.9 | 77.5      |
| 4h       | 4-CF₃   | 6        | Crystals/White   | 145.2–147.6 | 76.8      |
| 4i       | 2-NO₂   | 26       | Crystals/White   | 193.6–195.5 | 33.5      |
| 4j       | 3-NO₂   | 6        | Crystals/Yellowish | 177.5–178.1 | 75.2      |
| 4k       | 4-NO₂   | 25       | Crystals/White   | 231.7–236.9 | 96.7      |
| 4l       | 4-OCF₃  | 6        | Crystals/White   | 128.5–129.7 | 88.3      |
| 4m²      | 4-SCH₃  | 8        | Crystals/White   | 153.5–156.2 | 93.4      |
| 4n       | 3-OCH₃-4-F | 7    | Crystals/White   | 158.3–159.6 | 96.1      |
| 4o       | 3-OPh-4-F | 5   | Crystals/White   | 108.0–109.8 | 99.3      |
| 4p²      | 2-0H    | 1        | Crystals/White   | 195.6–196.9 | 99.6³     |

¹ Isolated yield. ² Compounds 4m and 4p showed fluorescence at 254 nm. ³ Using ultrasound.

The mechanism for the formation of the hybrid is depicted in Scheme 2 [20]. A possible explanation for the low yield observed in 4i may be due to the fact that the presence of the nitro group in the ortho position of the aldehyde will destabilize the formation of the oxyanion 5, due to electronic repulsions in the transition state. This effect is not observed in 4j (75.2%) and 4k (96.7%), which have the same substituent in meta and para positions respectively, neither in 4f (97.1%) that has a trifluoromethyl group in the ortho position. This suggests that the inductive and steric effects would not be responsible for the low yield observed. On the other hand, oxyanion 6 could be more stabilized by an intramolecular hydrogen bond between the hydroxyl and the nitro group, reducing the possibility of hydroxyl elimination to form 4i.
Scheme 2. Mechanism proposed for the synthesis of hybrids 1,2,3-triazole-chalcone derivatives 4a-p.

When salicylaldehyde (3p) was used for the reaction with 2, following the same methodology, no product was observed, and both TLC and NMR of the reaction crude, showed only the presence of starting materials. This result was expected since in the case of Claisen-Schmidt reaction using hydroxybenzaldehydes the use of protecting groups is required [21]. Several reports show that the use of ultrasound allows to increase the yield in the synthesis of chalcones [16,22,23], and also avoids the use of protective groups when hydroxy substituted benzaldehydes are used [24]. Following this methodology, the reaction for the synthesis of 4p was repeated at 50 °C using KOH as a base at a 1:10 ratio with compound 2, and methanol as solvent. After applying ultrasound for one hour, 1,2,3-triazole-chalcone 4p was obtained with an excellent yield (see Table 1).

Although a low-field NMR spectrometer was used, the most important signals of the compounds could be clearly observed, and all ¹H-NMR spectra are in agreement with the structures expected. The methoxy and the methyl groups are observed at 3.90 ppm and around 2.64 ppm respectively for all molecules. The protons of the α,β-unsaturated system appear as a pair of doublets at 7.78–8.42 ppm and 7.97–8.56 ppm. These protons have a coupling constant of around 16 Hz, indicating that the trans isomer was obtained.

In the case of fluorinated compounds, ¹⁹F-NMR spectra were run in addition to the proton spectra. Monosubstituted compounds 4b, 4c, and 4d, which present a simple fluorine atom in positions o, m, and p respectively, show chemical shifts at –115.60 ppm, –111.88 ppm, and –108.92 ppm respectively. The signal of disubstituted hybrids 4n and 4o, that both present a fluorine atom in para position, appear at 130.39 ppm, and -126.85 ppm respectively. All these signals appear as multiplets due to the coupling of the fluorine with the protons of the ring. On the other hand, the trifluoromethyl groups in 4f, 4g, and 4h, appear as a well-defined singlet shifted to –58.75 ppm (ortho), –62.85 ppm (meta), and –63.06 ppm (para) respectively, and the trifluoromethoxy group in 4l shows a chemical shift at –57.84 ppm. All signals for fluorine are observed in the expected ranges [25].

3.2. Biological Evaluation

The bioactivity of each compound was evaluated against promastigote of L. mexicana and RAW cells using a colorimetric assay that measures cell viability. Initially a screening was performed against L. mexicana at a concentration of 20 µM and with the compounds that showed less than 50% cell viability, the IC₅₀ was calculated against the two cell lines. The compounds in which the activity is reported as ND were not evaluated, because no
activity was observed in the screening, and in that case the compounds are considered as not active. Amphotericin B and saponin were used as positive control against the parasite and RAW cells, respectively.

