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

Sofía Vanessa Rodríguez-Gutiérrez [1], Olalla Barreiro-Costa [2], Christian David Alcívar León [1] and Jorge Heredia-Moya [2,*]

[1] Facultad de Ciencias Químicas, Universidad Central del Ecuador, Quito 170521, Ecuador; vrg101517@gmail.com (S.V.R.G.); cdalcivar@uce.edu.ec (C.D.A.L.)
[2] Centro de Investigación Biomédica (CENBIO), Facultad de Ciencias de la Salud Eugenio Espejo, Universidad UTE, Quito 170527, Ecuador; olalla.barreiro@ute.edu.ec
* Correspondence: jorgeh.heredia@ute.edu.ec; Tel.: +593-2-2990800 (ext. 2538)
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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 (2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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 IC_{50} between 1.0 and 29.2 μM.

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

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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), pentamidine, 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-inflammatories, local anesthetics, anticonvulsants, antineoplastic, antimalarial, antileishmania [9], antivirals, and anticancer among others [10]. Likewise, chalcones exhibit interesting biological properties, such as, antibacterial, antifungal, anti-inflammatory, anticancer, antidepressant, 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 (δ) were given in parts per million (ppm), relative to tetramethylsilane (TMS, δ = 0 ppm) for protons and trifluoroacetic acid (TFA, δ = −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*$_3$): δ 3.79 (s, 3H, OCH_3), 6.92 (m, 4H, Ar); ^{13}C-NMR (15 MHz, *CDCl*$_3$): δ 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 (0.7929 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*$_3$): δ 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.

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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) ; ^{1}H RMN (60 MHz, $CDCl_3$): δ 2.64 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 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.

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.64 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 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); ^{19}F RMN (56.17 MHz, *CDCl*$_3$): δ -115.60 (m, 1F).

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.64 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 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); ^{19}F RMN (56.17 MHz, *CDCl*$_3$): δ -111.88 (m, 1F).

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.64 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 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); ^{19}F RMN (56.17 MHz, *CDCl*$_3$): δ -108.92 (m, 1F).

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.63 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 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.

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.65 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 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); ^{19}F RMN (56.17 MHz, *CDCl*$_3$): δ -58.75 (s, CF_3).

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.65 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 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); ^{19}F RMN (56.17 MHz, *CDCl*$_3$): δ -62.85 (s, CF_3); FTIR (cm^{-1}): 1670, 1608, 1343, 1264, 1199, 1111, 989, 633.

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.64 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 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); ^{19}F RMN (56.17 MHz, *CDCl*$_3$): δ -63.06 (s, 3F, CF_3); FTIR (cm^{-1}): 1667, 1612, 1275, 1113, 996, 746.

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.65 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.06 (d, *J* = 9.1 Hz, 2H, Ar), 7.40 (d, *J* = 9.2, 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.

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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; ^{1}H RMN (60 MHz,

CDCl$_3$): δ 2.65 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.06 (d, *J* = 9.1 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*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-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; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.65 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.07 (d, *J* = 9.1 Hz, 2H, Ar), 7.41 (d, *J* = 9.1 Hz, 2H, Ar), 7.84 (d, *J* = 8.8 Hz, 2H, Ar), 7.86 (d, *J* = 15.8 Hz, 1H, CH=CH), 8.24 (d, *J* = 16.0 Hz, 1H, CH=CH), 8.29 (d, *J* = 8.9 Hz, 2H, Ar); FTIR (cm^{-1}): 1669, 1608, 1256, 1035.

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-3-[4-(trifluoromethoxy)-phenyl]-prop-2-en-1-one (**4l**): White crystals; yield: 88.3%; mp: 128.5–129.7 °C; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.64 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.06 (d, *J* = 9.0 Hz, 2H, Ar), 7.25 (d, *J* = 8.8 Hz, 2H, Ar), 7.40 (d, *J* = 9.1Hz, 2H, Ar), 7.76 (d, *J* = 8.8 Hz, 2H, Ar), 7.82 (d, *J* = 16.0 Hz, 1H, CH=CH), 8.13 (d, *J* = 15.9 Hz, 1H, CH=CH); ^{19}F RMN (56.17 MHz, *CDCl*$_3$): δ -84.82 (s, OCF_3).

