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Article

Synthesis and Evaluation of the Antileishmanial Activity of Novel Eugenol Analogs Containing 1,2,3-Triazole Fragments against Intracellular *Leishmania braziliensis*

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This investigation describes the synthesis of eugenol analogs presenting 1,2,3-triazole fragments and evaluation of their antileishmanial activity. The alkylation of guaiacol (**1**) with allyl bromide afforded 1-(allyloxy)-2-methoxybenzene (**2**) (93% yield). The Claisen rearrangement conducted with **1** gave *ortho* eugenol (**3**) (82% yield). Alkylation procedures performed with **3** produced 1-allyl-3-methoxy-2-(prop-2-yn-1-yloxy)benzene (**4**) (73% yield) and 1-allyl-3-methoxy-2-(pent-4-yn-1-yloxy)benzene (**6**) (53% yield). The copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reactions involving alkynes **4** and **6** with different benzylic azides afforded twenty-two eugenol analogs with 1,2,3-triazole functionalities (48-93% yield). We screened the compounds at 10 μmol L^{-1} against *Leishmania braziliensis* intracellular amastigotes during macrophage infection. The action of these compounds was compared with the known leishmanicidal drug amphotericin B. None of the analogs were toxic to macrophages at 10 μmol L^{-1}. The cytotoxic concentration at 50% (CC_{50}), effective concentration at 50% (EC_{50}), and selectivity index (SI) were determined to the best compounds 4-((2-allyl-6-methoxy)phenoxymethyl)-1-(4-chlorobenzyl)-1*H*-1,2,3-triazole (**8c**) and 4-((2-allyl-6-methoxy)phenoxymethyl)-1-(4-trifluoromethoxybenzyl)-1*H*-1,2,3-triazole (**8h**). They showed a significant leishmanicidal effect, with EC_{50} of 28.09 μmol L^{-1} (**8c**) and 52.03 μmol L^{-1} (**8h**). The SIs were 9.7 for **8c** and > 5.7 for **8h**. These compounds have the potential as new leishmanicidal agents against *L. braziliensis* and may represent a starting point for the development of alternative treatments for cutaneous leishmaniasis.

Keywords: leishmaniasis, eugenol analogs, ortho-eugenol, 1,2,3-triazoles, cutaneous leishmaniasis

Introduction

Leishmaniasis is a group of parasitic infections caused by at least 20 species of the *Leishmania* genus.[1] They are transmitted to mammal hosts during the bite of vector insects of the genera *Lutzomyia* and *Phlebotomus*, collectively known as sandflies and belonging to the order Diptera. The transmission occurs when infectious metacyclic promastigotes of the parasite in the gut of a female sandfly are inoculated into the host mammal during a blood meal.[2]

Three main forms of leishmaniasis, visceral, cutaneous, and mucocutaneous, are known, which depend on the

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virulence of the infecting protozoan, the susceptibility of the host, and co-infections.[3,4] The clinical manifestation of the disease may occur asymptomatically to the lethal form. In the tegumentary forms, the symptoms range from skin infections (cutaneous leishmaniasis), starting with a small lump at the site of protozoan inoculation, which may progress to plaque and ulcer formation, to nose and mouth mucosal deformations and disabilities in the mucosal form. In the most severe visceral form, hemorrhages and severe anemia occur leading to death if not treated.[1]

Leishmaniasis is among the top ten neglected tropical diseases. These diseases mainly affect low-income populations in developing countries, causing significant morbidity and mortality.[5,6] It is estimated that, globally, there are about 12 million people infected with leishmaniasis, 0.9 to 1.6 million new cases each year, between 20,000 and 30,000 deaths, and 350 million people at risk of infection.[6] Therefore, leishmaniasis is an important public health concern and deserves attention.

The treatment of leishmaniasis is carried out through drug administration, mainly with the use of pentavalent antimonials, such as meglumine antimoniate and sodium stibogluconate, which are regarded as the first-line drugs.[7,8] Severe side effects (ranging from injection site pain (administration is parenteral), anorexia, adynamia, and even cardiotoxicity), the need for daily parenteral administration, and drug resistance are important problems related to the use of pentavalent antimonials.[7,8]

Other alternative drugs for the treatment of leishmaniasis are different formulations of amphotericin B, pentamidine, and paromomycin, which are considered second-line drugs and used in case of antimonial resistance of parasites.[9,10] There are also problems associated with the use of these drugs such as the need for hospitalization, the high cost of some formulations, and side effects, including fever, renal dysfunction, nausea, abdominal pain, and hepatotoxicity.[9,10] Figure 1 shows the structures of the main drugs used for leishmaniasis treatment.

In view of the aforementioned problems related to the drugs currently utilized for leishmaniasis treatment, the search and development of alternatives is an important demand. In this sense, the use of compounds obtained from

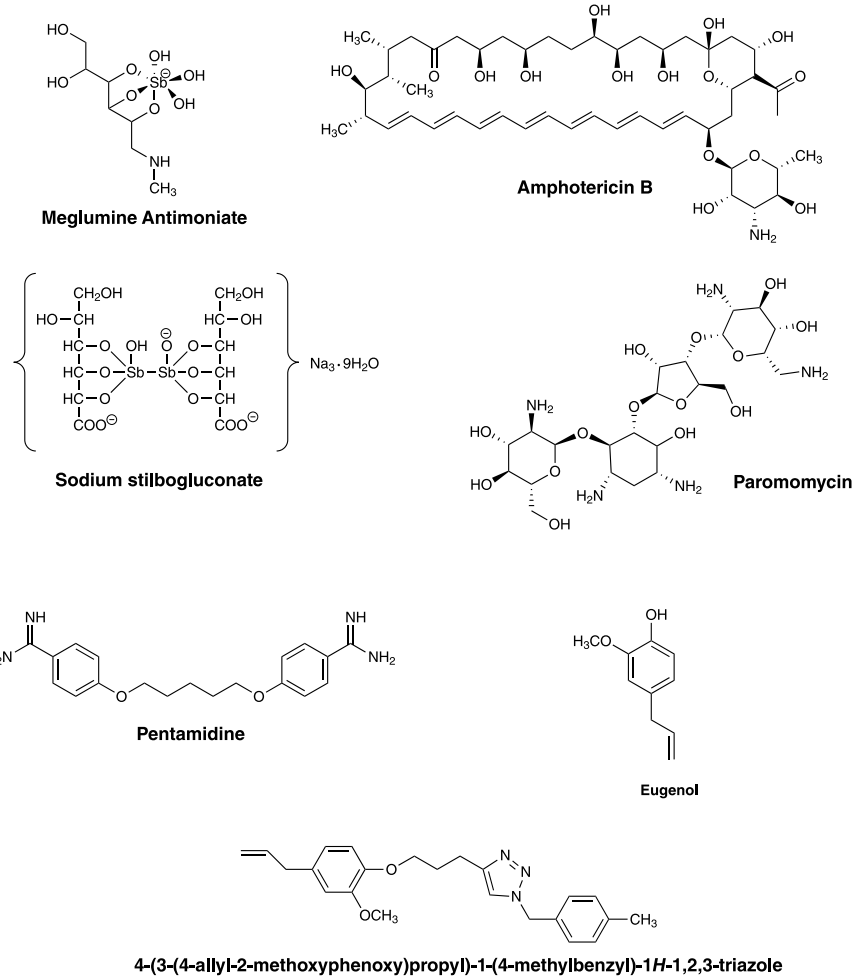


Figure 1. Structures of antileishmanial drugs, eugenol and eugenol derivative with 1,2,3-triazole functionality.

nature is an important approach.[11,12] The natural compounds can be used directly as drugs or chemically modified to improve their leishmanicidal effect. One of such natural products is eugenol (Figure 1).

Eugenol is a compound present in a variety of plants. However, *Eugenia caryophyllata* (= *Syzygium aromaticum*), known as clove, corresponds to the main natural source since generally 45 up to 90% of the oil obtained from this species is eugenol.[13] It is a very versatile substance that has several bioactivities, such as anti-inflammatory, antibacterial, antifungal, antiviral, insecticide, anticancer, analgesic, antioxidant, antimalarial, and leishmanicide.[13] Besides, eugenol is synthetically useful for the preparation of several organic compounds.[14] Our research group prepared a series of eugenol derivatives presenting 1,2,3-triazole fragments and evaluated their antileishmanial activity on *Leishmania amazonensis*.[15] It was found that compound 4-(3-(4-allyl-2-methoxyphenoxy)propyl)-1-(4-methylbenzyl)-1*H*-1,2,3-triazole (Figure 1) showed the highest efficacy (half maximal inhibitory concentration (IC_{50}) 7.4 μmol L^{-1}) against promastigote forms. This compound was selected for the evaluation of its effect against the intracellular amastigotes, with an IC_{50} 1.6 μmol L^{-1} and a macrophage selectivity index of 132.5.

Based on the premises and in continuation of our efforts to find useful compounds for leishmaniasis treatment by exploring the eugenol/1,2,3-triazole scaffold, it is described in the present investigation the preparation of novel eugenol analogs with 1,2,3-triazole fragments and the results concerning their antileishmanial activity evaluation now against *Leishmania braziliensis* which is the main species related with tegumentary leishmaniasis in the New World.

Experimental

Synthesis

Generalities

Solvents were purchased from F Maia (Mogi das Cruzes, SP, Brazil). Guaiacol, benzyl alcohols, pent-4-yn-1-ol, mesyl chloride, sodium azide, triethylamine, propargyl bromide, allyl bromide, tetrabutylammonium bromide, sodium ascorbate, copper(II) sulfate pentahydrate were procured from Sigma-Aldrich (St. Louis, MO, USA) and used as received. The nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury 300 instrument (Varian, Palo Alto, CA, USA) at 300 MHz (^{1}H) and 75 MHz (^{13}C), Bruker Avance DRX NMR (Billerica, Massachusetts, CA, USA) at 400 MHz (^{1}H) and 100 MHz (^{13}C) and Bruker Avance at 600 MHz (^{1}H) and 150 MHz (^{13}C), respectively, using $CDCl_3$ as solvent. NMR data are presented as follows: chemical shift (δ) in ppm, multiplicity, the number of hydrogens, *J* values in hertz (Hz), and hydrogen assignment. Multiplicities are shown as the following abbreviations: s (singlet), s_{ap} (apparent singlet), d (doublet), dd (doublet of doublets), dd_{ap} (apparent doublet of doublets), t (triplet), dq (doublet of quartets), ddt_{ap} (apparent doublet of doublets of triplets), t_{ap} (apparent triplet), dt (doublet of triplets), q (quartet), quint (quintet), sept (septet), m (multiplet). Fourier-transformed infrared (FTIR) spectra were obtained using Varian 660-IR (Palo Alto, CA, USA) equipped with GladiATR scanning from 4000 to 500 cm^{-1}. High-resolution mass spectra (HRMS) were recorded on a Q-Exactive (Thermo Scientific, Bremen, Germany). The spectra were acquired using the following conditions, ionization source: electron spray (+) and (−); spray voltage: 3.5 kV; capillary temperature: 275 ºC; sheath gas: 5 (arbitrary units); auxiliary gas: 0 (arbitrary units). For the mass spectrometry analyses, the samples were prepared as follows: a mass of 1 mg of the compound to be analyzed was dissolved in 1 mL of acetonitrile. Then, the solution was diluted with 1 mL of methanol so that the final concentration corresponded to 1 ppm. The resulting solution was directly injected into the Q-Exactive equipment at 5 μmL min^{-1}. The spectra were recorded in full MS mode. Melting points were uncorrected and obtained from an MQAPF-301 melting point apparatus (Microquimica, Palhoça, SC, Brazil). Analytical thin layer chromatography (TLC) analyses were conducted on aluminum-backed precoated silica gel plates using different solvent systems. After elution, the TLC plates were visualized using potassium permanganate solution and ultra-violet (UV) light. Column chromatography was performed using silica gel 60 (60-230 mesh). Solvents were dried using standard procedures described in the literature.[16]

