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Design, synthesis and antileishmanial in vitro activity of new series of chalcones-like compounds: A molecular hybridization approach

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ABSTRACT

The chalcone-like series **1a–1g** was efficiently synthesized from Morita–Baylis–Hillman reaction (52–74% yields). Compounds **1a–1g** were designed by molecular hybridization based on the anti-inflammatory drug methyl salicylate (**3**) and the antileishmanial moiety of the Morita–Baylis–Hillman adducts **2a–2g**. The **1a–1g** compounds were much more actives than precursor series **2a–2g,** for example, IC_{50} = 7.65 μM on *Leishmania amazonensis* and 10.14 μM on *Leishmania chagasi* (compound **1c**) when compared to IC_{50} = 50.08 μM on *L. amazonensis* and 82.29 μM on *L. chagasi* (compound **2c**). The IC_{50} values of compound **3** (228.49 μM on *L. amazonensis* and 261.45 μM on *L. chagasi)* and acryloyl salicylate **4** (108.50 μM on *L. amazonensis* and 118.83 μM on *L. chagasi)* were determined here, by the first time, on *Leishmania*.

1. Introduction

Leishmaniasis affects almost 12 million people in nearly 90 countries, representing a worldwide public health problem. More than 350 million people are currently at risk, and 2 million new cases are registered each year. An estimated 51,000 annual deaths for leishmaniasis have been reported.[1–3] Leishmaniasis infection causes many different clinical manifestations, such as cutaneous,[4] mucocutaneous[5] and a fatal infection of the liver and spleen (visceral leishmaniasis, also known as kalazar).[6,7] Human infection with *Leishmania chagasi,* the protozoan causing South American visceral leishmaniasis (VL), causes diverse sequelae ranging from subclinical infection to progressive fatal disease.[8] However, the cutaneous form is the most common. Both the cutaneous and mucocutaneous forms can cause severe disfigurement to patients, including ulcerative skin lesions and the destruction of the mucous membranes of the nose, mouth, and throat, leading to permanent disfigurement (diffuse cutaneous form of the disease) and frequently social ostracization. The most common species in the Americas and the most important causative agent of cutaneous and mucocutaneous leishmaniasis in Brazil is *Leishmania braziliensis*, while *Leishmania amazonensis* is the primary etiologic agent of the diffuse cutaneous form of the disease.[9]

For the last 60 years, the treatment options for leishmaniasis are limited and involve the administration pentavalent antimonial family agents, represented by Sodium stibogluconate (Pentostam®) and Meglumine antimoniate (Glucantime®). Second line drugs include pentamidine and Amphotericin B, but these drugs have not experienced widespread use due to the severe toxicities and high costs.[10] Pentamidine present several side effects, including renal and hepatic toxicity, pancreatitis, hypotension, dysglycemia, and cardiac abnormalities.[11] Amphotericin B is quite effective for visceral leishmaniasis being, however very expensive and do not appear to be suitable for treatment of non-visceral diseases.[11] Up to now, no vaccine approved for human use is available.[12,13] Therefore, there is an increasingly urgent need for the development of new, inexpensive, effective and safe drugs for the treatment of leishmaniasis and then, the discovery of new lead compounds for this disease is a pressing concern for global health programs.

A promising route towards development of improved therapeutic agents for diseases caused by human pathogens, such as parasitic protozoa, is the identification of key differences between the metabolism of the host and the parasite, and the development of inhibitors of parasite-specific enzymes.[14] The mechanisms by which different *Leishmania* species cause different pathologies are largely unknown. In a pioneering paper on this subject, Baiocco

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et al.[15] by first time disclosed the molecular mechanism of action of antimonial drugs against the parasite Sb(III), which is coordinated by the two redox-active catalytic cysteine residues (Cys52 and Cys57), one threonine residue (Thr335), and His461′ of the 2-fold symmetry related subunit in the dimer, strongly inhibits trypanothione reductase (TR).

Non-steroid anti-inflammatory drug NSAIDs are major drugs used in the treatment of inflammation and pain in a wide variety of disorders.[16] NSAIDs constitute a diverse group of chemicals, categorized according to their chemical structures that share the same therapeutic properties. Among the main compounds are aspirin and salicylate, diclofenac and flurbiprofen. The best-known mechanism of action of NSAIDs is the inhibition of prostaglandin synthesis secondary to their action on cyclooxygenases (COXs). However, data have been accumulating through the years indicating that NSAIDs also act on other targets to counteract pain[17,18] Since anti-inflammatory activities of natural[19] and non-natural[20] salicylate derivatives have been described and there is an important roles of COXs and PGE2 during Leishmaniasis infection,[21] the design of the new hybrid salicylate compounds **1a**–**1g** (Fig. 1) could be an attractive strategy for discover new antileishmania substances.

