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TREATMENT AND PROPHYLAXIS - ORIGINAL PAPER

Leishmanicidal activity of Morita-Baylis–Hillman adducts

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Abstract

Leishmaniasis is a neglected disease that affects millions of people, mostly in developing countries. Although this disease has a high impact on public health, there are few drug options to treat the different leishmaniasis forms. Additionally, these current therapies have various adverse effects, including gastrointestinal disturbances, headache, pancreatitis, and hepatotoxicity. Thus, it is essential to develop new drug prototypes to treat leishmaniasis. Accordingly, the present study aimed to evaluate the leishmanicidal activity of Morita-Baylis–Hillman adducts and their O-acetylates, carboxylic acid derivatives, and acid and ester derivatives of 2-methyl-phenylpropanoids against *Leishmania chagasi*. Initially, we evaluated the cytotoxicity of 16 derivatives (1-16G) against J774A.1 macrophages. Eight derivatives (2G, 4G, 5G, 7G, 9G, 10G, 13G, and 15G) showed no cytotoxicity at up to the maximum concentration tested (100 μM). When evaluated for antileishmanial effect against promastigote forms, 1G, 6G, 8G, 10G, 11G, 13G, 14G, 15G, and 16G displayed significant toxicity compared to the control (0.1% DMSO). Additionally, the compounds 1G, 5G, 7G, 9G, 11G, 13G, 14G, and 16G reduced macrophage infection by amastigotes. Thus, we conclude that these derivatives have antileishmanial effects, particularly 1G, which showed activity against promastigotes and amastigotes, and low toxicity against macrophages.

Keywords Leishmaniasis · Morita-Baylis–Hillman adducts · *L. chagasi*

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Introduction

Neglected tropical diseases (NTDs) are a group of communicable diseases that affect 149 countries, mainly the poorest ones, prevailing in tropical and subtropical regions and causing social and economic damage. Despite reaching humans for centuries, they were considered neglected due to lack of funding, research, and concern of health authorities during the twentieth century (Engelman et al. 2016; WHO 2015).

Leishmaniasis, one of 17 neglected diseases, is endemic in 98 countries. Its various forms cause deep and permanent scars or destroy the mucous membranes of the nose, mouth, and throat. In the most severe form, if untreated, it can lead to death rapidly, as it mainly affects the liver and spleen (WHO 2013, 2015). This complex of diseases is classified as tegumentary or visceral and caused by different species of the genus *Leishmania*, obligate intracellular parasites. They are among the ten priority endemics of the World Health Organization (WHO) because of their significant impact on public health. It is estimated that around 20 to 40 thousand people die each year from leishmaniasis and that 400 million are at risk of contracting the disease (Alvar et al. 2012); WHO 2009, 2013).

Currently, pentavalent antimonials (Sb^{+5}) are the drugs of choice for the treatment of all forms of leishmaniasis, followed by amphotericin B and pentamidine salts. As a second choice, if this treatment shows no response or if it cannot be applied, miltefosine and amphotericin B deoxycholate (antifungal antibiotic) are indicated. All these therapies have a high cost, adverse effects, and varied therapeutic response, which limits the patients' access to quality treatment. Currently, there is no approved vaccine available for humans (Sabbaga Amato et al. 2007; MacHado et al. 2015; WHO 2010).

The search for new drugs of natural or synthetic origin is a challenge for researchers nowadays (Neri et al. 2020; Ghodsian et al. 2020; Hzounda Fokou et al. 2021; Maaroufi et al. 2021). In this context, Morita-Baylis–Hillman adducts (MBHA) and their derivatives have been studied as promising antiparasitic drugs, showing activity against promastigote forms of *Leishmania brasiliensis*, *L. chagasi*, and *L. donovani*. (Barbosa et al. 2011; Lima-Junior and Vasconcellos 2012; Silva et al. 2016a, b; Xavier et al. 2016; Souza et al. 2017). Likewise, the phenylpropanoids are described in the scientific literature for their antiparasitic potential against trypanosomatids (Abreu et al. 2020; Sulistyowaty et al. 2021), including the leishmanicidal activity (Abdel-Mageed et al. 2012; Costa-Silva et al. 2015; Chauhan et al. 2019). Therefore, the hypothesis of this study is that the production and biological evaluation of MBHA of phenylpropanoids as new leishmanicidal derivatives is a promising strategy for the development of useful therapeutic alternatives for this neglected disease.

The Morita-Baylis–Hillman reaction provides polyfunctional, simple, and versatile molecules through carbon–carbon bond formation with high atom-economy. This sustainable transformation provides highly substituted small molecules, which can be used as valuable starting materials for the synthesis of natural products, heterocyclic compounds, and drugs (Das et al. 2006; Basavaiah et al. 2007; Basavaiah and Naganaboina 2018; Luna-Freire et al. 2011, 2014).

The low cost and ready availability of the reagents make the reaction advantageous and economical since all atoms are incorporated into the product. Moreover, it is considered green chemistry or a sustainable reaction, since it can be performed in aqueous media and in the absence of some solvents (Sheldon 2005).

According to WHO (2010, 2015), access to free and well-designed essential medicines is one of the ways to improve access to health for the most disadvantaged populations. Considering the exposed scenario, the search for new treatment alternatives is needed, mainly with greater effectiveness, less adverse effects, and better dosage forms. Thus, the present study aimed to evaluate the leishmanicidal activity of MBHA adducts and their O-acetylates, carboxylic acid derivatives, and acid and ester derivatives of 2-methyl-phenylpropanoids in the search for new drug prototypes, since these compounds contain chemical fragments privileged in relation to this biological activity.