Synthesis of 1-allyloxy-2-methoxybenzene (**2**)

A round bottom (100 mL) was charged with guaiacol (**1**) (2.25 mL, 20.0 mmol) and toluene (10.0 mL). The mixture was cooled in an ice bath and 10.0 mL of NaOH aqueous solution (35% m v^{-1}) and 0.389 g of tetrabutylammonium bromide (2.00 mmol) were added. The resulting mixture was kept under magnetic stirring for 1 h. Then, 2.07 mL of allyl bromide (24.0 mmol) were added. After that, the ice bath was removed and the reaction mixture was kept under stirring at room temperature. The completion of the reaction was confirmed after 3 h by TLC analysis. Then, brine (7.00 mL) was added to the reaction mixture, and the phases were separated. The aqueous phase was extracted with diethyl ether (3 × 20 mL). The organic extracts were combined and the resulting organic phase was washed with 1 mol L^{-1} NaOH aqueous solution, dried

under anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Compound **2** was obtained in 93% yield (3.12 g, 12.0 mmol) and was not subjected to any subsequent purification procedure. Its structure is supported by the following data.

Yellow oil; TLC: Rf = 0.53 (hexane-ethyl acetate 6:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3077, 3012, 2942, 2834, 1585, 1501, 1454, 1253, 1121, 1020, 922, 737, 576; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (s, 3H), 4.61 (dt, *J* 5.2, 1.4 Hz, 2H), 5.28 (dq, *J* 10.4, 1.4 Hz, 1H), 5.39 (dq, *J* 17.2, 1.4 Hz, 1H), 6.08 (ddt_{ap}, *J* 5.2, 10.4, 17.2 Hz, 1H), 6.84-6.95 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.1, 70.0, 111.9, 113.4, 117.5, 120.8, 121.2, 133.3, 148.0, 149.7; HRMS [M + Na^+] calcd. for $C_{10}H_{12}O_2Na$: 187.07350, found: 187.07299.

Synthesis of *ortho*-eugenol (**3**)

To a sealed tube, it was added 3.00 g (0.018 mol) of 1-allyloxy-2-methoxybenzene (**2**) under a nitrogen atmosphere. Compound **2** was kept under stirring at 200 °C for 10 h. After that, the system was cooled down to room temperature. Compound **3** was purified by silica gel column chromatography eluted with hexane-ethyl acetate (8:1 v v^{-1}) and obtained in 82% yield (2.45 g, 14.9 mmol).

Yellow oil; TLC: Rf = 0.54 (hexane-ethyl acetate 8:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3525 (broad band), 3081, 2840, 1638, 1616, 1592, 1481, 1436, 1353, 1270, 1214, 1068, 994, 908, 779, 735; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.39-3.43 (m, 2H), 3.83 (s, 3H), 5.02-5.10 (m, 2H), 5.71 (s, 1H, O<u>H</u>), 6.00 (ddt_{ap}, *J* 6.8, 10.0, 16.8 Hz, 1H), 6.71-6.81 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.6, 56.1, 108.4, 115.1, 119.3, 122.4, 125.7, 136.6, 143.5, 146.2; HRMS [M – H^+] calcd. for $C_{10}H_{11}O_{32}$: 163.07590, found: 163.07575.

Synthesis of 1-allyl-3-methoxy-2-(prop-2-yn-1-yloxy) benzene (**4**)

To a round bottom flask, it was added *ortho* eugenol (**3**) (1.15 g, 7.00 mmol) and toluene (10.0 mL). The reaction mixture was cooled in an ice bath. Then, NaOH aqueous solution 35% m v^{-1} (10.0 mL) and tetrabutylammonium bromide (0.225 g, 0.700 mmol) were added to the mixture. The resulting mixture was kept under magnetic stirring for 1 h. After that, propargyl bromide (0.726 mL, 8.40 mmol) was added, the ice bath was removed and the resulting mixture was stirred at room temperature for a further 4 h. Subsequently, toluene was removed under reduced pressure and saturated NaCl aqueous solution (7.00 mL) was added. The layers were separated and the aqueous phase was extracted with diethyl ether (3 × 20 mL). The organic extracts were combined and the resulting organic phase was dried under anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by silica gel column chromatography eluted with hexane-ethyl acetate (6:1 v v^{-1}). Compound **4** was obtained in 73% yield (0.798 g, 3.95 mmol).

Yellow oil; TLC: Rf = 0.65 (hexane-ethyl acetate 6:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3291, 3087, 3004, 2937, 2840, 2362, 2165, 2125, 1994, 1633, 1584, 1477, 1361, 1270, 1198, 1068, 998, 911, 782, 744, 628, 538; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (t, *J* 2.6 Hz, 1H), 3.48-3.52 (m, 2H), 3.84 (s, 3H), 4.70 (d, *J* 2.6 Hz, 2H), 5.02-5.11 (m, 1H), 5.97 (ddt_{ap}, *J* 6.6, 10.0, 16.8 Hz, 1H), 6.76-6.81 (m, 2H), 7.01 (t, *J* 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.2, 55.7, 59.7, 74.7, 79.6, 110.4, 115.7, 122.0, 124.0, 134.6, 137.2, 144.5, 152.5; HRMS [M + H^+] calcd. for $C_{13}H_{15}O_2$: 203.10720, found: 203.10675; [M + Na^+] calcd. for $C_{13}H_{14}O_2Na$: 225.08915, found: 225.08864.

Synthesis of pent-4-yn-1-yl methanesulfonate (**5**)

A round bottom flask (100 mL), under a nitrogen atmosphere, was charged with pent-4-yn-1-ol (1.68 g, 20.0 mmol) and 20.0 mL of dichloromethane. The reaction mixture was cooled to –50 °C and 5.60 mL of triethylamine (40.0 mmol) was added. Then, mesyl chloride (2.30 mL, 30.0 mmol) dissolved in 1.00 mL of dichloromethane was slowly added to the reaction mixture. The resulting mixture was stirred for 4 h and the completion of the reaction after this time was confirmed by TLC analysis. Subsequently, distilled water (10.0 mL) was added and the phases were separated. The organic phase was washed with 0.1 mol L^{-1} HCl aqueous solution (3 × 15.0 mL), followed by saturated $NaHCO_3$ aqueous solution (3 × 5.00 mL), dried under anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Compound **5** was purified by silica gel column chromatography eluted with hexane-ethyl acetate-dichloromethane (3:1:3 v v^{-1}). This procedure afforded compound **5** with 92% yield (3.00 g, 18.0 mmol).

Yellow oil; TLC: Rf = 0.76 (hexane-ethyl acetate-dicloromethane 3:1:3 v v^{-1}); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93 (quint, 2H, *J* 6.5 Hz), 1.99 (t, 1H, *J* 2.7 Hz), 2.33 (dt, 2H, *J* 6.8, 2.7 Hz), 3.00 (s, 3H), 4.32 (t, 2H, *J* 6.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.5, 27.6, 37.1, 68.2, 69.7, 82.0.

Synthesis of 1-allyl-3-methoxy-2-(pent-4-yn-1-yloxy)benzene (**6**)

To a round bottom flask, it was added 2-allyl-6-methoxyphenol (**3**) (1.40 g, 8.50 mmol) and toluene (10.0 mL). The reaction mixture was cooled in an ice bath. Subsequently, NaOH aqueous solution 35% m v^{-1} (10 mL) and tetrabutylammonium bromide (0.273 g,

0.850 mmol) were added and the resulting mixture was kept under magnetic stirring for 1 h. Then, pent-4-yn-1-ylmethanesulfonate (**5**) (1.65 mL, 10.2 mmol) was added, the ice bath was removed, and the mixture was stirred at room temperature for 24 h. Afterward, toluene was removed under reduced pressure, and a saturated NaCl aqueous solution (7.00 mL) was added. The phases were separated and the aqueous phase was extracted with diethyl ether (3 × 20 mL). The organic extracts were combined and the organic phase was dried under anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Compound **6** was purified by silica gel column chromatography eluted with hexane-ethyl acetate (6:1 v v^1) and obtained in 53% yield (1.00 g, 4.34 mmol).

Yellow oil; TLC: Rf = 0.69 (hexane-ethyl acetate 6:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3301, 3081, 3003, 2936, 2837, 2165, 2121, 1638, 1585, 1471, 1437, 1388, 1274, 1213, 1179, 1078, 1040, 998, 914, 830, 752, 630; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.95 (d, 1H, *J* 6.0 Hz), 1.98 (quint, 2H, *J* 6.0 Hz), 2.46 (dt, 2H, *J* 7.2, 2.4 Hz), 3.42 (d, 2H, *J* 6.0 Hz), 3.83 (s, 3H), 4.02 (t, 2H, *J* 6.0 Hz), 5.03-5.07 (m, 2H), 5.96 (ddt_{ap}, 1H, *J* 6.0, 12.0, 18.0 Hz), 6.77-6.78 (m, 2H), 6.98 (t, 1H, *J* 6.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 15.2, 29.3, 34.0, 55.8, 68.5, 71.4, 84.1, 110.6, 115.4, 122.1, 123.8, 134.0, 137.4, 146.0, 153.0; HRMS [M + H$^+$] calcd. for $C_{15}H_{19}O_2$: 231.13850, found: 231.13818; [M + Na$^+$] calcd. for $C_{15}H_{18}O_2Na$: 253.12045, found: 253.12002.

Preparation of benzyl azides

The organic azides benzylazide (**7a**), 4-fluorobenzylazide (**7b**), 4-bromobenzylazide (**7d**), 4-chlorobenzylazide (**7c**), 4-iodobenzylazide (**7e**), 4-nitrobenzylazide (**7f**), 4-methoxybenzylazide (**7g**), 4-trifluoromethoxybenzylazide (**7h**), 4-trifluoromethylbenzylazide (**7i**), 4-methylbenzylazide (**7j**), 4-isopropylbenzylazide (**7k**) utilized in preparation of eugenol analogs were obtained as previously described.[17]

General procedure for the preparation of compounds **8a-8k** and **9a-9k** exemplified by synthesis of compound 4-((2-allyl-6-methoxy)phenoxymethyl)-1-benzyl-1*H*-1,2,3-triazole (**8a**)

To a round bottom flask (10 mL), it was added 1-allyl-3-methoxy-2-(prop-2-yn-1-yloxy) benzene (**3**) (0.150 g, 0.740 mmol), benzylazide (**7a**) (0.0990 g, 0.740 mmol), sodium ascorbate (0.0590 g, 0.300 mmol), 1.00 mL ethanol and 1.00 mL water. Then, $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol) was added. The reaction mixture was kept under vigorous stirring at room temperature. The end of the reaction was confirmed by TLC analysis. The reaction mixture was extracted with dichloromethane (3 × 10.0 mL). The organic phases were combined, washed with saturated Na_2CO_3 solution, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Compound **8a** was purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}). The described procedure afforded compound **8a** in 54% yield (0.134 g, 0.402 mmol).