In our continuing search for bioactive substances[22–26] and in connection with our efforts towards reactivity of Morita–Baylis–Hillman reaction (MBHR) study (Scheme 1),[27–30] our research group described in 2006 the molluscicidal activities of simple aromatic Morita–Baylis–Hillman adducts (MBHA) against *Biomphalaria glabrata* (Say) snails, intermediate schistosomiasis host.[31] In sequence, some aromatic MBHA were presented as very active compounds against the *L. amazonensis* (cutaneous and mucocutaneous infections).[32] Following that work, we published the biological evaluation of aromatic MBHA against *L. chagasi* (visceral infections) parasites,[33] and in 2010, we have shown that the MBHA 3-hydroxy-2-methylene-3-(4-nitrophenyl) propanenitrile, a high antileishmania compound, is also a highly active compound against epimastigote and trypomastigote form of *Trypanosoma cruzi*, the parasite that causes Chagas disease.[34] In the same year, we presented an improved synthesis for 16 MBHA and their biological evaluation against *L. amazonensis* and *L. chagasi* and we proposed, at the first time, a Structure–Activity-Relationship (SAR) analysis for this class of new antiparasitic compounds.[35]

In this context, we present in this Letter the design, synthesis, inhibitory activities on antipromastigote form of *L. amazonensis* and *L. chagasi* of new congener hybrids **1a**–**1g** (Fig. 1). The design is based on the molecular hybridization[36] between the antileishmania compounds **2a**–**2g** presented by us in previous paper[35] and the methyl salicylate (**3**) (Fig. 1) (e.g., Metedic®). It is important to detach that the molecular hybridization is a relatively new concept in drug design and development based on the combination of pharmacophoric moieties of different bioactive substances to produce a new hybrid compound with improved bioactivity, when compared to the parent compounds.[36]

Methylsalicylate (MS) is a naturally occurring compound that is used as a major active ingredient of balms and liniments supplied as topical analgesics. Despite the common use of MS as a pain reliever, the underlying molecular mechanism is not fully understood. Recently, Ohta et al. showed that the inhibitory action of MS on transient receptor potential vanilloid subtype 1 (TRPV1) is one of the underlying mechanisms for its analgesic effects *in vivo*.[37]

In this Letter we also present, by the first time, the toxicity evaluation against promastigotes forms of *L. amazonensis* and *L. chagasi* of methyl salicylate (**3**) and the acryloyl salicylate (**4**) (Fig. 1). All molecular hybrids MBHA designed here are in accord with the Lipinski's rule of five[38] and also with the recent Reynisson's rule.[39]

We also should emphasize the structural similarity between compounds **1a–1g** designed here and the chalcones (Scheme 2)

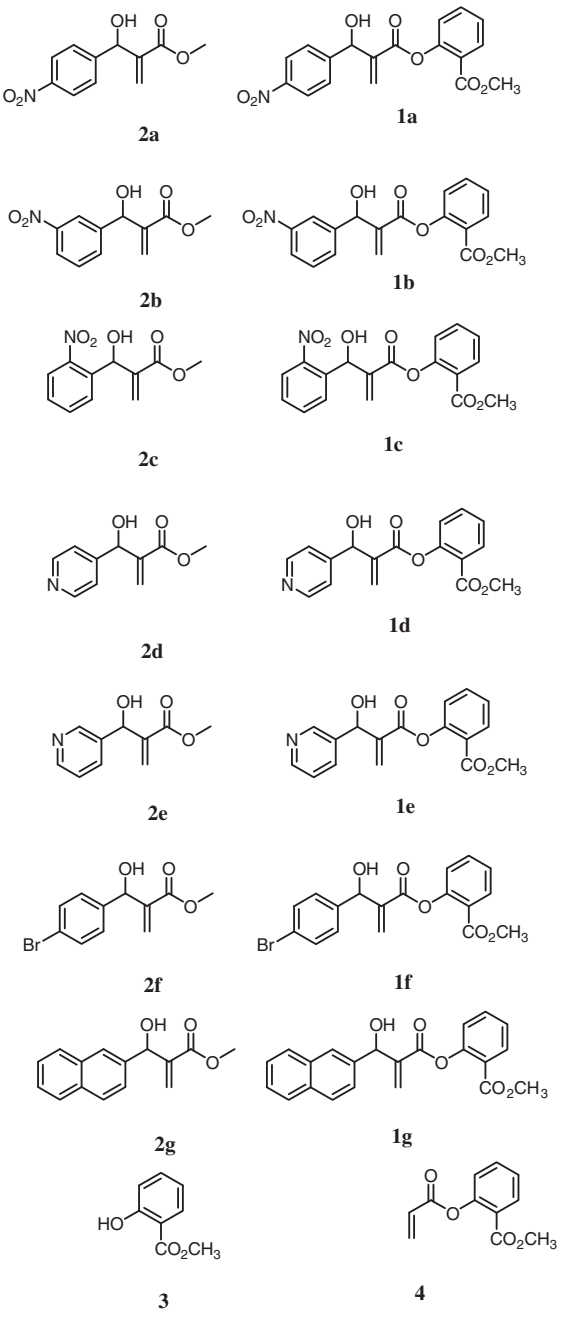
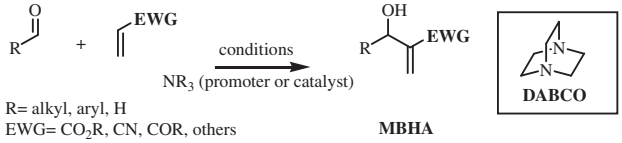
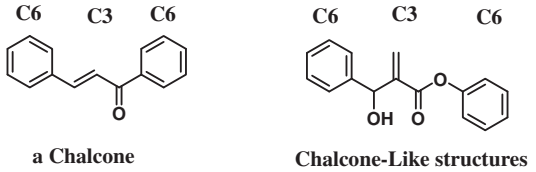


Figure 1. Previous antileishmanial compounds **2a**–**2g**, a new hybrid series of chalcone-like compounds synthesized here **1a**–**1g**, methyl salicylate **3**, and a new compound acryloyl salicylate **4**.