Material and methods

Synthesis of compounds

The compounds 1G–16G tested in this work (Fig. 1) were synthesized according to procedures described in previous works (Fig. 2) (Stork et al. 1978; Basavaiah et al. 1999; Amarante et al. 2010; Luna-Freire et al. 2011, 2014).

80% yield. Methyl 2-[(2-chloroquinolin-3-yl)(hydroxy) methyl]prop-2-enoate **(1G)—**FTIR (KBr, cm^{-1}): 3537, 3216, 3062, 2992, 2955, 1702, 1619, 1490, 1138, 1033, 759. ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) 3,8 (s, 3H); 5,65 (s, 1H); 6,1 (s, 1H); 6,40 (s, 1H); 7,5 (t, 1H); 7,75 (t, 1H); 7,8 (d, 1H), 8,0 (d, 1H); 8,4 (s, 1H). ^{13}C RMN (62,5 MHz, $CDCl_3$): δ (ppm) 52,51; 69,45; 127,40; 127,46; 127,97; 128,05; 128,37; 130,81; 132,89; 137,29; 140,42; 147,33; 149,49; 167,06.

92% yield. 2-[(2-Chloroquinolin-3-yl)(hydroxy)methyl] prop-2-enoic acid **(2G)—**FTIR (KBr, cm^{-1}): 3271, 1702, 1655, 1421, 1276, 1038, 957, 760. ^{1}H NMR (200 MHz, CD_3OD): δ (ppm) 5,85 (d, 1H, $J = 5{,}92$ Hz); 6,35 (d, 1H, $J = 5{,}90$ Hz); 7,28 (dd, 1H, $J = 7{,}85$ e 15,52 Hz); 7,50 (m,

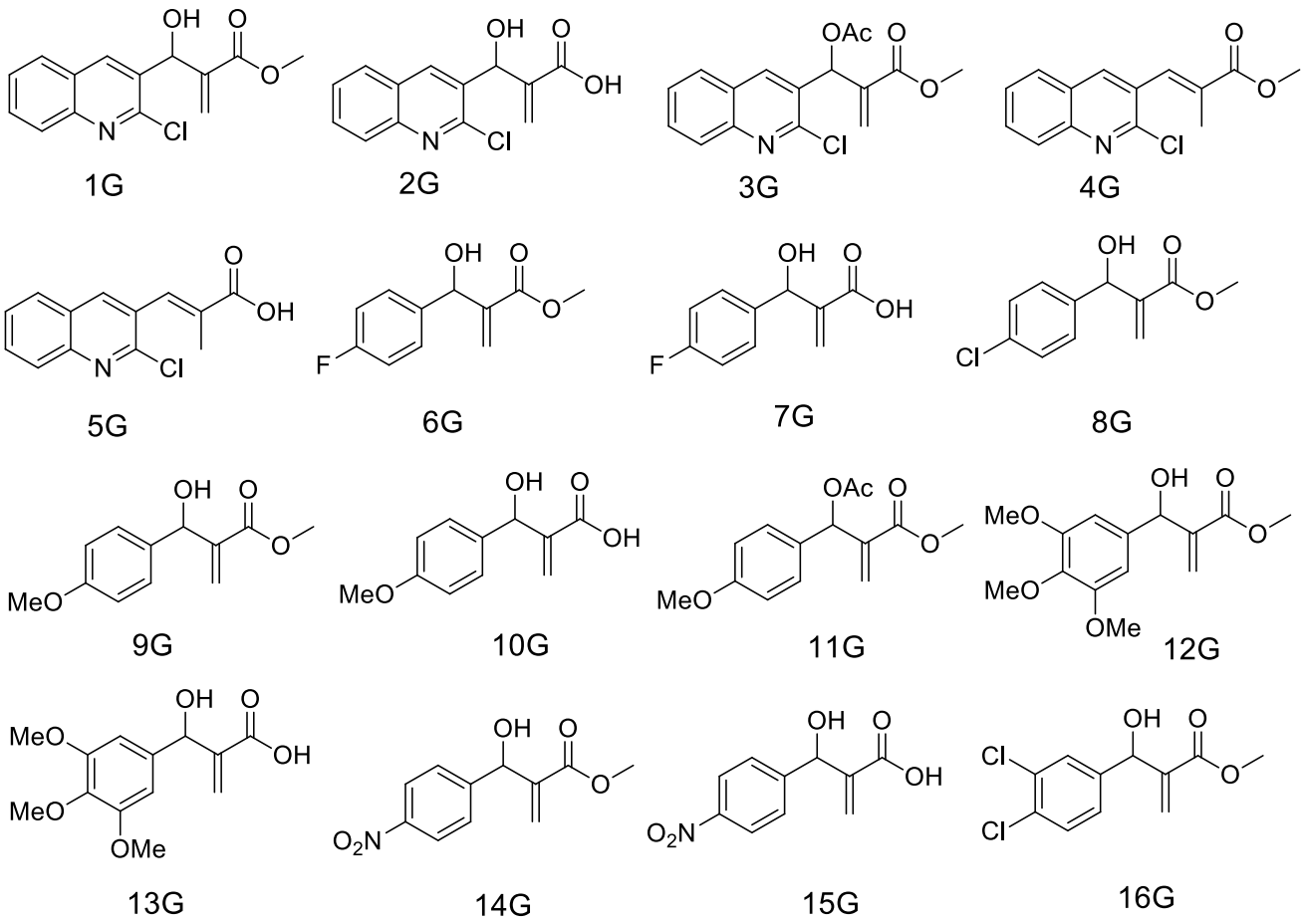


Fig. 1 Chemical structures of the antileishmanial synthetic compounds tested

1H); 7,64 (d, 1H, $J = 7{,}70$ Hz); 7,92 (d, 1H, $J = 3{,}01$). ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 67,29; 121,20; 123,90; 126,37; 129,20; 131,60; 138,18; 139,21; 143,39; 143,74; 169,17.