White solid, mp 76.2-78.2 ºC; TLC: Rf = 0.55 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3129, 3095, 3008, 2942, 2888, 2833, 2165, 1972, 1640, 1580, 1471, 1380, 1264, 1200, 1064, 988, 854, 765, 703, 651, 590, 461; ^{1}H NMR (400 MHz, $CDCl_3$) δ (d, *J* 6.4 Hz, 2H), 3.81 (s, 3H), 4.94-4.98 (m, 2H), 5.13 (s, 2H), 5.52 (s, 2H), 5.80-5.90 (m, 1H), 6.73-6.79 (m, 2H), 6.99 (t, *J* 8.0 Hz, 1H), 7.24-7.39 (m, 5H), 7.50 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.2, 54.3, 55.8, 66.3, 110.5, 115.7, 122.1, 122.8, 124.4, 128.2, 128.8, 129.2, 134.4, 134.8, 137.2, 145.3, 145.5, 152.8; HRMS [M + H$^+$] calcd. for $C_{20}H_{22}O_2N_3$: 336.17120, found: 336.17065; [M + Na$^+$] calcd. for $C_{20}H_{21}O_2N_3Na$: 358.15315, found: 358.15247; [2M + Na$^+$] calcd. for $(C_{20}H_{21}O_2N_3)_2Na$: 693.31652, found: 693.31460.

Compounds **8b**-**8k** and **9a**-**9k** were prepared using a procedure similar to that described for the synthesis of **8a**. The structures of these were confirmed by NMR (^{1}H and ^{13}C), FTIR and HRMS analyses. Information regarding the reactions involved in the preparation of the aforementioned compounds and data that support their structures are described below.

4-((2-Allyl-6-methoxy)phenoxymethyl)-1-(4-fluorobenzyl)-1*H*-1,2,3-triazole (**8b**)

White solid (0.227 g, 0.644 mmol) obtained in 87% yield from the reaction of alkyne (**3**) (0.150 g, 0.740 mmol), 4-fluorobenzylazide (**7b**) (0.111 g, 0.740 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate-dichloromethane (3:1:3 v v^{-1}), mp 62.0-63.3 ºC, TLC: Rf = 0.48 (hexane-ethyl acetate-dichloromethane 3:1:3 v v^{-1}) FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3117, 3077, 3003, 2888, 2844, 1994, 1901, 1639, 1594, 1511, 1477, 1257, 1205, 1125, 1060, 998, 914, 854, 765, 642, 526, 480; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.29-3.31 (m, 2H), 3.82 (s, 3H), 4.94-4.99 (m, 2H), 5.14 (s, 2H), 5.49 (s, 2H), 5.80-5.90 (m, 1H), 6.73-6.79 (m, 2H), 6.97-7.07 (m, 3H), 7.22-7.26 (m, 2H), 7.50 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.0, 53.3, 55.7, 66.1, 110.4, 115.6, 116.0 (d, *J* 22 Hz), 121.9, 122.5, 124.2, 129.8 (d, *J* 8 Hz), 130.5 (d, *J* 3 Hz), 134.2, 137.0, 145.2, 145.5, 152.6, 162.8 (d, *J* 246 Hz); HRMS [M + H$^+$] calcd. for $C_{20}H_{21}O_2N_3F$, 354.16178; found: 354.16148; [M + Na$^+$] calcd. for $C_{20}H_{20}O_2N_3FNa$: 376.14372, found: 376.14334.

4-((2-Allyl-6-methoxy)phenoxymethyl)-1-(4-chlorobenzyl)-1*H*-1,2,3-triazole (**8c**)

White solid, obtained in 68% yield (0.170 g, 0.460 mmol) from the reaction of alkyne (**3**) (0.137 g, 0.680 mmol), 4-chlorobenzylazide (**7c**) (0.111 g, 0.680 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate-dichloromethane (3:1:3 v v^{-1}), mp 72.7-75.0 ºC, TLC: Rf = 0.48 (hexane-ethyl acetate-dichloromethane 3:1:3 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3143, 3072, 2998, 2937, 2883, 2832, 1960, 1633, 1577, 1467, 1429, 1265, 1205, 1060, 961, 914, 857, 755, 664, 572, 496; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.29-3.31 (m 2H), 3.82 (s, 3H), 4.94-4.99 (m, 2H), 5.14 (s, 2H), 5.49 (s, 2H), 5.80-5.91 (m, 1H), 6.73-6.79 (m, 2H), 7.00 (t, *J* 8.0 Hz, 1H), 7.18 (d, *J* 8.4 Hz, 2H), 7.33 (d, *J* 8.4 Hz, 2H), 7.50 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.0, 53.3, 55.7, 66.0, 110.4, 115.6, 122.0, 122.6, 124.2, 129.3, 129.3, 133.2, 134.2, 134.7, 137.0, 145.2, 145.6, 152.6; HRMS [M + H^+] calcd. for $C_{20}H_{21}O_2N_3Cl$: 370.13223, found: 370.13199.

4-((2-Allyl-6-methoxy)phenoxymethyl)-1-(4-bromobenzyl)-1*H*-1,2,3-triazole (**8d**)

White solid, obtained in 74% yield (0.228 g, 0.551 mmol) from the reaction of alkyne (**3**) (0.150 g, 0.740 mmol), 4-bromobenzylazide (**7d**) (0.120 g, 0.740 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 82.2-83.2 ºC, TLC: Rf = 0.47 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3147, 3077, 2996, 2966, 2937, 2882, 2832, 2362, 2161, 1967, 1639, 1581, 1467, 1436, 1264, 1199, 1060, 981, 914, 792, 752, 651, 476; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.32-3.33 (m 2H), 3.85 (s, 3H), 4.97-5.02 (m, 2H), 5.16 (s, 2H), 5.50 (s, 2H), 5.84-5.92 (m, 1H), 6.76-6.82 (m, 2H), 7.02 (t, *J* 6.4 Hz, 1H), 7.14 (d, 2H, *J* 6.8 Hz), 7.51 (d, 2H, *J* 6.8 Hz), 7.53 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.0, 53.4, 55.7, 66.0, 110.4, 115.6, 122.0, 122.6, 122.8, 124.3, 129.6, 132.2, 133.7, 134.2, 137.0, 145.1, 145.6, 152.6; HRMS [M + H^+] calcd. for $C_{20}H_{21}O_2N_3Br$, 414.08171; found: 414.08171; [M + Na^+] calcd. for $C_{20}H_{20}O_2N_3BrNa$, 436.06366; found: 436.06322.

4-((2-Allyl-6-methoxy)phenoxymethyl)-1-(4-iodobenzyl)-1*H*-1,2,3-triazole (**8e**)

White solid, obtained in 75% yield (0.216 g, 0.469 mmol) from the reaction of alkyne (**3**) (0.128 g, 0.630 mmol), 4-iodobenzylazide (**7e**) (0.163 g, 0.630 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate-dichloromethane (3:1:3 v v^{-1}), mp 91.0-92.7 ºC, TLC: Rf = 0.53 (hexane-ethyl acetate-dichloromethane 3:1:3 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3143, 3077, 3002, 2937, 2894, 2840, 2161, 1976, 1639, 1581, 1488, 1477, 1429, 1312, 1264, 1195, 1053, 980, 913, 862, 793, 755, 651, 472; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.30-3.32 (m 2H), 3.83 (s, 3H), 4.95-5.01 (m, 2H), 5.15 (s, 2H), 5.47 (s, 2H), 5.81-5.91 (m, 1H), 6.74-6.80 (m, 2H), 6.98-7.03 (m, 3H), 7.51 (s, 1H), 7.70 (d, *J* 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.0, 53.5, 55.7, 66.0, 94.4, 110.4, 115.6, 121.9, 122.6, 124.2, 129.7, 134.2, 134.3, 137.0, 138.2, 145.1, 145.6, 152.6; HRMS [M + H^+] calcd. for $C_{20}H_{21}O_2N_3I$, 462.06784, found: 462.06762; [M + Na^+] calcd. for $C_{20}H_{20}O_2N_3INa$: 484.04979, found: 484.04935; [2M + Na^+] calcd. for $(C_{20}H_{20}O_2N_3I)_2Na$: 945.10981, found: 945.10565.

4-((2-Allyl-6-methoxy)phenoxymethyl)-1-(4-nitrobenzyl)-1*H*-1,2,3-triazole (**8f**)

White solid, obtained in 72% yield (0.203 g, 0.535 mmol) from the reaction of alkyne (**3**) (0.150 g, 0.740 mmol), 4-nitrobenzylazide (**7f**) (0.132 g, 0.740 mmol), sodium ascorbate (0 0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 40.0-42.0 ºC, TLC: Rf = 0.21 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3127, 3077, 3008, 2971, 2898, 2838, 2366, 2156, 1932, 1646, 1605, 1515, 1472, 1423, 1340, 1278, 1178, 1060, 976, 923, 809, 775, 727, 647, 453; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.34 (d, *J* 8.0 Hz, 2H), 3.85 (s, 3H), 4.97-5.01 (m, 2H), 5.19 (s, 2H), 5.67 (s, 2H), 5.85-5.93 (m, 1H), 6.76-6.82 (m, 2H), 7.03 (t, *J* 6.4 Hz, 1H), 7.39 (d, *J* 6.8 Hz, 2H), 7.63 (s, 1H), 8.23 (d, *J* 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.1, 53.0, 55.7, 65.9, 110.4, 115.7, 122.0, 123.0, 124.2, 124.3, 128.5, 134.1, 137.0, 141.7, 145.0, 146.0, 148.0, 152.6; HRMS [M + H^+] calcd. for $C_{20}H_{21}O_4N_4$: 381.15628, found: 381.15620; [M + Na^+] calcd. for $C_{20}H_{20}O_4N_4Na$: 403.13822, found: 403.13780.

4-((2-Allyl-6-methoxy)phenoxymethyl)-1-(4-methoxybenzyl)-1*H*-1,2,3-triazole (**8g**)

White solid, obtained in 72% yield (0.270 g, 0.740 mmol) from the reaction of alkyne (**3**) (0.150 g, 0.740 mmol), 4-methoxybenzylazide (**7g**) (0.120 g, 0.740 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 49.9-53.5 ºC, TLC: Rf = 0.50 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3129, 3074, 3007, 2936, 2835, 2165, 1976, 1731, 1643, 1608, 1581, 1506, 1479, 1436, 1243, 1175, 1064, 996,919, 850, 769, 645,

560, 516; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.29-3.31 (m, 2H), 3.80 (s, 3H), 3.81 (s, 3H), 4.93-4.99 (m, 2H), 5.12 (s, 2H), 5.45 (s, 2H), 5.80-5.90 (m, 1H), 6.71-6.79 (m, 2H), 6.89 (d, *J* 8.7 Hz, 2H), 6.99 (t, *J* 8 Hz, 1H), 7.21 (d, *J* 8.7 Hz, 2H), 7.47 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.0, 53.6, 55.3, 55.7, 66.2, 110.4, 114.4, 115.6, 121.9, 122.4, 124.2, 126.6, 129.6, 134.2, 137.0, 145.2, 145.3, 152.6, 159.9; HRMS [M + H$^+$] calcd. for $C_{21}H_{24}O_3N_3$: 366.18177, found: 366.18184; [M + Na$^+$] calcd. for $C_{21}H_{23}O_3N_3Na$: 388.16371, found: 388.16310; [M + K$^+$] calcd. for $C_{21}H_{23}O_3N_3K$: 404.13765, found: 404.13714; [2M + Na$^+$] calcd. for $(C_{21}H_{23}O_3N_3)_2Na$: 753.33765, found: 753.33569.