Scheme 1. The Morita–Baylis–Hillman reaction (MBHR).

that exhibit various biological activities, such as antibacterial, antifungal, antitumor, anti-inflammatory and antiparasitic properties.[40–42] Several chalcone-like compounds have been recently synthesized and also exhibit various biological activities.[43]



Scheme 2. The structural similarities between compounds **1a–1g** and chalcones.

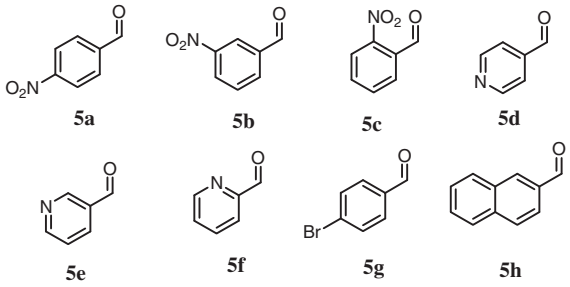


Figure 2. The aromatic aldehydes **5a–5h** used as electrophiles in this work.

2. Material and methods

2.1. Chemistry

Commercially available reagents were purchased from Aldrich® and Acros® and used without further purification. The solvents used in this study were purchased from Tedia®, the acryloyl chloride was purchased from Merck® and methyl salicylate was purchased from Dynamics®. All the new compounds were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, and HR-MS, using a NMR Varian Unitty Plus (300 MHz to ^{1}H and 75 MHz for ^{13}C), a NMR Varian Unmrs (400 MHz ^{1}H and 100 MHz for ^{13}C) in $CDCl_3$ using TMS as an internal standard. The Fourier Transform Infrared Spectroscopy (FT-IR) spectra were obtained using a spectrophotometer IR-Prestige-21 (Shimadzu). HR-MS spectra were obtained on a Shimadzu LC–MS-IT-TOF. Thin-layer chromatography was performed on 0.25 mm plates for TLC silica gel Kieselgel 60 (Whatman®) and spots were visualized with short wavelength UV light 254 nm.

2.1.1. Synthesis of methyl salicylate acrylic ester (4)

In a 125 mL flask, 40 mmol (6.08 g) of methyl salicylate (**3**) was dissolved in 20 mL of dichloromethane. Then, it was added 42 mmol of triethylamine. The resulting mixture was kept stirring for 15 min in an ice bath, shortly after this time was dripped into the flask a solution containing 42 mmol (3.5 mL) of acryloyl chloride in 10 mL of dichloromethane. The reaction mixture was kept under agitation and product formation was accompanied by TLC analysis using ethyl acetate/hexane (1:9 by volume). After 2 h of reaction, the mixture was put into a separation funnel washed with a 10% NH_4Cl solution (30 mL) to remove the triethylammonium salt and then preceded to the extraction with dichloromethane (3×15 mL). The organic phase was then dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure in rotaevaporatory. The reaction product was isolated by flash chromatography column on silica gel, using hexane as eluent (200 mL), and increasing slowly the polarity of eluent. The fractions were collected, and the product was obtained in the form of oil in 71% yield; yellow oil; IR (KBr): 2999, 2953, 1726, 1631, 1606, 1452, 1404, 1300, 1205, 1153, 1083, 756 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.82 (s, 3H), 6.02 (dd, 1H, J = 1.8/10.2 Hz), 6.35 (dd, 1H, J = 10.2/17.2 Hz), 6.61 (dd, 1H, J = 1.8/17.2 Hz), 7.11 (dd, 1H, J = 1.0/8.0 Hz), 7,29 (dt, 1H, J = 1.2/7.6 Hz) 7.54 (dt, 1H, J = 1.8/7.6 Hz/8.0 Hz) 8.00 (dd, 1H, J = 1.6/7.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 52.1; 123.2; 123.7; 126.0; 127.6; 131.7; 132.7; 133.8; 150.3; 164.6; 164.8. HR-MS-Mass calculated: 229.0477 [M+23], found: 229.0417; $C_{12}H_{13}NNa\ O_6$.

2.1.2. General protocols for the 1a–1g MBHA preparations

Reactions were carried out using the corresponding **5a–5h** (Fig. 2) aldehydes (1 mmol), the acrylate **4** (x mmol, Table 1), solvent-free condition or 1.0 mL of protic/nonprotic solvent (Table 1), 1 or 0.3 mmol of DABCO at 0 or 25 °C (Table 1) for x min h^{-1} day^{-1} (Table 1). After that, the reaction media was directly filtered through silica gel, using ethyl acetate/hexane (2:8; 3:7 or 4:6) as eluent and the reaction products were concentrated under reduced pressure. After purification by flash chromatography using ethyl acetate/hexane (1:9; 2:8 or 3:7) as eluent, the products were ready for in vitro bioevaluations.

2.1.2.1. Methyl-2-{2-[hydroxy(4-nitrophenyl)methyl])acryloyloxy}benzoate (1a). Seventy-one % yield; yellow oil; IR (KBr): 3478, 3001,2954, 1732, 1612, 1531, 1446, 1400, 1346, 972, 852, 825, 756, 732 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 33.76 (s, 3H), 4.06 (d, 1H, J = 4.0 Hz, CHOH), 5.79 (d, 1H, J = 3.6 Hz), 5.96 (s, 1H), 6.65 (s, 1H), 7.04 (dd, 1H, J = 0.8 Hz/8.0 Hz), 7.31 (ddd, 1H, J = 1.0 Hz/7.8 Hz), 7.54 (ddd, 1H, J = 1.8 Hz/7.6 Hz/7.9 Hz), 7.63 (d, 2H, J = 8.8 Hz) 8.01 (dd, 1H, J = 1.8 Hz/7.8 Hz) 8.19 (d, 2H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 52.3; 71.9, 122.4, 123.4 (2C), 123.6, 126.3; 127.5(2C), 129.5, 131.8, 134.1, 140.9, 147.2, 148.5, 145.0, 164.2, 164.7. HR-MS-Mass calculated: 380.0746 [M+23], found: 380.0701; $C_{18}H_{15}NNaO_7$.