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-3-[4-(methylsulfanyl)-phenyl]-prop-2-en-1-one (**4m**): White crystals; yield: 93.4%; mp: 153.5–156.2 °C; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.52 (s, 3H, SCH_3), 2.63 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 7.05 (d, *J* = 9.1 Hz, 2H, Ar), 7.25 (d, *J* = 6.4 Hz, 2H, Ar), 7.40 (d, *J* = 9.0 Hz, 2H, Ar), 7.65 (d, *J* = 8.6 Hz, 2H, Ar), 7.81 (d, *J* = 15.9 Hz 1H, CH=CH), 8.12 (d, *J* = 15.9 Hz, 1H, CH=CH); FTIR (cm^{-1}): 1657, 1584, 1554, 1406, 990.

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-3-(4-fluoro-3-methoxyphenyl)-prop-2-en-1-one (**4n**): White crystals; yield: 96.1%; mp: 158.3–159.6 °C; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.64 (s, 3H, CH_3), 3.90 (s, 3H, *p*-OCH_3), 3.97 (s, 3H, *m*-OCH_3), 7.06 (d, *J* = 9.1 Hz, 2H, Ar), 7.05–7.45 (m, 3H, Ar), 7.40 (d, *J* = 9.1 Hz, 2H, Ar), 7.78 (d, *J* = 15.7 Hz, 1H, CH=CH), 8.07 (d, *J* = 15.9 Hz, 1H, CH=CH); ^{19}F RMN (56.17 MHz, *CDCl*$_3$): δ -130.39 (m, *p*-F); FTIR (cm^{-1}): 1665, 1596, 1302, 996, 731.

(2*E*)-1-[1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-3-(4-fluoro-3-fenoxiphenyl)-prop-2-en-1-one (**4o**)**:** White crystals; yield: 99.3%; mp: 108.0–109.8 °C; ^{1}H RMN (60 MHz, $CDCl_3$): δ 2.62 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 6.88–7.66 (m, 12H, Ar), 7.76–8.00 (m, 2H, CH=CH); ^{19}F RMN (60 MHz, *CDCl*$_3$): δ -84.82 (m, *p*-F).

2.5. Synthesis of (E)-1-[1-(4-Methoxyphenyl)-5-methyl-1H-1,2,3-triazol-4-yl]-3-(2-hydroxyphenyl)-prop-2-en-1-One (**4p**)

The synthesis of **4p** was carried out via Claisen-Schmidt reaction under ultrasound irradiation [16]. To a solution of **2** (100.0 mg, 0.4324 mmol) in 0.5 mL of methanol, 2-hydroxybenzaldehyde (0.09180 mL, 0.8648 mmol), and slowly, 0.5 mL of an aqueous solution of potassium hydroxide (242.6 mg, 4.324 mmol) were added. The reaction mixture was sonicated at 50 °C for one hour. The reaction product was added dropwise to an ice-water mixture maintaining vigorous stirring, after which it was neutralized with HCl 0.3 M until reaching a pH of ~2, and then kept in a refrigerator overnight. The precipitate was vacuum filtered, washed with cold water and dried under vacuum. The reaction was monitored by TLC using EtOAc/Hexane 1:2. The product was recrystallized from ethanol, obtaining **4p** as white crystals with a yield of 99.6%; mp: 195.6–196.9 °C; ^{1}H RMN (60 MHz, *CDCl*$_3$): δ 2.67 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 6.72–7.31 (m, 3H, Ar), 6.92 (s, 1H, OH), 7.06 (d, *J* = 9.0 Hz, 2H, Ar), 7.40 (d, *J* = 9.0 Hz, 2H, Ar), 7.53–7.82 (m, 1H, Ar), 8.14 (d, *J* = 16.0 Hz, 1H, CH=CH), 8.42 (d, *J* = 16.0 Hz, 1H, CH=CH); FTIR (cm^{-1}): 1662, 1586, 1457, 1255, 1193, 1016.

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 × 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 (Bio-Tek, Winooski, VT, USA) spectrophotometer. Data were analyzed with the statistical software GraphPad Prism 7.02 (GraphPad Software, Corp.).