4-((2-Allyl-6-methoxy)phenoxymethyl)-1-(4-trifluoromethoxybenzyl)-1*H*-1,2,3-triazole (**8h**)

White solid, obtained in 61% yield (0.165 g, 0.394 mmol) from the reaction of alkyne (**3**) (0.128 g, 0.630 mmol), 4-trifluoromethoxybenzylazide (**7h**) (0.136 g, 0.630 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate-dichloromethane (3:1:3 v v^{-1}), mp 59.5-61.5 ºC, TLC: Rf = 0.56 (hexane-ethyl acetate-dichloromethane 3:1:3 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3120, 3073, 3004, 2923, 2840, 2169, 2011, 1976, 1643, 1585, 1511, 1481, 1257, 1205, 1151, 1068, 989, 923, 858, 765, 647, 507; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.30-3.31 (m, 2H), 3.82 (s, 3H), 4.94-4.99 (m, 2H), 5.15 (s, 2H), 5.53 (s, 2H), 5.81-5.91 (m, 1H), 6.73-6.79 (m, 2H), 7.00 (t, *J* 8.0 Hz, 1H), 7.21 (d, *J* 8.4 Hz, 2H), 7.27 (t, *J* 8.4 Hz, 2H), 7.52 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.0, 53.5, 55.7, 66.0, 110.4, 115.6, 121.5, 122.0, 122.6, 124.2, 129.5, 133.4, 134.2, 137.0, 145.1, 145.7, 149.4, 152.6; HRMS [M + H$^+$] calcd. for $C_{21}H_{21}O_3N_3F_3$: 420.15350, found: 420.15320; [M + Na$^+$] calcd. for $C_{21}H_{20}O_3N_3F_3Na$: 442.13545, found: 442.13471. [2M + Na$^+$] calcd. for $(C_{21}H_{20}O_3N_3F_3)_2Na$: 861.28112, found: 861.27889.

4-((2-Allyl-6-methoxy)phenoxymethyl)-1-(4-trifluoromethylbenzyl)-1*H*-1,2,3-triazole (**8i**)

White solid obtained in 63% yield (0.189 g, 0.468 mmol) from the reaction of alkyne (**3**) (0.150 g, 0.740 mmol), 4-trifluoromethylbenzylazide (**7i**) (0.223 g, 1.11 mmol), sodium (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 69.5-71.5 ºC, TLC: Rf = 0.45 (hexane-ethyl acetate-dichloromethane 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3135, 3074, 3009, 2938, 2837, 1976, 1646, 1591, 1476, 1382, 1330, 1270, 1199, 1126, 1057, 998, 920, 855, 775, 748, 651, 501; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.32-3.34 (m, 2H), 3.85 (s, 3H), 4.97-5.01 (m, 2H), 5.18 (s, 2H), 5.61 (s, 2H), 5.84-5.92 (m, 1H), 6.76-6.82 (m, 2H), 7.02 (t, *J* 6.4 Hz, 1H), 7.37 (d, *J* 6.4 Hz, 4H), 7.57 (s, 1H), 7.65 (d, *J* 6.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.0, 53.4, 55.6, 66.0, 110.2, 115.6, 121.9, 122.8, 124.3, 126.1 (q, *J* 3.0 Hz), 128.1, 131.0 (q, *J* 26.0 Hz), 134.2, 137.0, 138.6, 145.1, 145.8, 152.6, 123.8 (*J* 217.0 Hz); HRMS [M + H$^+$] calcd. for $C_{21}H_{21}O_2N_3F_3$: 404.15859, found: 404.15824; [M + Na$^+$] calcd. for $C_{21}H_{20}O_2N_3F_3Na$: 426.14053, found: 426.13975; [2M + Na$^+$] calcd. for $(C_{21}H_{20}O_2N_3F_3)_2Na$: 829.29129, found: 829.28877.

4-((2-Allyl-6-methoxy)phenoxymethyl)-1-(4-methylbenzyl)-1*H*-1,2,3-triazole (**8j**)

White solid, obtained in 88% yield (0.228 g, 0.652 mmol) from the reaction of alkyne (**3**) (0.150 g, 0.740 mmol), 4-methylbenzylazide (**7j**) (0.147 g, 1.00 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 64.5-66.5 ºC, TLC: Rf = 0.56 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3139, 3081, 3002, 2950, 2888, 2840, 2165, 1980, 1637, 1580, 1511, 1471, 1432, 1384, 1270, 1205, 1124, 1054, 988, 923, 858, 757, 650, 511, 460; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.37 (s, 3H), 3.32-3.33 (m, 2H), 3.84 (s, 3H), 4.97-5.01 (m, 2H), 5.15 (s, 2H), 5.50 (s, 2H), 5.84-5.92 (m, 1H), 6.75-6.81 (m, 2H), 7.01 (t, *J* 6.4 Hz, 1H), 7.19 (s, 4H), 7.50 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.1, 34.0, 53.9, 55.6, 66.1, 110.3, 115.6, 121.9, 122.6, 124.2, 128.1, 129.7, 131.6, 134.2, 137.0, 138.6, 145.2, 145.3, 152.6; HRMS [M + H$^+$] calcd. for $C_{21}H_{24}O_2N_3$: 350.18685; found: 350.18635. [M + Na$^+$] calcd. for $C_{21}H_{23}O_2N_3Na$: 372.16880; found: 372.16836.

4-((2-Allyl-6-methoxy)phenoxymethyl)-1-(4-isopropylbenzyl)-1*H*-1,2,3-triazole (**8k**)

White solid, obtained in 57% yield (0.159 g, 0.422 mmol) from the reaction of alkyne (**3**) (0.150 g, 0.740 mmol), 4-isopropylbenzylazide (**7k**) (0.130 g, 0.740 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 49.0-51.8 ºC, TLC: Rf = 0.59 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3122, 3077, 2954, 2867, 2156, 1972, 1643, 1577, 1511, 1477, 1316, 1265, 1199, 1127, 1054, 998, 910, 850, 765, 734, 642, 539; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (d, *J* 5.6 Hz, 6H), 2.93 (sept, *J* 5.6 Hz, 1H), 3.32-3.33 (m, 2H), 3.83 (s, 3H), 4.96-5.01 (m, 2H), 5.16 (s, 2H), 5.51 (s, 2H), 5.84-5.92 (m, 1H), 6.75-6.81 (m, 2H), 7.02 (t, *J* 6.2 Hz, 1H), 7.21-7.28 (m, 4H), 7.52 (s, 1H); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 23.9, 33.8, 34.0, 53.9, 55.6, 66.2, 110.3, 115.6, 121.9, 122.6, 124.2, 127.1, 128.2, 131.9, 134.3, 137.0, 145.2, 145.3, 149.6, 152.6; HRMS [M + H^+] calcd. for $C_{23}H_{28}O_2N_3$: 378.21815, found: 378.21757; [M + Na^+] calcd. for $C_{23}H_{27}O_2N_3Na$: 400.20010, found: 400.19908; [2M + Na^+] calcd. for $(C_{23}H_{27}O_2N_3)_2Na$: 777.41042, found: 777.40807.

4-(3-(2-Allyl-6-methoxyphenoxy)propyl)-1-benzyl-1*H*-1,2,3-triazole (**9a**)

Yellow oil, obtained in 73% yield (0.196 g, 0.540 mmol) from the reaction of alkyne (**5**) (0.170 g, 0.740 mmol), benzylazide (**7a**) (0.133 g, 1.00 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4{\cdot}5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), TLC: R_f = 0.61 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3136, 3073, 2942, 2840, 2165, 1633, 1585, 1477, 1270, 1213, 1060, 913, 782, 725, 572, 458; ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.93 (t, *J* 7.8 Hz, 2H), 2.10-2.14 (m, 2H), 3.36-3.37 (m, 2H), 3.76 (s, 3H), 3.95 (t, *J* 6.6 Hz, 2H), 4.99-5.02 (m, 2H), 5.49 (s, 2H), 5.88-5.95 (m, 1H), 6.74-6.76 (m, 2H), 6.96 (t, *J* 8.4 Hz, 1H), 7.25-7.26 (m, 3H), 7.34-7.36 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 22.3, 29.9, 34.0, 54.0, 55.6, 72.0, 110.4, 115.5, 120.4, 122.0, 123.7, 127.9, 128.6, 129.0, 133.9, 134.9, 137.2, 146.0, 148.2, 152.7; HRMS [M + H^+] calcd. for $C_{22}H_{22}O_2N_3$: 364.20250, found: 364.20183; [M + Na^+] calcd. for $C_{22}H_{25}O_2N_3Na$: 386.18445, found: 386.18364; [2M + Na^+] calcd. for $(C_{22}H_{25}O_2N_3)_2Na$: 749.37912; found: 749.37698.

4-(3-(2-Allyl-6-methoxyphenoxy)propyl)-1-(4-fluorobenzyl)-1*H*-1,2,3-triazole (**9b**)

White solid, obtained in 71% yield (0.199 g, 0.524 mmol) from the reaction of alkyne (**5**) (0.170 g, 0.740 mmol), 4-fluorobenzylazide (**7b**) (0.151 g, 1.00 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4{\cdot}5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 46.5-48.6 ºC, TLC: Rf = 0.41 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3108, 3055, 3002, 2950, 2885, 2836, 1643, 1598, 1608, 1467, 1432, 1383, 1344, 1275, 1216, 1170, 1054, 919, 848, 761, 665, 537, 480; ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.15 (quint, *J* 6.4 Hz, 2H), 2.95 (t, *J* 7.9 Hz, 2H), 3.39-3.40 (m, 2H), 3.80 (s, 3H), 3.98 (t, *J* 5.2 Hz, 2H), 5.01-5.02 (m, 2H), 5.48 (s, 2H), 5.89-5.99 (m, 1H), 6.78 (d, *J* 8.4 Hz, 2H), 6.99 (t, *J* 7.6 Hz, 1H), 7.07 (t, *J* 8.4 Hz, 2H), 7.25-7.28 (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 23.3, 30.0, 34.0, 53.2, 55.6, 72.0, 110.5, 115.5, 116.0 (d, *J* 22 Hz), 120.6, 122.0, 123.8, 129.8 (d, *J* 8 Hz), 130.8 (d, *J* 3 Hz), 133.9, 137.2, 146.1, 148.3, 152.7, 162.8 (d, *J* 247 Hz); HRMS [M + H^+] calcd. for $C_{22}H_{25}O_2N_3F$: 382.19308, found: 382.19243; [M + Na^+] calcd. for $C_{22}H_{24}O_2N_3FNa$: 404.17502, found: 404.17418; [2M + Na^+] calcd. for $(C_{22}H_{24}O_2N_3F)_2Na$: 785.36028, found: 785.35841.