2.1.2.2. Methyl 2-{2-[hydroxy(3-nitrophenyl)methyl])acryloyloxy}benzoate (1b). Seventy % yield; oil; IR (KBr): 3498, 3078, 3020, 2951, 1728, 1608, 1531, 1435, 1350, 1300, 1083, 806, 759, 690 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (s, 3H), 5.82 (d, 1H, J = 5.4 Hz), 5.97 (s, 1H), 6.69 (s, 1H), 7.07 (dd, 1H, J = 1.2/8.0 Hz), 7.33 (ddd, 1H, J = 1.2/7.6/7.8 Hz), 7.54 (t, 1H, J = 7.8/8.0 Hz), 7.57 (ddd, 1H, J = 1.8/2.0/7.8 Hz), 7.83 (d, 1H, J = 7.8 Hz), 8.04 (dd, 1H, J = 1.6 Hz/7.8 Hz), 8.16 (ddd, 1H, J = 0.8/1.2/2.2/7.8 Hz), 8.35 (sl, 1H).; ^{13}C NMR ($CDCl_3$, 7 MHz) δ 52.4, 72.1, 121.7, 122.6, 122.7, 123.7, 126.4, 129.3, 129.7, 131.9, 132.9, 134.1, 141.0, 143.4, 148.3, 150.1, 164.4, 164.8, HR-MS-Mass calculated: 380.0746 [M+23], found: 380.0692; $C_{18}H_{15}NNaO_7$.

2.1.2.3. Methyl 2-{2-[hydroxy(2-nitrophenyl)methyl])acryloyl oxy}benzoate (1c). Seventy-four % yield; oil; IR (KBr): 3479, 3078, 3001, 2954, 1732, 1612, 1531, 1446, 1350, 1300, 1087, 856, 736 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (s, 3H), 5.66 (s, 1H), 6.44 (s, 1H), 6.56 (s, 1H), 7.17 (dd, 1H, J = 0.8/1.2/8.0 Hz), 7.35 (ddd, 1H, J = 0.8/1.2/8.0 Hz), 7.50 (ddd, 1H, J = 1.2/1.6/8.0 Hz), 7.59 (ddd, 1H, J = 1.6/8.0 Hz), 7.71 (ddd, 1H, J = 1.2/7.6 Hz), 8.00 (d, 1H, J = 8.0 Hz), 8.03 (dd, 1H, J = 1.2/8.8 Hz), 8.05 (dd, 1H, J = 1.6/8.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.5, 67.7, 122.7, 124.0, 124.8, 126.3, 128.4, 128.7, 129.3, 132.0, 133.7, 134.1, 136.1, 141.2, 145.0, 150.4, 164.5, 165.1. HR-MS-Mass calculated: 380.0746 [M+23], found: 380.0675; $C_{18}H_{15}NNaO_7$.

2.1.2.4. Methyl 2-{2-[hydroxy(pyridin-4-yl)methyl]acryloyloxy})benzoate (1d). Sixty-seven % yield; oil; IR (KBr): 3113, 2951, 2850, 1728, 1708, 1600, 1454, 1296, 1199, 1130, 1056, 968. cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (s, 3H); 5.68 (s, 1H); 5.99 (s, 1H); 6.64 (s, 1H); 7.01 (dd, 1H, J = 1.0/8.2 Hz); 7.30 (ddd, 1H, J = 1.2/7.4/7.8 Hz); 7.38 (dd, 2H, J = 1.0/4.8 Hz); 7.53 (ddd, 1H, J = 1.4/1.6/8.0 Hz); 8.01 (dd, 1H, J = 1.6/8.0 Hz); 8.52 (dd, 2H, J = 1.4/4.4 Hz).; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 52.3, 71.7, 121.6 (2C), 122.7, 123.6, 126.3, 129.6, 131.9, 134.0, 140.9, 149.6 (2C), 150.0,

Table 1
Different experimental protocols and results in the preparation of **1a–1g** compounds (see Scheme 6)

Entry	Compd	Solvent	DABCO (equiv)	Temp (°C)	Time	Yield[a] (%)
1	**1a**	Solvent-free condition	1	0	3 h	27
2	**1b**	Solvent-free condition	1	0	3 h	15
3	**1c**	Solvent-free condition	1	0	6 h	26
4	**1a**	*t*BuOH/H_2O[b]	1	25	30 min	8[c]
5	**1a**	Methanol	1	25	30 min	0[d]
6	**1a**	Methyl salicylate	1	0	24 h	53
7	**1b**	Methyl salicylate	1	0	48 h	48
8	**1c**	Methyl salicylate	1	0	24 h	51
9	**1a**	Acryloyl salicylate (20 equiv)	0.3	0	3 h	70[e]
10	**1b**	Acryloyl salicylate (20 equiv)	0.3	0	24	61
11	**1c**	Acryloyl salicylate (20 equiv)	0.3	0	15	0
12	**1c**	Acryloyl Salicylate (20 equiv), methyl salicylate	0.3	0	48	74[e]
13	**1d**	CH_2Cl_2	0.3	0	20 min	67
14	**1e**	CH_2Cl_2	0.3	0	3 h	60
15	**1f**	Solvent-free condition	1	0	4 h	52
16	**1g**	Acetonitrile	1	0	24 h	65

[a] Purified yields.
[b] 9:1 *t*-BuOH/water.
[c] After 30 min a large amount of the acrylate **4** was transformed in the methyl salicylate (**3**).
[d] After 30 min the acrylate **4** was converted on the methyl acrylate (transesterifications reaction).
[e] Acryloyl salicylate (**4**) and methyl salicylate (**3**) excess could be easily recovery in high yields (>95%) by a simple filtration.