83% yield. Methyl 2-[(acetyloxy)(2-chloroquinolin-3-yl) methyl]prop-2-enoate **(3G)—**FTIR (KBr, cm^{-1}): 3079, 2949, 1726, 1623, 1435, 1246, 1053, 955, 750. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 2,31 (s, 3H); 3,89 (s, 3H); 5,96 (m, 1H); 6,72 (m, 1H); 7,72 (m, 1H); 7,89 (ddd, 1H, $J = 1{,}44$; 6,96 e 8,46 Hz); 7,97 (m, 1H); 8,17 (m, 1H); 8,28 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 20,9; 52,37; 70,11; 126,93; 127,50; 127,84; 128,39; 128,60; 129,90; 131,08; 137,55; 137,66; 147,43; 149,86; 165,14; 169,16.

75% yield. Methyl (2*E*)-3-(2-chloroquinolin-3-yl)-2-methylprop-2-enoate (**4G)—**FTIR (KBr, cm^{-1}): 3443, 2925, 2851, 1718, 1437, 1266, 1226, 1051, 750. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 2,23 (s, 3H); 3,89 (s, 3H); 7,75 (d, 1H, $J = 7{,}20$ Hz); 7,93 (dd, 3H, $J = 8{,}19$ e 19,13 Hz); 8,19 (m, 1H); 8,28 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 52,36; 79,1; 126,75; 126,92; 127,86; 128,41; 128,58; 129,86; 131,13; 131,89; 134,24; 137,56; 138,88; 147,42; 149,88; 169,22.

95% yield. (2*E*)-3-(2-Chloroquinolin-3-yl)-2-methylprop-2-enoic acid (**5G**)**—**FTIR (KBr, cm^{-1}):3476, 2961, 2924, 2871, 1690, 1624, 1268, 1056, 759. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 2,04 (s, 3H); 8,20 (m, 1H,); 8,38 (m, 1H); 8,63 (m, 1H); 8,86 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 14,15; 128,28; 128,51; 128,81; 129,30; 129,84; 132,40; 133,79; 134,76; 140,59; 148,02; 151,14; 170,77.

81% yield. Methyl 2-[(4-fluorophenyl)(hydroxy)methyl] prop-2-enoate (**6G**)**—**FTIR (KBr, cm^{-1}): 3452, 3003, 2954, 2901, 2848, 1713, 1604, 960, 834. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4,62 (s, 3H); 6,447 (s, 1H); 6,81 (s, 1H); 7,94 (t, 1H, $J = 8{,}65$ Hz); 8,26 (dd, 2H, $J = 5{,}59$ Hz, 8,36 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 51,81; 72,08; 114,94; 115,37; 125,76; 128,24; 128,44; 137,21; 142,01; 159,76; 164,68; 166,61.

89% yield. 2-[(4-Fluorophenyl)(hydroxy)methyl]prop-2-enoic acid (**7G**)- FTIR (KBr, cm^{-1}): 3358, 3878, 2547, 1689, 1605, 1511, 1235, 1028, 828. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 6,34 (s, 1H); 6,72 (s, 1H); 7,83 (t, 2H, $J = 8{,}38$); 8,12 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 72,12; 115,21; 115,64; 128,45; 128,60; 136,58; 136,63; 141,37; 160,01; 164,9; 179,7.