4-(3-(2-Allyl-6-methoxyphenoxy)propyl)-1-(4-chlorobenzyl)-1*H*-1,2,3-triazole (**9c**)

White solid, obtained in 66% yield (0.195 g, 0.490 mmol) from the reaction of alkyne (**5**) (0.170 g, 0.740 mmol), 4-chlorobenzylazide (**7c**) (0.370 g, 1.00 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4{\cdot}5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 37.5-38.5 ºC, TLC: Rf = 0.44 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3126, 3070, 2933, 1640, 1577, 1473, 1429, 1348, 1265, 1199, 1072, 1006, 915, 769, 657, 502; ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.12 (quint, *J* 6.6 Hz, 2H), 2.94 (t, *J* 7.2 Hz, 2H), 3.37 (d, *J* 6.6 Hz, 2H), 3.78 (s, 3H), 3.96 (t, *J* 6.0 Hz, 2H), 4.99-5.03 (m, 2H), 5.46 (s, 2H), 5.89-5.96 (m, 1H), 6.75-6.76 (m, 2H), 6.97 (t, *J* 7.8 Hz, 1H), 7.18 (t, *J* 8.4 Hz, 2H), 7.25 (s, 1H), 7.32-7.33 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 22.4, 30.0, 34.1, 53.2, 55.8, 72.1, 110.4, 115.6, 120.7, 122.1, 123.8, 129.3, 133.5, 133.9, 134.6, 137.4, 146.0, 148.7, 152.7; HRMS [M + H^+] calcd. for $C_{22}H_{25}O_2N_3Cl$: 398.1635, found: 398.1630; [M + Na^+] calcd. for $C_{22}H_{24}O_2N_3ClNa$: 420.1455, found: 420.1450; [M + K^+] calcd. for $C_{22}H_{24}O_2N_3ClK$: 436.1194, found: 436.1181. [2M + H^+] calcd. for $(C_{22}H_{24}O_2N_3Cl)_2H$: 795.3192, found: 795.3180; [2M + Na^+] calcd. for $(C_{22}H_{24}O_2N_3Cl)_2Na$: 817.3012, found: 817.2985.

4-(3-(2-Allyl-6-methoxyphenoxy)propyl)-1-(4-bromobenzyl)-1*H*-1,2,3-triazole (**9d**)

White solid, obtained in 64% yield (0.208 g, 0.471 mmol) from the reaction of the alkyne (**5**) (0.170 g, 0.740 mmol), 4-bromobenzylazide (**7d**) (0.210g, 1.00 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4{\cdot}5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 46.0-49.0 ºC, TLC: Rf = 0.37 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3118, 3070, 3016, 2931, 2840, 1643, 1577, 1473, 1432, 1261, 1208, 1072,1010, 907, 778, 738, 656, 493. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.11-2.18 (m, 2H), 2.96 (t, *J* 7.6 Hz, 2H), 3.39-3.40 (m, 2H), 3.80 (s, 3H), 3.98 (t, *J* 6.4 Hz, 2H), 5.01-5.06 (m, 2H), 5.47 (s, 2H), 5.90-6.00 (m, 1H), 6.78 (d, *J* 7.6 Hz, 2H), 6.99 (t, *J* 8.4 Hz, 1H), 7.14 (d, *J* 8.4 Hz, 2H), 7.27 (s, 1H), 7.50-7.52 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.3, 30.0, 34.0, 53.3, 55.7, 72.1,

110.6, 115.6, 120.7, 122.0, 122.7, 123.7, 129.5, 132.4, 133.9, 133.9, 137.2, 146.0, 152.8; HRMS [M + H$^+$] calcd. for $C_{22}H_{25}O_2N_3Br$: 442.1130, found: 442.1125; [M + Na$^+$] calcd. for $C_{22}H_{24}O_2N_3BrNa$: 464.0950, found: 464.0934; [M + K$^+$] calcd. for $C_{22}H_{24}O_2N_3BrK$: 480.0689, found: 480.0681.

4-(3-(2-Allyl-6-methoxyphenoxy)propyl)-1-(4-iodobenzyl)-1*H*-1,2,3-triazole (**9e**)

White solid, obtained in 93% yield (0.337 g, 0.690 mmol) from the reaction of alkyne (**5**) (0.170 g, 0.740 mmol), 4-iodobenzylazide (**7e**) (0.191 g, 0.740 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 57.0-58.0 ºC, TLC: Rf = 0.37 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3110, 3059, 2937, 2871, 2840, 1639, 1585, 1481, 1437, 1371, 1265, 1208, 1064, 1033, 1010, 907, 748, 647, 485; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.11-2.18 (m, 2H), 2.96 (t, *J* 7.2 Hz, 2H), 3.38-3.40 (m, 2H), 3.80 (s, 3H), 3.98 (t, *J* 6.4 Hz, 2H), 5.01-5.06 (m, 2H), 5.45 (s, 2H), 5.89-5.99 (m, 1H), 6.78 (d, *J* 8.2 Hz, 2H), 6.97-7.02 (m, 3H), 7.27 (s, 1H), 7.71 (d, *J* 8.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.3, 30.0, 34.0, 53.4, 55.7, 72.0, 94.3, 110.5, 115.5, 120.8, 122.0, 123.7, 129.7, 133.9, 134.6, 137.2, 138.2, 146.0, 152.7; HRMS [M + H$^+$] calcd. for $C_{22}H_{25}O_2N_3I$: 490.09915, found: 490.09891.

4-(3-(2-Allyl-6-methoxyphenoxy)propyl)-1-(4-nitrobenzyl)-1*H*-1,2,3-triazole (**9f**)

Yellow oil, obtained in 68% yield (0.205 g, 0.503 mmol) from the reaction of alkyne (**5**) (0.170 g, 0.740 mmol), 4-nitrobenzylazide (**7f**) (0.178 g, 1.00 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), TLC: Rf = 0.19 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3139, 3074, 2939, 2832, 1629, 1606, 1585, 1519, 1457, 1446, 1344, 1261, 1213, 1120, 1054, 919, 857, 787, 731, 651, 524; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.13-2.20 (m, 2H), 2.99 (t, *J* 7.6 Hz, 2H), 3.39 (d, *J* 6.4 Hz, 2H), 3.81 (s, 3H), 4.00 (t, *J* 6.4 Hz, 2H), 5.01-5.06 (m, 2H), 5.63 (s, 2H), 5.89-5.99 (m, 1H), 6.78 (d, *J* 8.4 Hz, 2H), 7.00 (t, *J* 7.6 Hz, 1H), 7.36 (s, 1H), 7.39 (d, *J* 8.6 Hz, 2H), 8.22 (d, *J* 8.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4, 29.9, 34.0, 52.9, 55.7, 72.0, 110.5, 115.6, 121.1, 122.0, 123.8, 124.2, 128.4, 133.9, 137.2, 142.1, 146.0, 148.0, 148.8, 152.7; HRMS [M + H$^+$] calcd. for $C_{22}H_{25}O_4N_4$: 409.1876, found: 409.1873; [M + Na$^+$] calcd. for $C_{22}H_{24}O_4N_4Na$: 431.1695, found: 431.1690; [M + K$^+$] calcd. for $C_{22}H_{24}O_4N_4K$: 447.1435, found: 447.1425.

4-(3-(2-Allyl-6-methoxyphenoxy)propyl)-1-(4-methoxybenzyl)-1*H*-1,2,3-triazole (**9g**)

White solid, obtained in 67% yield (0.194 g, 0.495 mmol) from the reaction of alkyne (**5**) (0.170 g, 0.740 mmol), 4-methoxybenzylazide (**7g**) (0.163 g, 1.00 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 61.7-63.0 ºC, TLC: Rf = 0.45 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3112, 3064, 3012, 2937, 2832, 1639, 1612, 1577, 1515, 1472, 1440, 1371, 1282, 1246, 1176, 1065, 1024, 1020, 910, 822, 744, 682, 555, 507. ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.11 (quint, *J* 6.6 Hz, 2H), 2.91 (t, *J* 7.8 Hz, 2H), 3.36 (d, *J* 6.6 Hz, 2H), 3.77 (s, 3H), 3.80 (s, 3H), 3.95 (t, *J* 6.0 Hz, 2H), 4.99-5.02 (m, 2H), 5.40 (s, 2H), 5.89-5.95 (m, 1H), 6.75 (d, *J* 8.1 Hz, 2H), 6.88 (d, *J* 8.4 Hz, 2H), 6.96 (t, *J* 8.1 Hz, 1H), 7.20-7.22 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 22.3, 30.2, 34.0, 53.5, 55.3, 55.6, 72.1, 110.4, 114.4, 115.6, 120.7, 122.1, 123.7, 126.9, 129.5, 134.0, 137.4, 146.3, 148.2, 152.7, 159.9; HRMS [M + H$^+$] calcd. for $C_{23}H_{28}O_3N_3$: 394.2131, found: 394.2124; [M + Na$^+$] calcd. for $C_{23}H_{27}O_3N_3Na$: 416.1950, found: 416.1936. [M + K$^+$] calcd. for $C_{23}H_{27}O_3N_3K$: 432.1689, found: 432.1671; [2M + H$^+$] calcd. for $(C_{23}H_{27}O_3N_3)_2H$: 787.4183, found: 787.4169; [2M + Na$^+$] calcd. for $(C_{23}H_{27}O_3N_3)_2Na$: 809.4002, found: 809.3997.

4-(3-(2-Allyl-6-methoxyphenoxy)propylyl)-1-(4-trifluoromethoxybenzyl)-1*H*-1,2,3-triazole (**9h**)

Yellow oil, obtained in 51% yield (0.169 g, 0.377 mmol) from the reaction of alkyne (**5**) (0.170 g, 0.740 mmol), 4-trifluoromethoxybenzylzide (**7h**) (0.217 g, 1.00 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), TLC: Rf = 0.40 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3139, 1078, 2942, 2836, 1638, 1581, 1510, 1471, 1261, 1213, 1164, 1051, 1010, 914, 779, 744, 659, 608, 528; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.12-2.19 (m, 2H), 2.97 (t, *J* 7.2 Hz, 2H), 3.39-3.40 (m, 2H), 3.80 (s, 3H), 3.99 (t, *J* 6.4 Hz, 2H), 5.01-5.06 (m, 2H), 5.52 (s, 2H), 5.90-6.00 (m, 1H), 6.78 (d, *J* 7.8 Hz, 2H), 7.00 (t, *J* 7.8 Hz, 1H), 7.22-7.24 (m, 2H), 7.28-7.30 (m, 3H); HRMS [M + H$^+$] calcd. for $C_{23}H_{25}O_3N_3F_3$: 448.1848, found: 448.1843; [M + Na$^+$] calcd. for $C_{23}H_{25}O_3N_3F_3Na$: 470.1668, found: 470.1655; [M + K$^+$] calcd. for $C_{23}H_{25}O_3N_3F_3K$: 486.1407, found: 486.1401; [2M + H$^+$] calcd. for $(C_{23}H_{24}O_3N_3F_3)_2H$: 895.3618, found: 895.3598; [2M + Na$^+$] calcd. for $(C_{23}H_{24}O_3N_3F_3)_2Na$: 917.3437, found: 917.3430.