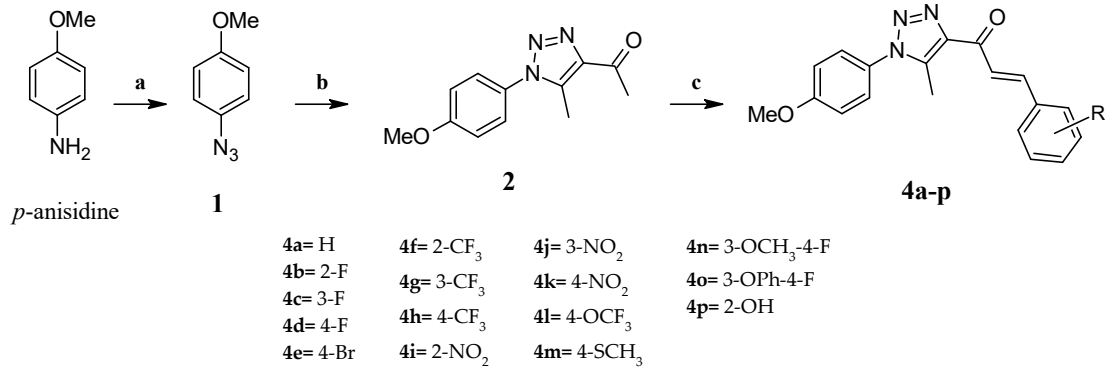
2.7. Evaluation of the Cell Viability

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% CO_2. 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 × 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.



Scheme 1. General reaction for the synthesis of 1,2,3-triazole-chalcones **4a-p**. Reagents and conditions: (**a**) i. $KHSO_4$, ii. $NaNO_2$, iii NaN_3, r.t., (**b**) acetylacetone, K_2CO_3, 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.

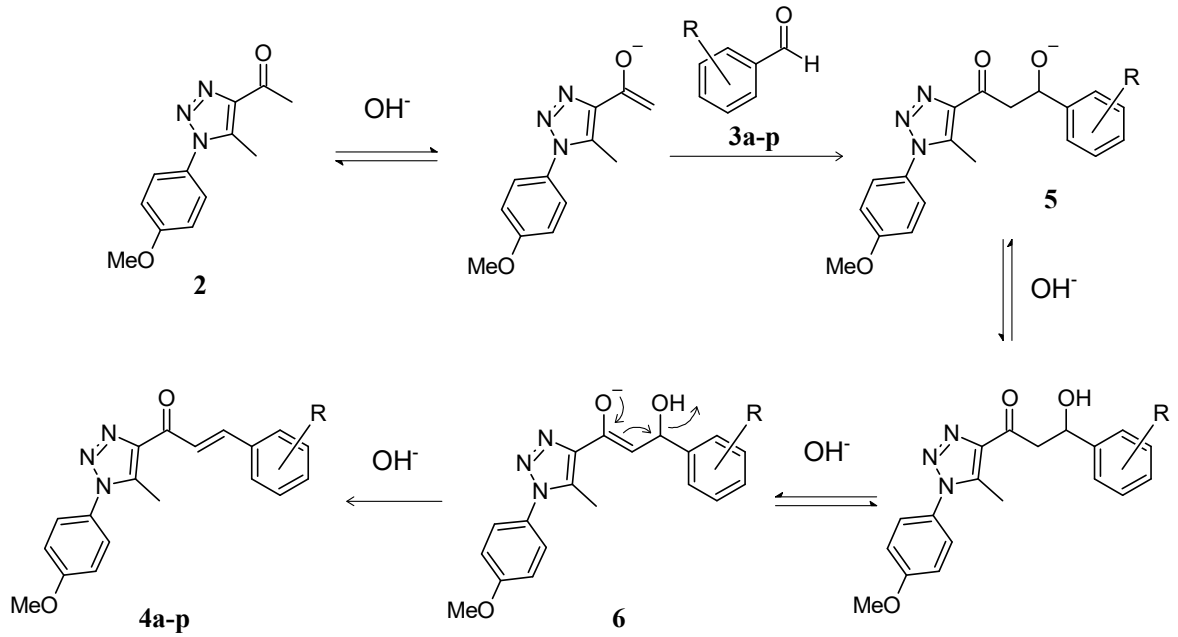
Thereby, using this synthetic route the hybrids 1,2,3-triazole-chalcones **4** were synthesized between 54% and 73% overall yields from **1**, except for **4i** which was obtained in 24% overall yield.