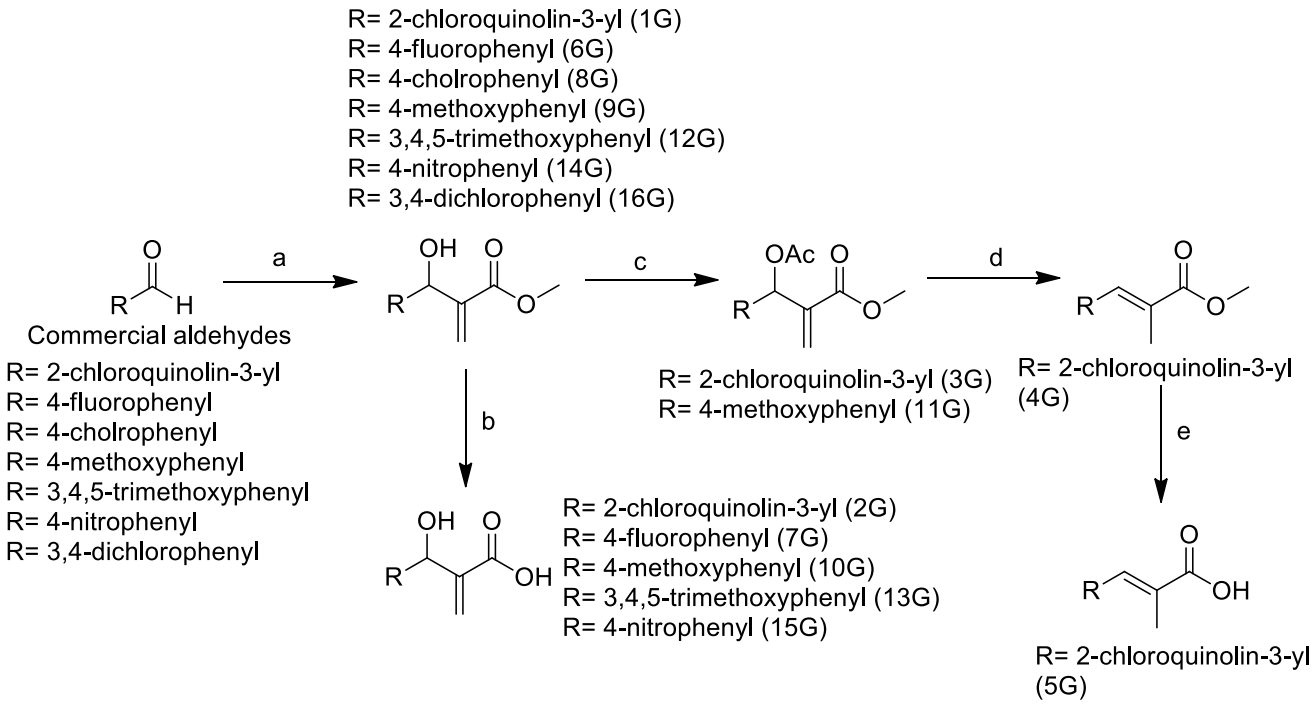


Fig. 2 Scheme — Reagents and conditions: (a) methyl acrylate, DABCO, r.t.; (b, e) LIOH, acetonitrile:water (1:1), 50–60 °C; (c) acetic anhydride, pyridine, DMAP, DCM, r.t.; (d) $NaBH_4$, t-BuOH, r.t

78% yield. Methyl 2-[(4-chlorophenyl)(hydroxy)methyl] prop-2-enoate (**8G**)—FTIR (KBr, cm^{-1}): 3359, 3102, 3018, 2959, 1718, 1635, 1437, 1275, 1037, 813, 744. ^{1}H NMR (200 MHz $CDCl_3$): δ (ppm) 3,71 (s, 3H); 5,5 (s, 1H); 5,83 (s, 1H); 6,33 (s, 1H), 7,30 (s, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 52,17; 72,67; 126,43; 128,08; 128,65; 133,64; 139,89; 141,69; 166,70.

60% yield. Methyl 2-[hydroxy(4-methoxyphenyl)methyl] prop-2-enoate (**9G**)—FTIR (KBr, cm^{-1}): 3358, 3001, 2952, 2833, 1717, 1512, 1259, 1023, 826, 579. ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) 2,94 (d, 1H); 3,6 (s, 3H); 3,79 (s, 3H); 5,53 (d, 1H); 5,85 (s, 1H); 6,32 (s, 1H); 6,85 (d, 2H); 7,29 (d, 2H). ^{13}C NMR (62,5 MHz, $CDCl_3$): δ (ppm) 52,10; 55,45; 72,96; 114,03; 125,80; 128,09; 133,66; 142,38; 159,43; 166,99.

92% yield. 2-[Hydroxy(4-methoxyphenyl)methyl]prop-2-enoic acid (**10G**)—FTIR (KBr, cm^{-1}): 3465, 2959, 2838, 1700, 1609, 1513, 1252, 1175, 1032, 829, 580. ^{1}H NMR (450 MHz, MeOD): δ (ppm) 3,73(s, 3H), 5,08 (s, 1H); 5,84 (s, 1H), 6,27 (s, 1H); 6,84 (d, 2H, $J = 9,5$); 7,22 (d, 2H, $J = 9,5$). ^{13}C NMR (112,5 MHz, MeOD): δ (ppm) 55,67, 81,76; 114,59; 130,00; 132,98; 136,63; 143,20; 145,91; 160,7; 173,42.

77% yield. Methyl 2-[(acetyloxy)(4-methoxyphenyl) methyl]prop-2-enoate (**11G**)—FTIR (KBr, cm^{-1}): 3461, 3008, 2954, 2839, 1739, 1611, 1514, 1230, 1030, 828, 563. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 2,71 (s, 3H); 4,32 (s, 3H); 4,41 (s, 3H); 6,50 (s, 1H); 7,00 (s, 1H); 7,49 (d, 2H, $J = 8,73$ Hz); 7,93 (d, 2H, $J = 8,68$ Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 21,25; 52,09; 55,34; 72,95; 113,03; 125,23; 129,27; 129,88; 139,79; 159,71; 165,56; 169,59.

77% yield. Methyl 2-[hydroxy(3,4,5-trimethoxyphenyl) methyl]prop-2-enoate (**12G**)—FTIR (KBr, cm^{-1}): 3491, 2997, 2943, 2839, 1716, 1126, 1003, 838, 683. ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) 3,14 (s, 1H); 3,74 (s, 3H); 3,83 (s, 9H); 5,50 (s, 1H); 5,82 (s, 1H); 6,32 (s, 1H); 6,58 (s, 2H). ^{13}C NMR (62,5 MHz, $CDCl_3$): δ (ppm) 52,14; 56,19; 60,89; 73,31; 103,69; 126,29; 136,98; 137,55; 141,97; 153,31; 166,97.

71% yield. 2-[Hydroxy(3,4,5-trimethoxyphenyl)methyl] prop-2-enoic acid (**13G**)—FTIV (KBr, cm^{-1}): 3458; 3371; 2974; 2941; 2841; 2561; 1709; 822; 685; 667. ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) 3,83 (s, 9H); 5,50 (s, 1H); 5,90 (s, 1H); 6,45 (s, 1H); 6,57 (s, 2H). ^{13}C NMR (62,5 MHz, $CDCl_3$): δ (ppm) 56,24; 60,95; 73,01; 103,81; 128,44; 136,73; 141,57; 153,37; 170,75.