As observed in Table 2, only nine compounds were active against *L. mexicana* and six of them were fluorinated compounds. Compound *4j* is the most active with an IC₅₀ of 1.0 µM, while compound *4o* was the least active (IC₅₀ = 29.2 µM). All compounds tested were at least ≥ 5.8-fold less active against *L. mexicana* promastigotes than the control drug amphotericin (IC₅₀ = 0.172 µM). Based on their potency, the compounds can be grouped in moderate- (IC₅₀ 1–10 µM, *4j, 4p, 4g, 4h,* and *4b*), low- (IC₅₀ 11–30 µM, *4c, 4a, 4l,* and *4o*) or non-active (*4d, 4e, 4f, 4i, 4k, 4m,* and *4n*).

### Table 2. Leishmanicidal and cytotoxicity activity against *L. Mexicana* and RAW cells, respectively, of compounds 4a-p.

| Compound | Leishmanicidal Activity IC₅₀ (µM) | RAW Cytotoxicity CC₅₀ (µM) | SI Index |
|----------|-------------------------------|-----------------------------|----------|
| 4a       | 15.7                          | 20.1                        | 1.3      |
| 4b       | 7.9                           | 13.7                        | 1.7      |
| 4c       | 14.4                          | 26.2                        | 1.8      |
| 4d       | NA                            | 44.3                        | ND       |
| 4e       | NA                            | 22.4                        | ND       |
| 4f       | NA                            | 23.2                        | ND       |
| 4g       | 3.9                           | 11.3                        | 2.9      |
| 4h       | 4.9                           | 19.5                        | 4.0      |
| 4i       | NA                            | 4.6                         | ND       |
| 4j       | 1.0                           | 3.6                         | 3.7      |
| 4k       | ND                            | ND                          | ND       |
| 4l       | 27.0                          | >100                        | >3.7     |
| 4m       | NA                            | ND                          | ND       |
| 4n       | NA                            | 16.2                        | ND       |
| 4o       | 29.2                          | 1.7                         | 0.1      |
| 4p       | 1.3                           | 7.3                         | 5.7      |
| Amphotericin B | 0.172                      | >5                          | ND       |
| Saponin  | ND                            | 0.163 *                     | ND       |

ND: not determined, NA: not active, *: mg/mL.

In general, para substituted compounds were less active than compounds substituted in other positions, however, when evaluating the effect of the substituent, no relationship could be observed between the position of the substituent on the ring, and/or its electronic nature, with the leishmanicidal activity. In compounds with groups NO₂ and CF₃, more activity is observed when the substituent is in para position, while, in monofluorinated compounds, the more active one was the ortho substituted *4b* with IC₅₀ = 7.9 µM. The same trend is observed in cytotoxicity against RAW cells. In this case, all compounds were less toxic compared to the activity observed against parasites, except in the case of *4o*, which was 17.2-fold more active against *L. mexicana* promastigotes.

In comparison to amphotericin (CC₅₀ > 5 µM), compounds *4j* and *4p* have similar cytotoxicity against murine macrophages with CC₅₀ 3.6 µM and 7.3 µM respectively, all the other compounds displaying moderate to low anti-leishmanial activity, excepted for *4o* (CC₅₀ = 1.7 µM), proved to be at least 2.3 to 5.2-folds less cytotoxic against RAW cells. On the other hand, *4o* proved to be at least 2.9-fold more cytotoxic than amphotericin and *4l* did not show cytotoxicity against macrophages at the concentrations evaluated. Compounds classified as moderate inhibitors of *L. mexicana* proliferation presented SI values
between 1.7 and 5.7, while, with the exception of 4l and 4o, low inhibitors have SI values of 1.3 and 1.8. Among them, the hybrid 4p, with an o-hydroxyl group, had the best SI (Table 2).

4. Conclusions

The synthesis of the molecular hybrids 1,2,3-triazole-chalcones 4 can be easily carried out through three reactions. In the first reaction, 1-azido-4-methoxybenzene (1) was obtained with a good yield starting from p-anisidine. In the second reaction, the 1,2,3-triazole 2 was prepared with a good yield through a 1,3-dipolar cycloaddition of 1 with acetylacetone, and finally in the third reaction the hybrids (2E)-1,2,3-triazole-chalcones 4 were synthesized stereoselectively using a Claisen–Schmidt condensation of several substituted benzaldehydes 3 with 2 in basic medium. Thus, Hybrids 1,2,3-triazole-chalcone 4 were synthesized in moderate to good overall yield from p-anisidine, except for 4l which was obtained in low overall yield due to the low yield observed in the Claisen–Schmidt reaction.

In general, the synthesized compounds showed good leishmanicidal activity in vitro against promastigotes of Leishmania mexicana, and 9 of the 16 evaluated compounds showed to be active with IC₅₀ in the range 1.0–29.2 µM. However, no effect of the substituent could be found in this activity. Although 4j was the compound most active against the parasite, the best SI is observed for compound 4p, which is 5.7 times more toxic against L. mexicana compared to macrophage cells.

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