4-(3-(2-Allyl-6-methoxyphenoxy)propyl)-1-(4-trifluoromethylbenzyl)-1*H*-1,2,3-triazole (**9i**)

Yellow oil, obtained in 48% yield (0.154 g, 0.357 mmol) from the reaction alkyne (**5**) (0.170 g, 0.740 mmol), 4-trifluoromethylbenzylazide (**7i**) (0.201 g, 1.00 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), TLC: Rf = 0.45 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3143, 3077, 2950, 2836, 1625, 1585, 1477, 1436, 1265, 1163, 1126, 1072, 1006, 919, 817, 752, 593, 494; HRMS [M + H^+] calcd. for $C_{23}H_{25}O_2N_3F_3$: 432.1899, found: 432.1887; [M + Na^+] calcd. for $C_{23}H_{24}O_2N_3F_3Na$: 454.1718, found: 454.1703; [M + K^+] calcd. for $C_{23}H_{24}O_2N_3F_3K$, 470.1458, found: 470.1474; [2M + H^+] calcd. for $(C_{23}H_{24}O_2N_3F_3)_2H$: 863.3720, found: 863.2698.

4-(3-(2-Allyl-6-methoxyphenoxy)propyl)-1-(4-methylbenzyl)-1*H*-1,2,3-triazole (**9j**)

White solid, obtained in 66% yield (0.183 g, 0.485 mmol) from the reaction of alkyne (**5**) (0.170 g, 0.740 mmol), 4-methylbenzylazide (**7j**) (0.147 g, 1.00 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 34.5-36.8 ºC, TLC: Rf = 0.50 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3126, 3077, 3016, 2942, 2844, 1633, 1585, 1516, 1477, 1378, 1266, 1205, 1064, 998, 910, 817, 744, 659, 532, 471; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.08-2.15 (m, 2H), 2.34 (s, 3H), 2.91 (t, *J* 7.2 Hz, 2H), 3.36-3.38 (m, 2H), 3.77 (s, 3H), 3.95 (t, *J* 6.0 Hz, 2H), 4.99-5.03 (m, 2H), 5.44 (s, 2H), 5.87-5.97 (m, 1H), 6.75 (d, *J* 8.0 Hz, 2H), 6.96 (t, *J* 8.0 Hz, 1H), 7.16 (s, 4H), 7.22 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.3, 22.5, 30.1, 34.2, 54.0, 55.8, 72.2, 110.7, 115.6, 120.8, 122.2, 123.9, 128.2, 129.9, 132.1, 134.1, 137.5, 138.7, 146.3, 148.3, 152.9; HRMS [M + H^+] calcd. for $C_{23}H_{28}O_2N_3$: 378.21815, found: 378.21807; [M + Na^+] calcd. for $C_{23}H_{27}O_2N_3Na$: 400.20010, found: 400.19962.

4-(3-(2-Allyl-6-methoxyphenoxy)propyl)-1-(4-isopropylbenzyl)-*1H*-1,2,3-triazole (**9k**)

White solid, obtained in 61% yield (0.184 g, 0.454 mmol) from the reaction of alkyne (**5**) (0.170 g, 0.740 mmol), 4-isopropylbenzylazide (**7k**) (0.175 g, 1.00 mmol), sodium ascorbate (0.0590 g, 0.300 mmol) and $CuSO_4 \cdot 5H_2O$ (0.0370 g, 0.150 mmol). Purified by silica gel column chromatography eluted with hexane-ethyl acetate (2:1 v v^{-1}), mp 35.5-37.0, TLC: Rf = 0.50 (hexane-ethyl acetate 2:1 v v^{-1}); FTIR (ATR) $\bar{\nu}_{max}$ / cm^{-1} 3116, 3074, 2964, 2875, 2835, 1646, 1581, 1477, 1388, 1265, 1205, 1064, 906, 854, 782, 752, 665, 541; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (d, *J* 6.8 Hz, 6H), 2.08-2.16 (m, 2H), 2.85-2.95 (m, 3H), 3.36-3.38 (m, 2H), 3.76 (s, 3H), 3.95 (t, *J* 6.4 Hz, 2H), 4.98-5.03 (m, 2H), 5.45 (s, 2H), 5.87-5.97 (m, 1H), 6.75 (d, *J* 8.0 Hz, 2H), 6.96 (t, *J* 8.0 Hz, 1H), 7.17-7.21 (m, 4H), 7.24 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.5, 24.0, 30.1, 34.0, 34.2, 54.0, 55.8, 72.2, 110.7, 115.7, 120.9, 122.2, 123.9, 127.3, 128.2, 132.5, 134.1, 137.5, 146.3, 148.3, 149.7, 152.9; HRMS [M + H^+] calcd. for $C_{25}H3_2O_2N_3$: 406.24945, found: 406.24909.

Biological assays

Mammalian cells and parasite strain

Raw 264.7 macrophages (ATCC, Gaithersburg, MD, USA) were kept in RPMI medium (RPMI-1640, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% inactivated fetal calf serum (LGC Biotecnologia, Cotia, SP, Brazil), penicillin (100 µg mL^{-1}) (USB Corporation, Cleveland, OH, USA) and L-glutamine (2 mmol L^{-1}) (Serva Electrophoresis & Life Science Products, Heidelberg, Germany), pH 7.2, filtered through a 0.22 µm membrane and incubated at 37 °C in a humid atmosphere containing 5% CO_2 (Forma Series II Water-Jacketed CO_2 Incubators, Thermo Fisher Scientific, Waltham, MA, USA). The promastigote forms of *L. braziliensis* - M2904-GFP (this cell line constitutively expresses the Green Fluorescent Protein "GFP") were maintained in Grace's medium (Grace's Insect medium, Sigma-Aldrich, St. Louis, MO, USA),[18] supplemented with 10% inactivated fetal calf serum (LGC Biotecnologia, Cotia, SP, Brazil), penicillin (100 µg mL^{-1}) (USB Corporation, OH, USA) and L-glutamine (2 mmol L^{-1}) (Serva Electrophoresis & Life Science Products, Heidelberg, Germany), pH 6.5, filtered through a 0.22 µm membrane and incubated in a B.O.D incubator (B.O.D 411D Incubator, New Ethics, São Paulo, SP, Brazil) at 25 ºC.

Dilution of eugenol analogs

The eugenol analogs were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) to obtain a concentration of 10 mmol L^{-1}. Then, these solutions were diluted with sterilized ultrapure water to prepare 0.30 mmol L^{-1} solutions that were stored at –20 ºC until use.

Cytotoxicity to mammalian cells

The evaluation of the cytotoxic action of the compounds was performed using the resazurin method in 96 well microplates as previously described.[18] Positive and

negative controls were amphotericin B (3.125 μg mL^{-1}; 3.4 μmol L^{-1}) (Sigma-Aldrich, St. Louis, MO, USA) and DMSO (0.1% v v^{-1}) (Neon Comercial Reagentes Analíticos Ltda, Suzano, SP, Brazil), respectively. The concentration of 0.1% (v v^{-1}) of DMSO in the control samples is the same final amount of DMSO used in the assays with eugenol analogs. The eugenol analogs were assayed at a final concentration of 10 μmol L^{-1}. The choice of this concentration was based on the guidelines in a hit and lead criteria in drug discovery for infectious diseases.[19] For the cytotoxic concentration at 50% (CC_{50}) determination assay, the compounds were tested at concentrations of 300, 270, 240, 210, 180, 80, 40, 30, 20, 10, 5, 2.5, and 1 μmol L^{-1}. Experiments were performed independently at least 3 times in quadruplicates. GraphPad Prism version 6[20] was used for eugenol analogs CC_{50} determination.

Macrophage infection assay

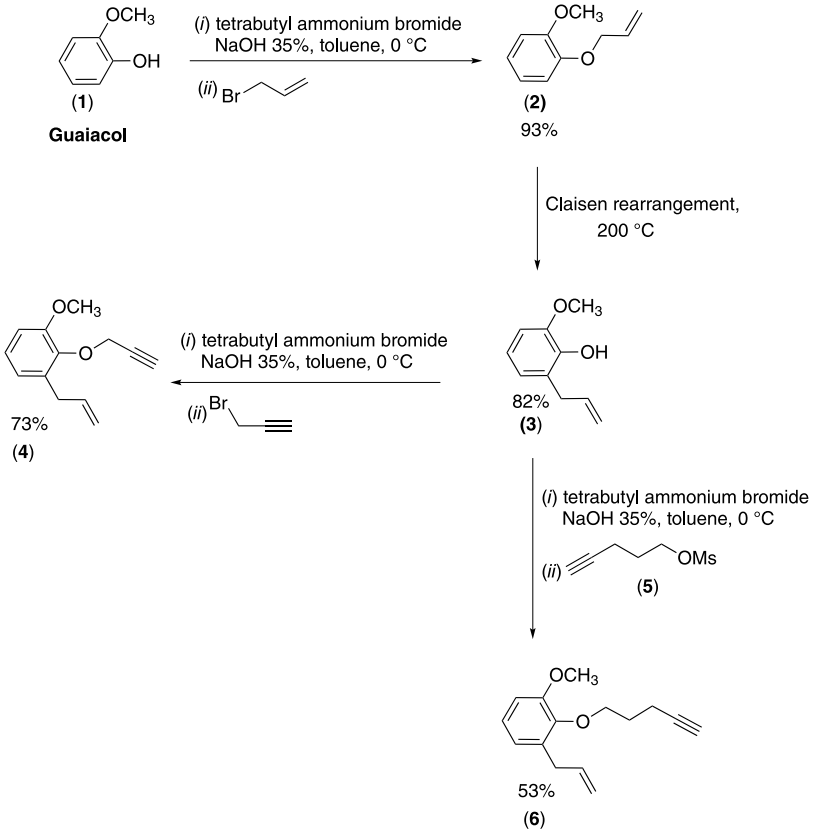
The macrophage infection assays were performed using a previously described methodology based on *L. braziliensis*-M2904-GFP.[18] All eugenol analogs were tested at 10 μmol L^{-1}, based on the guidelines in a hit and lead criteria in drug discovery for infectious diseases and the best compounds were used for determination of the effective concentration at 50% (EC_{50}).[19] The EC_{50} was determined using the concentrations: 80, 40, 30, 20, 10, 5, 2.5, and 1 μmol L^{-1}, according to the percentage of live cells after screening at 10 μmol L^{-1}. Positive and negative controls were amphotericin B 3.125 μg mL^{-1} (3.4 μmol L^{-1}) and 0.1% (v v^{-1}) DMSO, respectively. Experiments were performed independently at least 3 times in quadruplicates. GraphPad Prism version 6[20] was used for eugenol analogs EC_{50} determination.