90% yield. Methyl 2-[hydroxy(4-nitrophenyl)methyl] prop-2-enoate (**14G**)—FTIR (KBr, cm^{-1}): 3511, 3104, 2958, 1708, 1529, 1348, 1146, 1044, 984, 751. ^{1}H NMR (250 MHz, $CDCl_3$): δ (ppm) 3,29 (d, 1H); 3,75 (s, 3H); 5,65 (d, 1H); 5,87 (s, 1H); 6,40 (s, 1H); 7,58 (d, 2H); 8,25 (d, 2H); ^{13}C NMR (62,5 MHz, $CDCl_3$): δ (ppm) 52,20; 72,8; 123,6; 127,3; 140,9; 147,4; 148,6; 166,4.

98% yield. 2-[Hydroxy(4-nitrophenyl)methyl]prop-2-enoic acid (**15G**)—FTIR (KBr, cm^{-1}): 3568, 3456, 2917, 2848, 1690, 1513, 1350, 1038, 752. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 5,53 (s,1H); 5,84 (s, 1H); 6,30 (s, 1H); 7,5 (d, 2H); 8,04 (d, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 71,72; 123,30; 126,52; 127,5; 141,91; 147,03; 149,73; 167,76.

85% yield. Methyl 2-[(3,4-dichlorophenyl)(hydroxy) methyl]prop-2-enoate (**16G**)—FTIR (KBr, cm^{-1}):1432, 2952, 2867, 1719, 1629, 1469, 1151, 1031, 961, 820. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4,63 (s, 3H); 6,38 (s, 1H); 6,78 (s, 1H); 8,10 (d, 1H, $J = 8{,}19$ Hz); 8,32 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 52,17; 71,82; 128,04; 128,66; 128,64; 130,30; 131,63; 132,40; 141,60; 141,73; 166,40.

In vitro pharmacological tests

Promastigote culture The parasite of the Gonçalo Moniz—Fiocruz—BA Research Center, provided by Dr. Valéria de Matos Borges (MCAN/BR/89/BA262), was used to culture the *L. chagasi* promastigotes. These promastigotes were maintained in vitro in Schneider's medium supplemented with 10% fetal bovine serum (FBS) and 2% male human urine at 27 °C in a biochemical oxygen demand (BOD) incubator. For the experiments, the parasites were placed in Falcon tubes and centrifuged at 3500 rpm for 10 min. The supernatant was then discarded, and the pellet was resuspended in Schneider's medium. The parasites were then counted in a Neubauer chamber for plating and thus the subsequent assays.

Macrophage culture J774.A1 macrophages were maintained in culture flasks containing 10 mL of RPMI medium supplemented with 10% FBS, 2 mM L-glutamine, non-essential amino acids, and pyruvate. For the experiments, the cells were counted, adjusted in RPMI medium supplemented with 10% FBS, and then plated on a culture dish.

Macrophage viability assay For the study of cell viability of macrophages exposed to acyloxymethyl derivatives, we performed the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay (Mosmann 1983; Hussain et al. 1993). J774A.1 macrophages were seeded in 96-well plates for 1 h in (5×10^4/well) and exposed to different concentrations of the diluted test compounds in supplemented RPMI medium (0.1, 1, 10, and 100 µM) for 24 h in a 37 °C incubator with a humid 5% CO_2 atmosphere. Control wells contained cells with only culture medium or cells exposed to the diluent (0.1% DMSO). After incubation, the supernatant was discarded and 100 µL of the MTT solution (5 mg/10 mL) was then added. The plates were incubated again for 1 h in a 37 °C incubator with a 5% CO_2 atmosphere. After removing the supernatant, 100 µL of DMSO was added and the plate read with a spectrophotometer at 550 nm. The cell viability of the wells treated with the synthesized compounds was compared to that of the death standard in the control cultures.

Promastigote viability assay Promastigote forms of *L. chagasi* at a density of 1×10^6 parasites/well in a volume of 100 µL were grown in triplicate in 96-well plates using Scnheider's medium supplemented with 10% FBS, 2 mM L-glutamine, and 2% human urine. Different concentrations (0.1, 1, 10, and 100 µM) of the derivatives and controls (pentamidine, glucantime, RPMI medium, and 0.1% DMSO) were added to the wells containing the promastigote forms up to a final volume of 200 µL. The plate was incubated in a BOD incubator at 27 °C for 48 h. Afterwards, 20 µL of MTT solution was added to the wells, and the plates were placed in a CO_2 incubator for 2 h. The MTT solution was then removed, and isopropyl alcohol was added to the wells to dissolve the formazan product. The plates were then read in a spectrophotometer at 550 nm. Cell viability of the wells treated with the synthesized compounds was compared to the death standard obtained in the control cultures.

Macrophage infection with *L. chagasi* assay J774.A1 macrophages were seeded at a density of 5×10^4 cells/well in 24-well plates with coverslips for 1 h with supplemented RPMI medium. Macrophages were incubated overnight and subsequently infected with *L. chagasi* promastigotes, at a proportion of 10 parasites:1 macrophage. The plate was incubated for 6 h at 37 °C in a humid 5% CO_2 atmosphere. Six hours after infection, the macrophages were washed with phosphate-buffered saline (PBS, pH 7.2), to remove the non-internalized parasites. Macrophages were cultured with RPMI supplemented with 10% FBS, 2 mM L-glutamine, 2 mM non-essential amino acids, and 2 mM sodium pyruvate, in the presence or absence of derivatives at a concentration of 10 µM, and were kept in a 37 °C incubator with a humid 5% CO_2 atmosphere for 24 h. Afterwards, the wells containing the coverslips were washed with PBS, and the cells were fixed with methanol, stained with May-Grünwald-Giemsa, and mounted on slides. The number of infected macrophages and the number of amastigotes in 100 macrophages were evaluated using a light microscope with 100× oil-immersion objective (Nunes et al. 2005).