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 [1] (%)
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-CF_3	24	Crystals/White	137.6–144.6	97.1
4g	3-CF_3	6	Crystals/White	140.5–142.9	77.5
4h	4-CF_3	6	Crystals/White	145.2–147.6	76.8
4i	2-NO_2	26	Crystals/White	193.6–195.5	33.5
4j	3-NO_2	6	Crystals/Yellowish	177.5–178.1	75.2
4k	4-NO_2	25	Crystals/White	231.7–236.9	96.7
4l	4-OCF_3	6	Crystals/White	128.5–129.7	88.3
4m [2]	4-SCH_3	8	Crystals/White	153.5–156.2	93.4
4n	3-OCH_3-4-F	7	Crystals/White	158.3–159.6	96.1
4o	3-OPh-4-F	5	Crystals/White	108.0–109.8	99.3
4p [2]	2-OH	1	Crystals/White	195.6–196.9	99.6 [3]

[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 ^{1}H-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, ^{19}F-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 IC_{50} 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 IC_{50} of 1.0 μM, while compound **4o** was the least active (IC_{50} = 29.2 μM). All compounds tested were at least ≥ 5.8-fold less active against *L. mexicana* promastigotes than the control drug amphotericin (IC_{50} = 0.172 μM). Based on their potency, the compounds can be grouped in moderate- (IC_{50} 1–10 μM, **4j**, **4p**, **4g**, **4h**, and **4b**), low- (IC_{50} 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 IC_{50} (μM)	RAW Cytotoxicity CC_{50} (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 NO_2 and CF_3, 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 IC_{50} = 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 (CC_{50} > 5 μM), compounds **4j** and **4p** have similar cytotoxicity against murine macrophages with CC_{50} 3.6 uM and 7.3 uM respectively, all the other compounds displaying moderate to low anti-leishmanial activity, excepted for **4o** (CC_{50} = 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 a *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 (2*E*)-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 **4i** which was obtained in low overall yield due to the low yield observed in the Claisen–Schmidt reaction.

In general, the synthetized compounds showed good leishmanicidal activity in vitro against promastigotes of *Leishmania mexicana*, and 9 of the 16 evaluated compounds showed to be active with IC_{50} 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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References

1. Arenas, R.; Torres-Guerrero, E.; Quintanilla-Cedillo, M.R.; Ruiz-Esmenjaud, J. Leishmaniasis: A review. *F1000 Rev.* **2017**, *6*, 750.
2. Braga, S.S. Multi-target drugs active against leishmaniasis: A paradigm of drug repurposing. *Eur. J. Med. Chem.* **2019**, *183*, 111660.
3. Akhoundi, M.; Kuhls, K.; Cannet, A.; Votýpka, J.; Marty, P.; Delaunay, P.; Sereno, D. A Historical Overview of the Classification, Evolution, and Dispersion of Leishmania Parasites and Sandflies. *PLoS Negl. Trop. Dis.* **2016**, *10*, e0004349.
4. Kobets, T.; Grekov, I.; Lipoldova, M. Leishmaniasis: Prevention, Parasite Detection and Treatment. *Curr. Med. Chem.* **2012**, *19*, 1443–1474, doi:10.2174/092986712799828300.
5. World Health Organization. Leishmaniasis. Available online: https://www.who.int/en/news-room/fact-sheets/detail/leishmaniasis (accessed on 2 June 2020).
6. Croft, S.L.; Sundar, S.; Fairlamb, A.H. Drug resistance in leishmaniasis. *Clin. Microbiol. Rev.* **2006**, *19*, 111–126.
7. World Health Organization. *Control of the Leishmaniasis: Report of a Meeting of the WHO Expert Committee on the Control of Leishmaniases*; WHO Press: Geneve, Switzerland, 2010; Volume 949, p. 54-73.
8. Gomtsyan, A. Heterocycles in drugs and drug discovery. *Chem. Heterocycl. Compd.* **2012**, *48*, 7–10, doi:10.1007/s10593-012-0960-z.
9. Temraz, M.G.; Elzahhar, P.A.; Bekhit, A.E.D.A.; Bekhit, A.A.; Labib, H.F.; Belal, A.S.F. Anti-leishmanial click modifiable thiosemicarbazones: Design, synthesis, biological evaluation and in silico studies. *Eur. J. Med. Chem.* **2018**, *151*, 585–600, doi:10.1016/j.ejmech.2018.04.003.