150.6, 164.3, 164.8. HR-MS-Mass calculated: 336.0848 [M+23], found: 336.0814; $C_{17}H_{15}NNaO_5$.

2.1.2.5. Methyl 2-{2-[hydroxy(pyridin-3-yl)methyl]acryloyloxy})benzoate (1e). Sixty % yield; oil; IR (KBr): 3151, 3055, 1728, 1608, 1454, 1431, 1203, 1053, 964, 732 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.68 (s, 3H); 5.74 (s, 1H); 6.20 (s, 1H); 6.67 (s, 1H); 7.00 (d, 1H, J = 8.0 Hz); 7.24 (d, 1H, J = 7.8 Hz,); 7.28 (ddd, 1H, J = 1,0/7.8 Hz); 7.51 (ddd 1H, J = 1,8/7.8 Hz); 7.81 (dd, 1H, J = 7.8 Hz); 7.98 (dd, 1H, J = 1.6/7.8 Hz); 8.39 (d, 1H, J = 4.6 Hz); 8.56 (d, 1H, J = 1.8 Hz).; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 51.9, 69.9, 122.8, 123.3, 123.5, 126.0, 128.0, 131.6, 133.8, 134.9, 137.6, 141.5, 148.2 (2C), 149.9, 164.1, 164.7. HR-MS-Mass calculated: 336.0848 [M+23], found: 336.0791; $C_{17}H_{15}NNaO_5$.

2.1.2.6. Methyl 2-{2-[(4-bromophenyl-hydroxy)methyl]acryloyl oxy}benzoate (1f). Fifty-two % yield; oil; IR (KBr): 3502, 3078, 3001, 2951, 1716, 1604, 1485, 1435, 1404, 1300, 1041, 960, 844, 810, 756, 729 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.76 (s, 3H); 5.68 (s, 1H); 5.92 (s, 1H); 6.61 (s, 1H); 7.05 (dd, 1H, J = 1.2/8.0 Hz); 7.31 (1H) 7.33 (d, 2H, J = 8.6 Hz); 7.48 (d, 2H, J = 8.0 Hz); 7.55 (ddd, 1H, J = 1.6/7.6 Hz); 8.02 (dd, 1H, J = 1.8/7.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 52.3, 72.3, 121.7, 122.8, 123.7, 126.3, 128.5 (2C), 128.8, 131.4 (2C), 131.8, 134.1, 140.1, 141.6, 150.1, 164.6, 164.8. HR-MS-Mass calculated: 390.0103 [M], found: 390.0138; $C_{18}H_{15}BrO_5$.

2.1.2.7. Methyl 2-{2-[hydroxy(naphthalen-2-yl)methy])acryloyl loxy}benzoate (1g). Sixty-five % yield; oil; IR (KBr): 3498, 3055, 2951, 1732, 1604, 1485, 1446, 1300, 1087, 1041, 960, 856, 817, 752 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.69 (s, 3H); 5.90 (sl, 1H); 5.97 (s, 1H); 6.64 (s, 1H); 7.02 (dd, 1H, J = 0.8/8.0 Hz); 7.28 (ddd, 1H, J = 0.8/7.8 Hz); 7.50 (m, 4H); 7.84 (m, 3H); 7.95 (sl, 1H); 8.01 (dd, 1H, J = 1.6/7.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 52.3, 72.8, 123.0, 123.8, 124.8, 125.8, 126.0, 126.2, 126.2, 127.7, 128.1, 128.2, 128.8, 131.9, 133.1, 133.3, 133.9, 138.4, 142.0, 150.2, 164.8, 165.0 HR-MS-Mass calculated: 385,1052 [M+23], found: 385.0976; $C_{17}H_{15}NNaO_5$.

2.1.2.8. Bis(2-(methoxycarbonyl)phenyl)-2-methylenepentane dioate (25). Fifty-one % yield; oil; IR (KBr): 2999, 2953, 1726, 1631, 1606, 1452, 1404, 1300, 1205, 1153, 1083, 756. cm $^{-1}$; ^1H NMR (300 MHz, $CDCl_3$) δ 2.96 (m, 4H), 3.84 (sl, 3H), 3.85 (sl, 3H), 5.94 (s, 1H), 6,54 (s, 1H), 7,10 (dd, 1H, J = 1.0 Hz/8,0 Hz), 7,17 (dd, 1H, J = 1.0 Hz/8,0 Hz), 7,32 (m, 1H), 7,56 (m, 1H), 8.05 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.1, 32.8, 52.2 (2C), 123.2 e 123.4 (2C), 123.8 (2C), 126.0 (2C), 128.2, 131.7, 131.8, 133.81 (2C), 138.1, 150.6 (2C), 165.0 (2C), 165.3, 171.4 HR-MS-Mass calculated: 435.1056 [M+23], found: 435.0965; $C_{22}H_{20}O_8$.