Statistical analysis All data are expressed as the mean ± SEM and were analyzed by GraphPad Prism 8.0 software using one-way analysis of variance (ANOVA) followed by Dunnett's test. Differences were considered statistically significant when $p < 0.05$.

Table 1 Effect of the test derivatives on the cell viability of J774.A1 macrophages in the MTT assay after 24 h

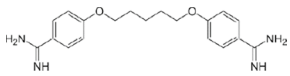
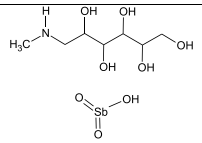
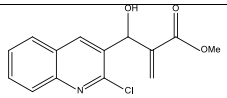
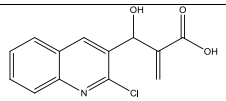
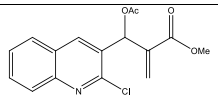
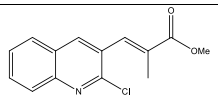
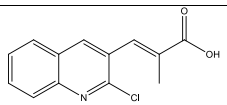
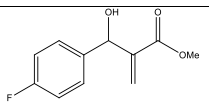
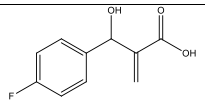
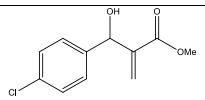
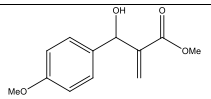
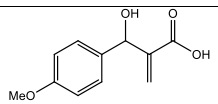
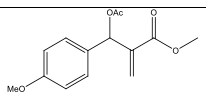
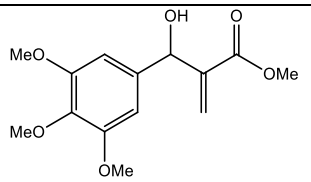
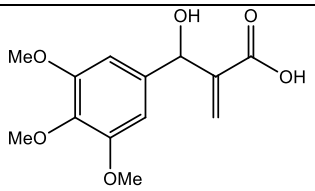
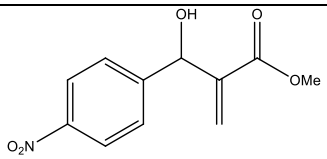
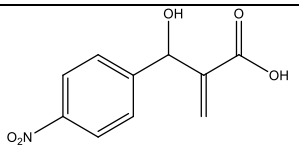
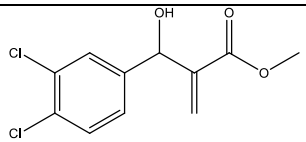
Derivative	Structure	IC_{50} (μM)[a]	Maximum cytotoxicity (%)[b]	Cytotoxicity 10 μM (%)[c]
Pentamidine		50.0 ± 2.8	89.7 ± 0.3***	16.4 ± 3.7***
Glucantime		> 100	25.4 ± 1.4 **	21.1 ± 0.2***
1G		> 100	48.6 ± 0.8***	NT
2G		> 100	NT	NT
3G		93.3 ± 1.2	53.3 ± 0.7***	NT
4G		> 100	NT	NT
5G		> 100	NT	NT
6G		> 100	79.1 ± 1.2***	NT
7G		> 100	NT	NT
8G		> 100	30.7 ± 1.8***	NT
9G		> 100	NT	10.2 ± 1.8*
10G		> 100	NT	NT
11G		> 100	75.2 ± 0.5***	62.5 ± 1.1***

Table 1 (continued)

12G		> 100	63.4 ± 3.2***	NT
13G		> 100	NT	NT
14G		93.3 ± 7.8	59.8 ± 4.7***	13.6 ± 1.6 *
15G		> 100	NT	NT
16G		> 100	75.1 ± 0.5***	71.4 ± 1.3***

The results refer to as follows: [a]concentration required to kill 50% of macrophages (IC_{50}) determined by concentration–response curves; [b]mean ± standard error of the mean showing maximum cytotoxicity in triplicates of a representative experiment; and [c]cytotoxicity at 10 µM. *NT*, substance did not show significant lethal activity against macrophages at the concentrations of 100, 10, 1, or 0.1 µM compared to the DMSO group. Cytotoxicity was considered significant when ***$p < 0.001$, **$p < 0.01$, or *$p < 0.05$ compared to the 0.1% DMSO group

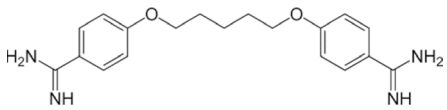
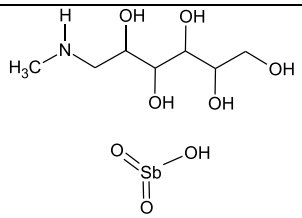
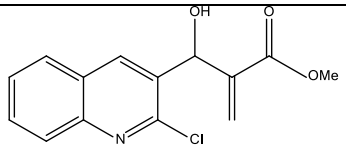
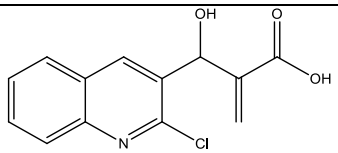
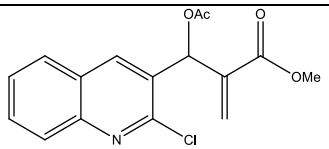
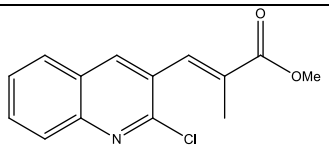
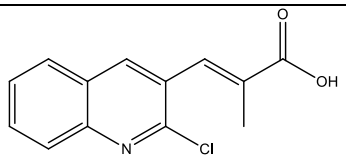
Results and discussion

Leishmaniasis is a complex of diseases in which the cells of the mononuclear phagocytic system serve as host and replication site for the parasite as well. The parasite in turn resists lysis by the lysosomes and resides mainly in macrophages in the spleen and liver. It is important to note that the use of pentavalent antimonials is the first choice for the treatment of leishmaniasis in Brazil. However, they have a high cost and are hepatotoxic and nephrotoxic as well, which justifies the search for new therapeutic options (Ayres et al. 2007; Jain and Jain 2013).