Selectivity index

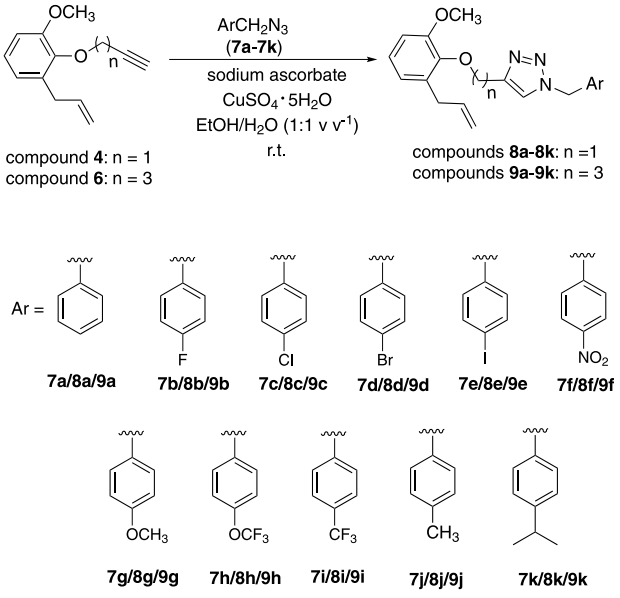
The selectivity indexes (SI) were calculated as the ratio obtained on raw macrophages/*L. braziliensis* values (CC_{50}/EC_{50}).

Results and Discussion

The transformations used to synthesize the eugenol analogs **8a-8k** and **9a-9k** are depicted in Schemes 1 and 2. As shown in Scheme 1, the preparation of terminal alkynes **4** and **6** started with alkylation of guaiacol (**1**), using allyl bromide, affording compound **2** in 93% yield. Then, the sigmatropic Claisen rearrangement[21] was conducted with compound **2** and gave *ortho* eugenol (**3**) in 82% yield. Subsequently, the alkylation of **3** with propargyl bromide or mesilate **5** provided compounds **4** (73% yield) and **6** (53%



Scheme 1. Steps involved in the preparation of alkynes **4** and **6**.



Scheme 2. CuAAC reaction involved in the preparation of eugenol analogs **8a-8k** and **9a-9k**.

yield), respectively. The preparation of mesylate **5** has been previously described.[15] The alkylation procedures were carried out using the phase transfer catalysis approach,[22-24] which gave the alkylated compounds with satisfactory yields.

The compounds **4** and **6** were submitted to the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reaction[25-29] with different benzyl azides (Scheme 2) affording the eugenol analogs **8a**-**8k** and **9a**-**9k** with yields ranging from 48 to 93%. In general, the reactions lasted about two hours. The benzyl azides **7a-7k** were prepared according to the previous published procedure.[17] They were chosen since their employment in the preparation of 1,2,3-triazole derived from eugenol afforded compounds with leishmanicidal activity.[15]

All eugenol analogs **8a-8k** and **9a-9k** were characterized by ^{1}H and ^{13}C NMR, FTIR, and high-resolution mass spectrometry. In ^{1}H NMR spectra, the signals for hydrogens present in the triazole ring were observed within the range of 7.20-7.63 ppm. Allylic hydrogens were observed as doublets, while hydrogen atoms of the methylene groups bound to nitrogen or oxygen were observed as singlets in the ^{1}H NMR spectra. In IR spectra, the expected bands for the functional groups were observed. The molecular formulas of eugenol analogs were confirmed by high-resolution mass spectrometry analyses.

Once prepared, the compounds **8a**-**8k** and **9a**-**9k** were submitted to biological assays to evaluate their toxicity to macrophages and antileishmanial activity against *L. braziliensis* during *in vitro* infection of macrophages.

Macrophages are the main mammalian host cells of *Leishmania* and so they are used in the evaluation of *in vitro* infection assays with *L. braziliensis*.[30] To be used in infection assays, all the eugenol analogs with 1,2,3-triazole fragments were previously tested at 10 μmol L^{-1} to evaluate their cytotoxicity on macrophages. The results are shown as survival rates after 48 h of treatment with the compounds (Figure 2). Amphotericin B was used as an anti-leishmanial control drug and DMSO was used as a negative control. As expected, the amphotericin B (3.125 μg mL^{-1}; 3.4 μmol L^{-1}) and the negative control (0.1% v v^{-1} DMSO) were not significantly toxic to macrophages. In addition, none of the twenty-two compounds tested were significantly cytotoxic to macrophages at 10 μmol L^{-1} concentration.

Amastigotes are the parasitic forms that persist in the host and are responsible for the symptoms caused by the disease. Therefore, this should be the main chemotherapeutic target in *in vitro* studies of new leishmanicidal agents.[31] Based on that, all compounds were evaluated against *L. braziliensis* amastigotes in *in vitro* macrophage infection. Figure 3 shows the parasite's survival rate after 48 h of treatment with the compounds.

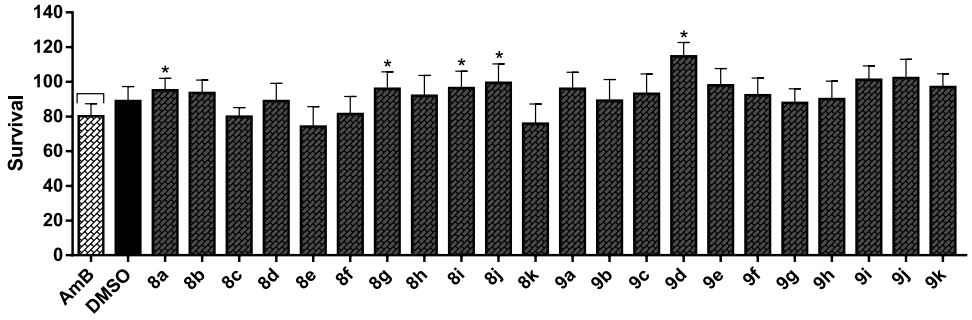


Figure 2. Effect of the compounds against macrophages Raw 264.7 compared to amphotericin B. Cells were treated with 10 μmol L^{-1} of the compounds for 48 h. The data are representative of the mean and the standard deviation of at least three independent experiments that were performed with internal quadruplicates. The amphotericin B (AmB) bar (positive control) represents cells treated with amphotericin B (3.125 μg mL^{-1}; 3.4 μmol L^{-1}) and the dimethyl sulfoxide (DMSO) bar (negative control) represents cells treated with DMSO at the same concentration used in the dilution of compounds. The data were subjected to one-way analysis of variance (ANOVA) followed by Tukey test using GraphPad Prism version 6.[20] The asterisks mean the statistical difference ($p < 0.05$) between the treatment and the AmB.

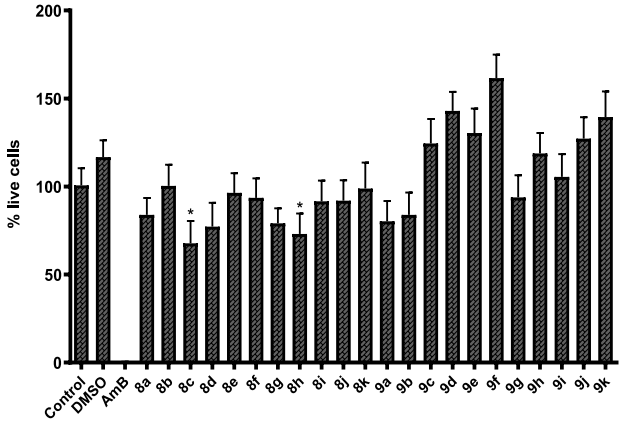


Figure 3. Effect of the eugenol analogue compounds against intracellular amastigotes of *Leishmania braziliensis*. Cells were treated with 10 μmol L^{-1} of the compounds for 48 h following the *L. braziliensis*-GFP infection assay methodology previously described.[18] The data represent the mean and standard deviation of at least three independent experiments. The data were subjected to one-way analysis of variance (ANOVA) followed by Tukey test using GraphPad Prism version 6.[20] The asterisks mean statistical difference with $p < 0.05$. The asterisks mean the difference between the treatment and the dimethyl sulfoxide (DMSO).

As can be seen, the compounds **8c** and **8h** showed a significant leishmanicidal effect, **8c** (33%) and **8h** (27%).

Since compounds **8c** and **8h** were non-toxic to macrophages and displayed the most significant leishmanicidal effect upon *L. braziliensis* intracellular amastigotes, they were selected to be used in further assays. Thus, the CC_{50} was determined for macrophage and the EC_{50} was determined against *Leishmania* intracellular amastigote for both compounds (Table 1). For macrophages, the CC_{50} of **8c** was 274.5 μmol L^{-1} and of **8h** higher than 300 μmol L^{-1}, respectively. The EC_{50} were 28.09 and 52.3 μmol L^{-1}, respectively (Table1).

The selectivity index (SI) is an indication of how much a compound is most effective against the parasite over the host mammalian cells. The higher the SI, the more selective the drug is on the parasite and less toxic to mammalian cells. For **8c** and **8h,** the SI were 9.7 and greater than 5.7, respectively (Table 1).

Table 1. Effective concentration at 50% (EC_{50}) and cytotoxic concentration at 50% (CC_{50}) for the selected compounds **8c** and **8h** to *L. braziliensis* intracellular amastigotes and for macrophages

Compound	CC_{50} MØ / (μmol L^{-1})	EC_{50} *L. braziliensis* amastigotes (infection assay) / (μmol L^{-1})	Selectivity index (SI)
8c	274.5	28.09	9.7
8h	> 300	52.03	> 5.7

The cytotoxic concentration at 50% (CC_{50}) and effective concentration at 50% (EC_{50}) were determined after 48 h by resazurin assay to macrophages and by GFP fluorescence methodology to the infection assay.[18] GraphPad Prism version 6[20] was used for compounds CC_{50} and EC_{50} performing a nonlinear fitting and data representing the median and standard deviation of at least two independent assays with internal quadruplicates for each of them. MØ: macrophages. Selective index (SI) = CC_{50} (MØ)/EC_{50} (*L. braziliensis* amastigotes).

As quoted above, the objective of this investigation was to continue exploring the eugenol/1,2,3-triazole scaffold towards the discovery of new potential antileishmanial agents. Previously, our research group demonstrated the leishmanicidal activity of eugenol derivatives bearing 1,2,3-triazole, showing that these compounds represent a scaffold that can be explored for the development of new agents for the treatment of leishmaniasis.[15,32] In this regard, the preparation of *ortho* eugenol, a constitutional isomer of eugenol, gave us the opportunity to evaluate new compounds within the aforementioned scaffold. *Ortho* eugenol was linked to 1,2,3-triazole functionality affording new eugenol analogs. The 1,2,3-triazoles are heterocycles that have attracted great scientific interest because they have a wide field of applications, including in medicinal chemistry. There are several biological activities reported for 1,2,3-triazole derivatives,[33-42] among them the leishmanicidal activity.[43] The activity of the 1,2,3-triazole nucleus against *Leishmania* parasites has been compared to other trypanocidal sterols, such as azasterols, which have been synthesized as inhibitors of sterol methyltransferase, an enzyme that has been validated as a target for leishmanicidal and trypanocidal drugs.[44,45]

Among the twenty-two synthesized compounds, we identified the compounds 4-((2-allyl-6-methoxy) phenoxymethyl)-1-(4-chlorobenzyl)-1*H*-1,2,3-triazole (**8c**) and 4-((2-allyl-6-methoxy)phenoxymethyl)-1-(4-trifluoro-methoxybenzyl)-1*H*-1,2,3-triazole (**8h**) as the most actives. They present as a common feature the presence of an electron-withdrawing group attached to the *para* position of the benzyl ring. In addition, they displayed low toxicity to macrophages and selective index values that showed that the compounds are more toxic to the parasite than to mammalian cells. These are important features considering the research and development of new antileishmanial agents.