2.2. Biology[33]

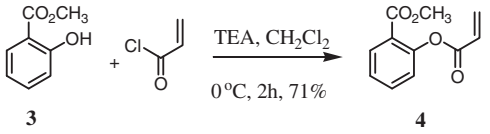
Logarithmic phase promastigotes (1×10^6 parasites mL^{-1}) of *L. amazonensis* (MHOM/IFLA/BR/67/PH8) and *L. chagasi* (MCAN/BR/99/JP15) were incubated at 26 °C in Schneider's *Drosophila* medium supplemented with 20% of fetal bovine serum, streptomycin (100 μg mL^{-1}), and penicillin (100 U mL^{-1}), in the presence or absence of different concentrations of new hybrids chalcones-like **1a–1g** MBHA, methyl salicylate acrylic ester (**4**), methyl salicylate (**3**) and methyl salicylate acrylic ester dimer **6** The reference drugs Glucantime® and Amphotericin B® were used as control. After 72 h, parasites were collected, fixed in isotonic solution (10.5 g citric acid, 7.0 g NaCl, 5.0 mL formalin and 1000 mL distilled water) and examined under light microscopy. The inhibitory effect of the compounds on cell growth was estimated by cell counting using a Neubauer chamber. The IC_{50}/72 h, concentration responsible for a 50% reduction in culture growth after 72-hour incubation was determined by the probity regression model using the software SPSS 8.0 for Windows. All experiments were performed in triplicate and repeated at least three times. Statistical analysis of data was done by means of one-way ANOVA. P values of 0.05 or less were considered significant.

3. Results and discussion

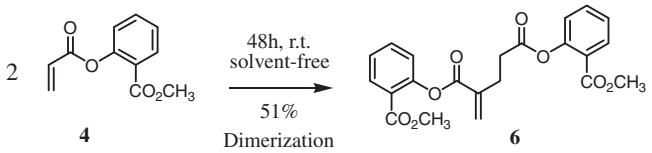
3.1. Chemistry

We chose as strategy the use of MBHR[44] between the unpublished acrylate **4** (Scheme 3) and the commercials aldehydes **5a–5h** (Fig. 2) as key-step of the MBHA **1a–1g** *one-pot* syntheses.

The synthesis of **4** was carried out in good yield reacting methyl salicylate (**3**) and commercial acryloyl chloride on CH_2Cl_2 as solvent at 0 °C for 2 h (71%, Scheme 3). The acrylate **4** is stable for more than 30 days at low temperature (−4 °C/−20 °C) or stable at room temperature by addition of BHT as antipolymerization. However, a new acrylate **6** was formed after 48 h at room



Scheme 3. Synthesis of acrylate **4**.



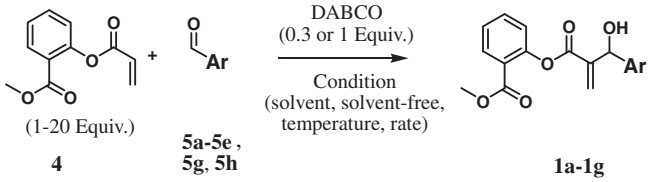
Scheme 4. Spontaneous dimerization of acrylate **4**. Synthesis of acrylate **6**.

temperature in solvent-free condition, from a spontaneous dimerization reaction (Scheme 4).

Our first protocol aim to prepare **1a**–**1g** MBHA was at low temperature and on solvent-free condition.[35] When the reaction between excess of acrylate **4** and aldehyde **5a** was performed under 1 equiv of DABCO as promoter, the expected adduct **1a** was obtained in low yield (Table 1, entry 1) and the principal product of reaction was the dioxanone **7** (Scheme 5), that can be understood based on nonprotic mechanism proposed by McQuade.[45] When the reactions were carried out with aldehydes **5b** and **5c** (on the same conditions) the **1b** and **1c** adducts were obtained in low yields and over again the corresponding dioxanones were obtained as principal products (Table 1, entries 2 and 3, see also Scheme 6).

Aiming to minimize the dioxanones formation, the MBHR with aldehyde **5a** was carried out in the presence of protic *t*-butanol/water (9:1) as solvent, modifying the nonprotic to protic mechanism, as proposed by Aggarwal.[46] Both, protic and the nonprotic mechanisms to the MBHR were recently corroborated by Coelho.[47] As expected, there was a total disappearance of **7**. Unfortunately, due the salicylate moiety on MBHA **1a** to be a good leaving group, the adduct **1a** was also obtained in low yield, and the methyl salicylate (**3**) was the principal compound obtained of this reaction (Table 1, entry 4). The use of anhydrous methanol as solvent presented to be also inefficient producing the methyl acrylate from a transesterification reaction (Table 1, entry 5). However, when the methyl salicylate itself was used as protic solvent, the **1a**, **1b**, and **1c** MBHA could be obtained in moderated yields (Table 1, entries 6, 7, and 8).

As reported by McQuade, excess of aldehydes under a prolonged reaction time leads to the formation of dioxanones like **7**.[48] Therefore, we realize to used a larger amount of acrylate **4** (20 equiv) aim to minimize the formation of dioxanones. Besides that, in diluted reaction medium, the formation of dioxanones would be less favored, after the proton transfer step (rate-determining step, RDS). We also decrease the DABCO quantity to



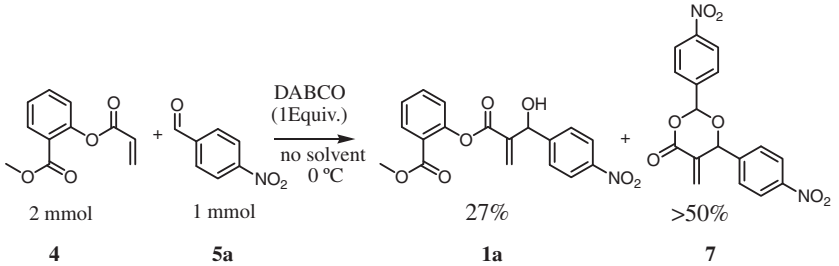
Scheme 6. Morita–Baylis–Hillman reaction on the **1a**–**1g** preparations (see Table 1).