The Morita-Baylis–Hillman reaction has proved to be effective, is low cost, and provides highly functional derivatives. Morita-Baylis–Hillman derivatives have been studied since 1999 from a pharmacological point of view. They have a range of activities, including antifungal, antimalarial, antineoplastic, molluscicide, antileishmanial, and antichagasic action (Lima-Junior and Vasconcellos 2012).

First, the compounds synthesized were evaluated for their effect on cell viability of J774A.1 macrophages using the MTT assay. The results showed that only the derivatives 2G, 4G, 5G, 7G, 9G, 10G, 13G, and 15G were not cytotoxic at up to the maximum concentration tested (100 µM). The other compounds (1G, 3G, 6G, 8G, 11G, 12G, 14G, and 16G) were cytotoxic when compared to the control (0.1% DMSO). When cytotoxicity was determined at a concentration of 10 µM, only 9G, 11G, 14G, and 16G showed a significant cytotoxic effect against macrophages (Table 1). As expected, 0.1% DMSO had no deleterious

Table 2 Effect of the test derivatives on the viability of *L. chagasi* promastigotes after 48 h of treatment

Derivative	Structure	IC_{50} (µM)[a]	Maximum effect (%)[b]
Pentamidine		2.0 ± 1.0	97.5 ± 1.2***
Glucantime		2.0 ± 0.5	51.4 ± 0.3***
1G		0.6 ± 0.3	61.3 ± 2.6***
2G		> 100	NA
3G		> 100	NA
4G		> 100	NA
5G		> 100	NA

effect when compared to the negative control (RPMI culture medium). A similar assay was used by Silva et al. 2011 in evaluating the cytotoxicity of MBHA in peritoneal murine macrophages.

After determination of cytotoxicity in macrophages, the viability of promastigote forms treated with the derivatives and controls was evaluated by the MTT reduction assay. The results showed that the 1G, 6G, 8G, 10G, 11G, 13G,

Table 2 (continued)

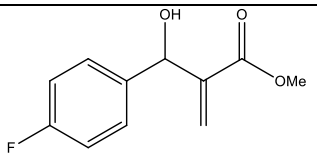
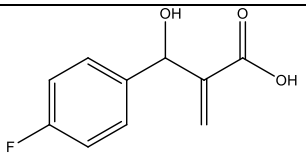
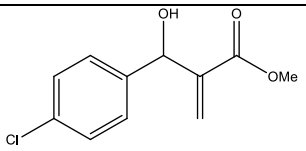
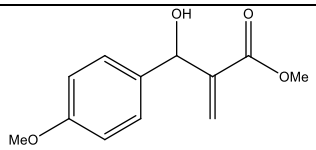
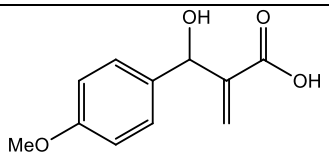
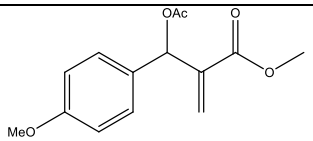
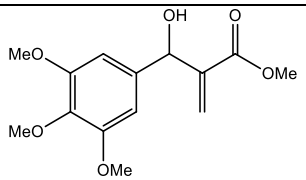
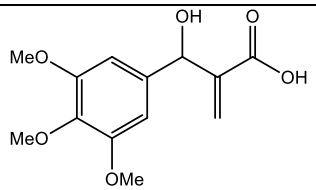
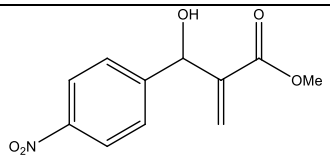
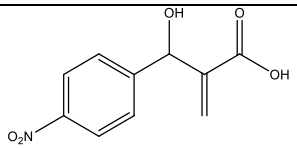
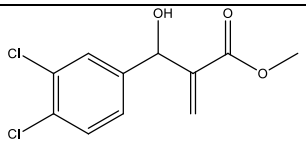
6G		66.3 ± 9.0	62.3 ± 2.9***
7G		> 100	NA
8G		0.6 ± 0.3	67.0 ± 4.1***
9G		> 100	NA
10G		> 100	25.9 ± 6.2***
11G		58.3 ± 3.4	88.1 ± 4.4***
12G		> 100	NA
13G		> 100	16.9 ± 3.4**

Table 2 (continued)

14G		> 100	22.5 ± 1.8*
15G		> 100	22.2 ± 1.5***
16G		4.3 ± 0.3	77.4 ± 1.3***

The results refer to as follows: [a]concentration required to kill 50% of the promastigote forms (IC_{50}) determined by concentration–response curves and expressed as mean ± standard error of the mean; [b]maximum effect (ME) which is expressed as mean maximum toxicity ± standard error of mean for triplicate values of a representative experiment. ME values were considered significant when ***$p < 0.001$, **$p < 0.01$, or *$p < 0.05$ compared to the 0.1% DMSO group. *NA*, substance had no significant lethal activity for *L. chagasi* promastigotes at the concentrations tested compared to the DMSO group