Conclusions

By using a four-step synthetic route, it was possible to synthesize twenty-two new eugenol analogs, which had their structures firmly confirmed. The evaluation of these compounds against *L. braziliensis*, which is the main species related with tegumentary leishmaniasis in the New World, revealed that they are not cytotoxic on macrophages. Besides, it was found two compounds that present significant activity and selectivity against amastigote forms of *L. braziliensis*. The data described in the present

investigation point to the fact that these eugenol analogs are promising leishmanicidal candidates against *L. braziliensis* and may represent a starting point for further development of alternative treatments for Cutaneous Leishmaniasis.

Supplementary Information

Supplementary information (spectroscopic data of eugenol analogs) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Roberta S. Evangelista was responsible for data curation, investigation, validation, writing original draft, writing-review and editing; Larissa C. Pereira for data curation, investigation, validation, writing-review and editing; Luciana Â. de Souza for data curation, investigation, validation, writing original draft, writing-review and editing; Adilson V. Costa for investigation, validation, visualization, writing-review and editing; Danilo A. da Silva for data curation, investigation, validation, writing original draft, writing-review and editing; Fabrício M. de Oliveira: investigation, validation, visualization, writing-review and editing; Boniek G. Vaz for data curation, investigation, validation, visualization, writing-review and editing; Gustavo C. Bressan for data curation, investigation, validation, writing-review and editing; Juliana L. R. Fietto for data curation, investigation, validation, writing-review and editing; Róbson R. Teixeira for conceptualization, data curation, formal analysis funding acquisition, investigation, project administration, validation, visualization, writing original draft, writing-review and editing.

References

1. Burza, S.; Croft, S. L.; Boelaert, M.; *Lancet* **2018**, *392*, 951. [Crossref]
2. Serafim, T. D.; Coutinho-Abreu, I. V.; Dey, R.; Kissinger, R.; Valenzuela, J. G.; Oliveira, F.; Kamhawi, S.; *Trends Parasitol.* **2021**, *37*, 976. [Crossref]
3. Kobets, T.; Grekov, I.; Lipoldová, M.; *Curr. Med. Chem.* **2012**, *19*, 1443. [Crossref]
4. Ashford, R. W.; *Int. J. Parasitol.* **2000**, *30*, 1269. [Crossref]
5. Feasey, N.; Wansbrough-Jones, M.; Mabey, D. C. W.; Solomon, A. W.; *Br. Med. Bull.* **2010**, *93*, 179. [Crossref]
6. Pan American Healt Organization, https://www.paho.org/en/topics/leishmaniasis, accessed in May 2023.
7. Frézard, F.; Demicheli, C.; Ribeiro, R. R.; *Molecules* **2009**, *14*, 2317. [Crossref]
8. Haldar, A. K.; Sen, P.; Roy, S.; *Mol. Biol. Intern.* **2011**, *2011*, 571242. [Crossref]
9. Wiwanitkit, V.; *Ther. Clin. Risk Manage.* **2012**, *8*, 323. [Crossref]
10. Lindoso, J. A. L.; Costa, J. M. L.; Queiroz, I. T.; Goto, H.; *Res. Rep. Trop. Med.* **2012**, *3*, 69. [Crossref]
11. Rocha, L. G.; Almeida, J. R. G. S.; Macêdo, R. O.; Barbosa-Filho, J. M.; *Phytomedicine* **2005**, *12*, 514. [Crossref]
12. Gervazoni, L. F. O.; Barcellos, G. B.; Ferreira-Paes, T.; Almeida-Amaral, E. E.; *Front. Chem.* **2020**, *8*, 579891. [Crossref]
13. Kamatou, G. P.; Vermaak, I.; Viljoen, A. M.; *Molecules* **2012**, *17*, 6953. [Crossref]
14. Kaufman, T. S.; *J. Braz. Chem. Soc.* **2015**, *26*, 1055. [Crossref]
15. Teixeira, R. R.; Gazolla, P. A. R.; Silva, A. M.; Borsodi, M. P. G.; Bergmann, B. R.; Ferreira, R. S.; Vaz, B. G.; Vasconcelos, G. A.; Lima, W. P.; *Eur. J. Med. Chem.* **2018**, *146*, 274. [Crossref]
16. Perrin, D. D.; Armarego, W. L. F.; *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon: Oxford, U.K., 1988.
17. Borgati, T. F.; Alves, R. B.; Teixeira, R. R.; de Freitas, R. P.; Perdigão, T. G.; da Silva, S. F.; dos Santos, A. P.; Bastidas, A. J.; *J. Braz. Chem. Soc.* **2013**, *24*, 953. [Crossref]
18. Bastos, M. S.; de Souza, L. A.; Onofre, T. S.; Silva-Júnior, A.; de Almeida, M. R.; Bressan, G. C.; Fietto, J. L. R.; *Mem. Inst. Oswaldo Cruz* **2017**, *112*, 155. [Crossref]
19. Katsuno, K.; Burrows, J. N.; Duncan, K.; Hooft van Huijsduijnen, R.; Kaneko, T.; Kita, K.; Mowbray, C. E.; Schmatz, D.; Warner, P.; Slingsby, B. T.; *Nat. Rev. Drug Discovery* **2015**, *14*, 751. [Crossref]
20. *GraphPad Prism*, version 6.0; GraphPad Software Inc., San Diego, California, USA, 2012.
21. Freitas, J. J. R.; Avelino, R. A.; Mata, M. M. S.; Santos, C. S.; Almeida, C. L. A.; Freitas, J. C. R.; Freitas Filho, J. R.; *Rev. Virtual Quim.* **2017**, *9*, 1597. [Crossref]
22. Lucchese, A. M.; Marzorati, L.; *Quim. Nova* **2000**, *23*, 641. [Crossref]
23. Lima, A. M. A.; Teixeira, R. R.; da Silva, B. F.; Siqueira, R. P.; da Silva, I. E. P.; Santos, E. G.; Fernandes, M. C.; Gonçalves, V. H. S.; Bressan, G. C.; Mendes, T. A. O.; de Paula, S. O.; Costa, A.V. C.; dos Santos, M. H.; *Quim. Nova* **2019**, *42*, 473. [Crossref]
24. de Sousa, S. M.; Teixeira, R. R.; Costa, A. V.; de Aguiar, A. R.; Fonseca, V. R.; Lacerda Jr., V.; Romão, W.; Oliveira, L. A. M.; Ribeiro, I. M. L.; Nogueira, K. O. P. C.; do Nascimento, C. J.; Junker, J.; *Quim. Nova* **2021**, *44*, 1268. [Crossref]
25. Kolb, H. C.; Finn, M. G.; Sharpless, K. B.; *Angew. Chem., Int. Ed.* **2001**, *40*, 2004. [Crossref]
26. Rostovtsev, V. V.; Green, L. G.; Fokin V. V.; Sharpless K. B.; *Angew. Chem., Int. Ed.* **2002**, *41*, 2596. [Crossref]

27. Tornϕe, C. W.; Christensen, C.; Meldal, M.; *J. Org. Chem.* **2002**, *67*, 3057. [Crossref]
28. Singh, M. S.; Chowdhury, S.; Koley, S.; *Tetrahedron* **2016**, *72*, 5257. [Crossref]
29. Meldal, M.; Diness, F.; *Trends Chem.* **2020**, *2*, 569. [Crossref]
30. Tomiotto-Pellissier, F.; Bortoleti, B. T. D. S.; Assolini, J. P.; Gonçalves, M. D.; Carloto, A. C. M.; Miranda-Sapla, M. M.; Conchon-Costa, I.; Bordignon, J.; Pavanelli, W. R.; *Front Immunol*. **2018**, *9*, 2529. [Crossref]
31. de Morais, S. M.; Vila-Nova, N. S.; Bevilaqua, C. M. L.; Rondon, F. C.; Lobo, C. H.; Moura, A. A. A. N.; Sales, A. D.; Rodrigues, A. P. R.; de Figuereido, J. R.; Campello, C. C.; Wilson, M. E.; de Andrade Jr., H. F.; *Bioorg. Med. Chem.* **2014**, *22*, 6250. [Crossref]
32. Teixeira, R. R.; Gazolla, P. A. R.; Borsodi, M. P. G.; Ferreira, M. M. C.; Costa, M. C. A.; Costa, A. V.; Grijó, B. C. A.; Bergmann, B. R.; Lima, W. P.; *Exp. Parasitol.* **2022**, *238*, 108269. [Crossref]
33. Dheer, D.; Singh, V.; Shankar, R.; *Bioorg. Chem.* **2017**, *71*, 30. [Crossref]
34. Bozorov, K.; Zhao, J.; Aisa, H. A.; *Bioorg. Med. Chem.* **2019**, *27*, 3511. [Crossref]
35. Tron, G. C.; Pirali, T.; Billington, R. A.; Canonico, P. L.; Sorba, G.; Genazzani, A. A.; *Med. Res. Rev.* **2008**, *28*, 278. [Crossref]
36. Zhang, B.; *Eur. J. Med. Chem.* **2019**, *168*, 357. [Crossref]
37. Agalave, S. G.; Maujan, S. R.; Pore, V. S.; *Chem. Asian J.* **2011**, *6*, 2696. [Crossref]
38. Kabi, A. K.; Sravani, S.; Gujjarappa, R.; Garg, A.; Vodnala, N.; Tyagi, U.; Kaldhi, D.; Singh, V.; Gupta, S.; Malakar, C. C. In *Nanostructured Biomaterials*; Swain, B. P., ed.; Springer Nature: Singapore, 2022, ch. 11.
39. Jiang, X.; Hao, X.; Jing, L.; Wu, G.; Kang, D.; Liu, X.; Zhan, P.; *Expert Opin. Drug Discovery* **2019**, *14*, 779. [Crossref]
40. Alam, M. M.; *Arch. Pharm.* **2021**, *355*, e2100158. [Crossref]
41. Xu, Z.; Zhao, S.-J.; Liu, Y.; *Eur. J. Med. Chem.* **2019**, *183*, 111700. [Crossref]
42. Forezi, L. S. M.; Lima, C. G. S.; Amaral, A. A. P.; Ferreira, P. G.; Souza, M. C. B. V.; Cunha, A. C.; da Silva, F. C.; Ferreira, V. F.; *Chem. Rec.* **2021**, *21*, 2782. [Crossref]
43. Razzaghi-Asl, N.; Sepehri, S.; Ebadi, A.; Nejatkhan, N.; Johari-Ahar, M.; *Mol. Diversity* **2020**, *24*, 525. [Crossref]
44. Ferreira, S. B.; Costa, M. S.; Boechat, N.; Bezerra, R. J. S.; Genestra, M. S.; Canto-Cavalheiro, M. M.; Kover, W. B.; Ferreira, V. F.; *Eur. J. Med. Chem.* **2007**, *42*, 1388. [Crossref]
45. Porta, E. O. J.; Carvalho, P. B.; Avery, M. A.; Tekwani, B. L.; Labadie, G. R.; *Steroids* **2014**, *79*, 28. [Crossref]

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