0.3 equiv trying to minimize the dimers formation between the acrylates. Satisfactorily, this experimental modification was efficient and we could to prepare the **1a** and **1b** MBHA in good yields (Table 1, entries 9 and 10). Curiously, MBHA **1c** could not be prepared using this methodology even after fifteen days of reaction (Table 1, entry 11). Fortunately, in this case, the addition of methyl salicylate (**3**) as protic solvent and using 20 equiv of **4** was efficient for the MBHA **1c** preparation in good yield (Table 1, entry 12). The acrylate **4** could be easily recovery in high yields (>95%) by a simple filtration. These efficient results can be understood based on the very recent Cantillo and Kappe work.[49] They proven that the use of phenols as a solvent or an addictive can improve rate and yields on MBH reaction by change it slow determination rate (RDS).[49]

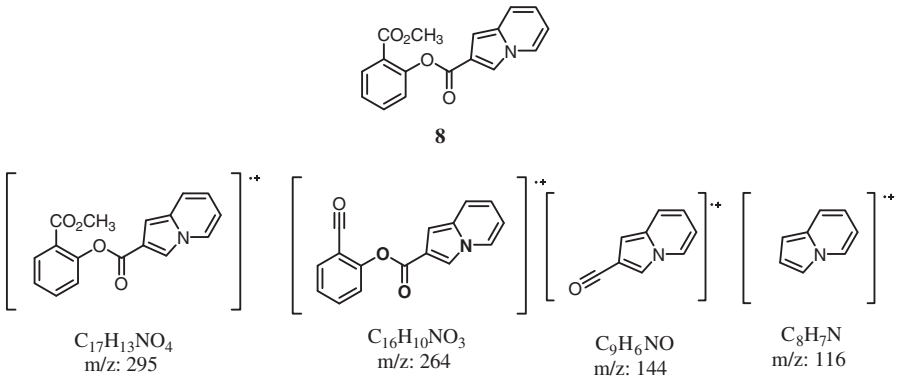
For the preparation of the **1d** and **1e** pyridine MBHA derivatives, reaction were carried out between the aldehydes pyridine-4-carboxaldehyde (**5d**) or pyridine-3-carboxaldehyde (**5e**) with the acrylate **4**, on dichloromethane as solvent. In these cases, the reactions mixtures showed low solubilities in solvent-free conditions. Dichloromethane also has the advantage being easily evaporated and do not contained significant traces of water, which could hydrolyze the MBHA. These experimental protocols produced **1d** and **1e** in moderated or good yields (Table 1, entries 13 and 14).

Unlike of the expected, reaction between pyridine-2-carboxaldehyde (**5f**) and acrylate **4** produced several byproducts and the corresponding chalcone-like compound could not be isolated (not shown). Based on characteristics fragmentation obtained by CG–MS, we speculate that principal byproducts is the indolizine **8** (m/z = 295, m/z = 264, m/z = 144 and m/z = 116, Scheme 7). Unfortunately due to the existence of several byproducts of similar polarities of **8**, we cannot isolate pure indolizine **8** and than its complete characterization was not done. Basavaiah et al. established that several indolizines can be synthesized from MBHR.[50] Several conditions (not shown) were investigated aiming to prepare **8** efficiently, but without any success.

For the adduct **1f** preparation (Fig. 1) was used solvent-free condition at 0 °C (Table 1, entry 15, and 52% yield). Unfortunately, the use of other protocols not improved yield on **1f** preparations. Finally, we prepared, in improved possible way, the MBHA **1g** in moderated yield (65% yields, Table 1, entry 16) by use acetonitrile as solvent. Curiously, in this last case the solvent-free condition, protic and phenol addition as solvent not improved yields.



Scheme 5. Dioxanone **7** as the principal product of MBH reaction on solvent-free condition.



Scheme 7. Characteristics fragmentation of **8**.

Table 2
[a,b] Antileishmanial in vitro activity of **3**, **1a–1g**, **4** and dimer **6** and Glucantime® as reference compound

Compd	IC_{50} ($\mu g\ mL^{-1}$) *L. amazonensis*	IC_{50} (μM) *L. amazonensis*	IC_{50} ($\mu g\ mL^{-1}$) *L. chagasi*	IC_{50} (μM) *L. chagasi*
3	34.73	228.49	39.74	261.45
1a	4.05	11.34	20.48	57.34
1b	8.16	22.86	14.51	40.64
1c	2.73	7.65	3.62	10.14
1d	7.46	23.83	10.44	33.35
1e	9.75	31.15	12.11	38.69
1f	8.58	22.00	16.78	43.03
1g	3.26	9.00	14.98	41.38
4	22.35	108.50	24.48	118.83
6	9.25	22.45	20.22	49.08
Glucantime®	>4000	Variable	>4000	Variable
Amphotericin B®	0.11	0.12	0.64	0.69

[a] IC_{50} values obtained from a minimum of three separate experiments performed in triplicate are shown.
[b] The reference drugs Glucantime® and Amphotericin B® were used in this study and *in vitro* activities.