10. Holla, B.S.; Mahalinga, M.; Karthikeyan, M.S.; Poojary, B.; Akberali, P.M.; Kumari, N.S. Synthesis, characterization and antimicrobial activity of some substituted 1,2,3-triazoles. *Eur. J. Med. Chem.* **2005**, *40*, 1173–1178, doi:10.1016/j.ejmech.2005.02.013.
11. Arlindo Pascual, Z.; Carrera González, S.; Francisco González Matilla, J. Synthesis of Chalcones: Privilegial Structures in the Synthesis of Heterocycles with Biological Activity. *Psychol. Lat. Copyr.* **2018**, 20–23, doi:10.1016/j.bmc.2007.04.047.
12. Viegas-Junior, C.; Danuello, A.; da Silva Bolzani, V.; Barreiro, E.J.; Fraga, C.A. M. Molecular Hybridization: A Useful Tool in the Design of New Drug Prototypes. *Curr. Med. Chem.* **2007**, *14*, 1829–1852, doi:10.2174/092986707781058805.
13. Dyall, L.K.; Suffolk, P.M.; Dehaen, W.; L'Abbé, G. Factors affecting the rates of thermal decomposition of azidothiophenes. *J. Chem. Soc. Perkin Trans. 2* **1994**, *10*, 2115–2118, doi:10.1039/p29940002115.
14. Kharb, R.; Shahar Yar, M.; Sharma, P.C. New Insights into Chemistry and Anti-Infective Potential of Triazole Scaffold. *Curr. Med. Chem.* **2011**, *18*, 3265–3297, doi:10.2174/092986711796391615.
15. Dong, H.S.; Wang, H.C.; Gao, Z.L.; Li, R.S.; Cui, F.H. Tandem Michael addition/imino-nitrile cyclization synthesis of 2-amino-6-(1-aryl-5-methyl-1H-1,2,3-triazol-4yl)-4-phenylpyridine-3-carbonitrile. *J. Heterocycl. Chem.* **2010**, *47*, 389–395, doi:10.1002/jhet.336.
16. Li, J.T.; Yang, W.Z.; Wang, S.X.; Li, S.H.; Li, T.S. Improved synthesis of chalcones under ultrasound irradiation. *Ultrason. Sonochem.* **2002**, *9*, 237–239, doi:10.1016/S1350-4177(02)00079-2.
17. Nikzad, S.; Baradaran-Ghahfarokhi, M.; Nasri, P. Dose-response modeling using MTT assay: A short review. *Life Sci. J.* **2014**, *11*, 1097–8135, doi:10.15171/jrb.2014.04.
18. Gorlushko, D.A.; Filimonov, V.D.; Krasnokutskaya, E.A.; Semenischeva, N.I.; Go, B.S.; Hwang, H.Y.; Cha, E.H.; Chi, K.W. Iodination of aryl amines in a water-paste form via stable aryl diazonium tosylates. *Tetrahedron Lett.* **2008**, *44*, 1243–1244, doi:10.1016/j.tetlet.2007.11.192.
19. Nelson, R.; Kesternich, V.; Pérez-Fehrmann, M.; Jaldin, S.; Marcourt, L.; Christen, P. Regiospecific synthesis of 1,4,5-trisubstituted 1,2,3-triazoles via enolate-azide cycloaddition between 1,3-dicarbonyl compounds and aryl azides. *J. Chem. Res.* **2016**, *40*, 453–457, doi:10.3184/174751916 × 14656662266973.
20. Perrin, C.L.; Chang, K.L. The Complete Mechanism of an Aldol Condensation. *J. Org. Chem.* **2016**, *81*, 5631–5635, doi:10.1021/acs.joc.6b00959.
21. Daskiewicz, J.B.; Comte, G.; Barron, D.; Di Pietro, A.; Thomasson, F. Organolithium mediated synthesis of prenylchalcones as potential inhibitors of chemoresistance. *Tetrahedron Lett.* **1999**, *40*, 7095–7098, doi:10.1016/S0040-4039(99)01461-6.
22. Xin, Y.; Zang, Z.H.; Chen, F.L. Ultrasound-promoted synthesis of 1,5-diarylpenta-2,4-dien-1-ones catalyzed by activated barium hydroxide. *Synth. Commun.* **2009**, *39*, 4062–4068, doi:10.1080/00397910902883686.
23. Jin, H.; Xiang, L.; Wen, F.; Tao, K.; Liu, Q.; Hou, T. Improved synthesis of chalconoid-like compounds under ultrasound irradiation. *Ultrason. Sonochem.* **2008**, *15*, 681–683, doi:10.1016/j.ultsonch.2008.01.006.
24. Bui, T.H.; Nguyen, N.T.; Dang, P.H.; Nguyen, H.X.; Nguyen, M.T.T. Design and synthesis of chalcone derivatives as potential non-purine xanthine oxidase inhibitors. *Springerplus* **2016**, *5*, 1789, doi:10.1186/s40064-016-3485-6.
25. Dolbier, W.R. An Overview of Fluorine NMR. In *Guide to Fluorine NMR for Organic Chemists*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2008; pp. 9–34.