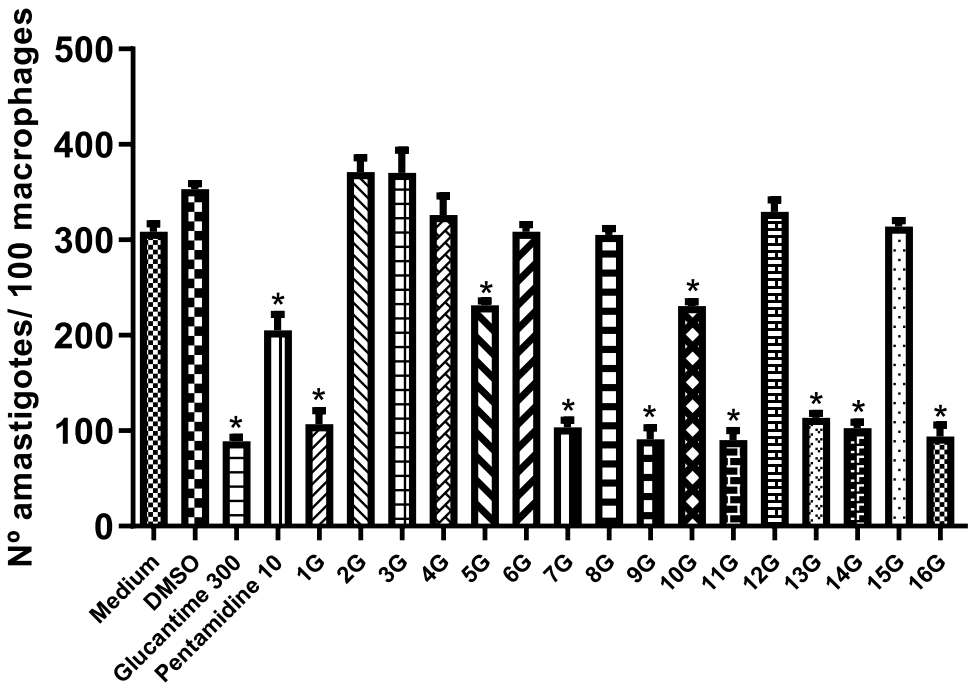


Fig. 3 Leishmanicidal effect of the derivatives (10 µM) against the amastigote forms of *Leishmania chagasi*. The results refer to the mean ± standard error of the mean of triplicates of a representative experiment. The values were considered significant when *$p < 0.05$, when compared to the 0.1% DMSO group

14G, 15G, and 16G derivatives had a significant cytotoxic effect compared to the control (0.1% DMSO) (Table 2).

The direct leishmanicidal activity of MBHA has been previously described. Barbosa et al. (2011) evaluated the in vitro leishmanicidal activity of MBHA compounds in promastigotes of *L. chagasi* and *L. amazonesis*, using the molecular hybridization strategy, but without evaluating cytotoxicity in macrophages. Additionally, it was previously observed that others MBHA had an antileishmanial effect against promastigote forms of *L. chagasi* after 72 h of treatment (Junior et al. 2010).

After cytotoxicity evaluation in macrophages and promastigote forms, the derivatives were evaluated for leishmanicidal activity in amastigote forms of *L. chagasi* at

a concentration of 10 µM. The results obtained demonstrated that the test compounds 1G, 5G, 7G, 9G, 11G, 10G, 13G, 14G, and 16G caused a significant reduction in the number of amastigotes in 100 macrophages. On the other hand, the derivatives 2G, 3G, 4G, 6G, 8G, 12G, and 15G did not display leishmanicidal activity against intracellular parasites (Fig. 3).

Sandes et al. 2014 evaluated the effect of an MBHA on epimastigote forms of *Trypanosoma cruzi.* The group found that the 3-hydroxy-2-methylene-3-(4-nitrophenylpropanenitrile) adduct induced parasite cell death by necrosis, in a mitochondria-dependent manner. A range of therapeutic targets for the treatment of leishmaniasis has been emerging (Sundar and Singh 2018; Raj et al. 2020). The exact mechanism of action MBHA in *Leishmania* targets remains to be elucidated. However, it is possible that MBHA acts inhibiting Leishmania proteases (da Silva et al. 2016a, b), which are virulence factors related to the pathogenesis of this protozoan (Machado et al. 2019).

The compounds 1G, 11G, 13G, 14G, and 16G present antileishmanial activity against both amastigotes and promastigotes forms. However, 11G, 14G, and 15G derivatives showed significant cytotoxicity against macrophages, being 1G and 13G derivatives safer for mammalian cells based on this preliminary assay. Thus, in molecular prospecting for leishmanicidal compounds, this study demonstrates that derivative 1G is the most promising for drug development, since the compound was highly active against promastigotes, with IC_{50} lower than standard drugs and 13G derivative, and amastigotes of *L. chagasi.* In addition, it showed low toxicity against macrophages.

Considering that MBHA compounds are easy to synthesize and readily available, their use becomes an advantage when compared to the currently available drugs, since leishmaniosis is a neglected diseases and affects less fortunate populations (WHO 2010, 2015).

Conclusion

On the basis of the results obtained, it can be concluded that some MBHA compounds, their O-acetylates, carboxylic acid derivatives, and acid and ester derivatives of 2-methylphenylpropanoids possess leishmanicidal activity in vitro against promastigotes and amastigotes of *L. chagasi*. Derivative 1G, which was active against promastigotes and amastigotes, showed low toxicity against the macrophage cell line J774A.1. Thus, it is a strong candidate for further pharmacological studies aimed at the treatment of leishmaniasis.

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Declarations

Conflict of interest The authors declare no competing interests.

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