3.2. Biology

Our biological data are described in Table 2 and a few comments about these results deserve be made. Initially, the IC_{50} evaluation of methyl salicylate (**3**) and acryloyl salicylate (**4**) on *L. amazonensis* and *L. chagasi* have shown a moderated antipromastigote activities (Table 2) when compared to the designed new compounds **1a–1g**. Secondly, we can note that the new hybrids **1a–1g** have higher toxicity on *L. amazonensis* than on *L. chagasi*. Additionally, the leishmanicidal activities of compounds **2a–2g** (IC_{50} = 50.08–731.37 μM on *L. amazonensis* and IC_{50} = 52.92–724.59 μM on *L. chagasi*) presented in our previous article.[33] are much smaller than the hybrids 1a–1g of this new series of chalcones-like compounds (Table 2). This experimental observation confirmed the importance of the salicylate moiety in **1a–1g**, to increase the leishmanicidal activity of this new series, may be also a consequence of anti-COXs activity, the best known mechanism of action to salicylate derivatives.[17,18,21,51] Thus, as idealized by us, the molecular hybridization is critical to increase the leishmanicidal activity.

We can also notice in Table 2 that the *ortho* nitro compound **1c** presents the most leishmanicidal activity on both *L. amazonensis* and *L. chagasi* in the **1a–1g** series. We recently described that the presence of the *ortho* nitro group also increases antileishmania activity being the compound **2c** (Fig. 1) the most active in the **2a–2g** series.[35] We can identify on geometric shapes of the **1a** and **1c** calculated conformationals minima (Fig. 3) that, different from that occurs on compound **1a**, the NO_2 group in **1c** is not completely conjugated with the aromatic moiety. This conformational modification caused by the *o*-nitro group can change the interaction between ligand–enzyme or/and change the reduction potential of these compounds modifying, for example, the redox system TR/trypanothione that is vital for parasite survival within the host cell.[52–54] Even if, up to now, the correct biological mechanism of action of these MBHA is not known, it is reasonable to speculate for these different biological activities on **1a**, **1b**, and **1c** compounds in this series.

Further, the position 4 or 3 of the nitrogen atom in the pyridine rings into the corresponding **1d** and **1e** compounds did not significantly alter these bioactivities (Table 2). The same tendency was observed in the previous studied **2a–2g** series,[35] (IC_{50} = 488.98 μM on *L. amazonensis*; IC_{50} = 494.81 μM on *L. chagasi* to the compound **2d** and IC_{50} = 448.50 μM on *L. amazonensis*; IC_{50} = 350.57 μM on *L. chagasi* to the compound **2e**).[35] However, the **1d** and **1e** (Table 2) were almost 20 times more active than the corresponding compounds **2d** and **2e**.

The antileishmania activity of compound **1f** is similar than **1d** and **1e** (Table 2), differently that determinate to **2f**,[35] which is almost 5 times more active than the corresponding compounds **2d** and **2e** in the **2a–2g** series (shown in the previous paragraph).

It should be noted that the dimer **6** (Scheme 4), which also is an analogues of chalcones, has a significant activity against the *L. amazonensis* and *L. chagasi*, unlike the corresponding monomer **4**. This remark suggests once again the importance of the presence of two the aromatic groups in the **1a–1g** series, one of the

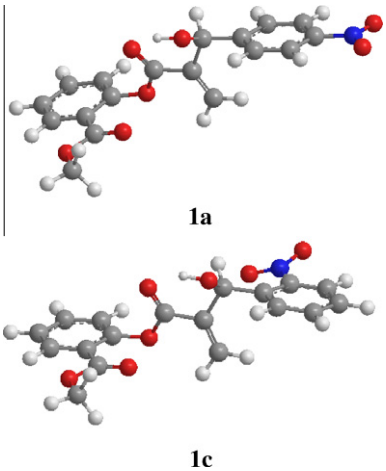


Figure 3. Conformationals minima of **1a** and **1c**, calculated by B3LYP/6-311++G (d,p) as calculation level from GAUSSIAN 09 (versions for Linux).

characteristics of chalcones. Finally, compound **1g** is a second more active in this new series (Table 2) of hybrid compounds. It is reasonable to propose in this case that, since compound **1g** is the most lipophilic into this new series (calculated from Pharma-algorithms®, Log $P = 4.54 \pm 0.51$),[55] lipophilicity it could be an important parameter for it bioactivity.

4. Conclusion

In this work, we successfully applied the current mechanistic knowledge of MBHR, to improve synthetic results.[45–49] Our data also are useful to corroborate these mechanistic proposals for this fine reaction.

Moreover, in this work, we idealized, synthesized and show with success one more example of molecular hybridization, a very important strategy in Medicinal Chemistry.[36]

Seven new chalcone-like compounds **1a–1g** were designed using molecular hybridization strategy, efficiently synthesized, completely characterized and bioevaluated as a new series of in vitro leishmanicides. Amphotericin B presented a higher in vitro activity compared with compounds **1a–1g** and **2a–2g**. However, Amphotericin B is well-known for its severe and potentially lethal side effects and very high cost to production.[56] In addition, preparation of **1a–1g** have advantages in terms of yield and operational simplicity.

Finally, all these new original series of compounds **1a–1g** were active against the promastigotes form of *L. amazonensis* and *L. chagasi* and much more active than the previous bioevaluated series **2a–2g**.[35] This prospective work needed to study the real mechanism of action of these new compounds. Then, the study of toxicities in macrophages and the biological mechanisms of action of these new compounds will now be investigated.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.05.055.

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