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Original article

Synthesis, evaluation against *Leishmania amazonensis* and cytotoxicity assays in macrophages of sixteen new congeners Morita–Baylis–Hillman adducts

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ABSTRACT

We report the design, synthesis, *in vitro* evaluation against *Leishmania amazonensis* (IC_{50}), cytotoxicity assays in macrophages (CC_{50}), and selectivity index ($SI=CC_{50}/IC_{50}$) of sixteen new congeners aromatic Morita–Baylis–Hillman adducts **1**–**16**. The **1**–**16** were prepared in good to excellent yields (58%–97%) from the "one pot" Morita–Baylis–Hillman Reaction between the aldehydes **29**–**36** and the acrylates **27** or **28** under DABCO as promoter. The MBHA 2-[Hydroxy(2-nitrophenyl)propyl] propanoate (**1**, IC_{50} = 7.52 μg/mL or 28.38 μM; CC_{50} = 35.77 μg/mL or 134.98 μM; SI = 4.75) and 2-[Hydroxy(2-nitrophenyl)hydroxyethyl] propanoate (**9**, IC_{50} = 5.48 μg/mL or 20.52 μM; CC_{50} = 29.81 μg/mL or 111.64c μM and, SI = 5.43) were the most effective and safe evaluated compounds.

1. Introduction

Neglected tropical diseases (NTDs) have traditionally ranked low on national and international health agendas [1]. Leishmaniasis are a complex of NTDs, caused by different species of *Leishmania* sp. protozoan parasite, which have significant impact on the world, especially in developing countries with infections spread over several hundred million people and a significant cause of morbidity and mortality [1]. Leishmaniasis affect 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 [2]. Increased travel and migration within the tropics, subtropics, Middle East and Southern Europe as well as global climate and environmental changes are making leishmaniasis a considerable risk for populations in geographic regions previously unaffected by the disease. As a result, there has been a progressive expansion of leishmaniasis endemic regions as well as a concomitant increase in the total number of reported leishmaniasis cases, often in epidemic proportions [3]. Leishmaniasis infection causes many different clinical manifestations, such as cutaneous [4], mucocutaneous [5] and visceral (VL), also called Kala Azar. Human infection with *Leishmania chagasi*/*Leishmania infantum*, the protozoan causing South American VL, ranges from sub-clinical infection to progressive fatal disease [6] 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 [7]. 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 [8]. The treatment options for leishmaniasis are limited and involve the administration of pentavalent antimonial agents as first line drugs: sodium stibogluconate (Pentostan®) and meglumine antimonite (Glucantime®). The amphotericin B and pentamidine are second choice drugs [9]. However, the chemotherapy with the second-line drug pentamidine is also far from satisfactory due to several side effects including renal and hepatic toxicity,

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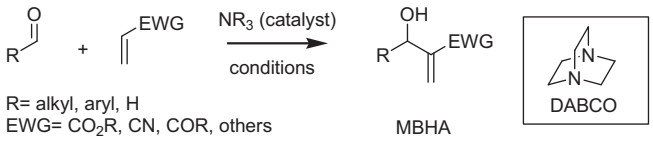


Scheme 1. General Morita–Baylis–Hillman reactions with *e.g.* aldehydes.

pancreatitis, hypotension, dysglycemia, and cardiac abnormalities [10]. Although amphotericin B and its lipid complex, are quite effective for visceral leishmaniasis, they are expensive and do not appear to be suitable for treatment of non-visceral diseases [10]. Reproducible evidence of protective efficacy has not emerged from clinical trials of first generation leishmaniasis vaccines. So far no vaccine approved for human use is available [11]. 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.

The Morita–Baylis–Hillman reaction (MBHR) is an important way for C–C bond formation [12,13]. It involves the coupling of alkenes containing electron-withdrawing groups (EWG) with aldehydes, ketones or imines, among others. Tertiary amines are used as nucleophilic catalysts of which 1,4-diazabicyclo[2.2.2] octane (DABCO) is the most widely used (Scheme 1). The Morita–Baylis–Hillman adducts (MBHA) have been extensively used as starting materials in organic synthesis for a variety of applications, many of which have biological activity [14–17]. An inconvenience associated with this reaction is, in several examples, the long reaction times. There are reports of reactions that, last up to 65 days [14]. However, due to the synthetic utility of these MBHA adducts, several protocols have been described to improve the reaction time and yields, such as the use of microwaves [18], ultrasound [19], high pressures [20], use of ionic liquids [21], change of reaction temperature [22] and catalyst [23], and other experimental protocols [17].

The first biological evaluation of the MBHA prepared from "one pot" MBHR was described against *Plasmodium falciparum*, the etiologic agent of malaria [24,25]. In 2006, we described the molluscicidal activities of simple aromatic MBHA against *Biomphalaria glabrata* (Say) snails, the intermediate host of schistosomiasis [26]. In the same year the antiproliferative effects of some MBHA and a new phthalide derivative on human tumor cell lines was described. In this article Kohn et al. reinforce what is known, the higher biological activity of aromatic compounds when compared to aliphatic compounds [27].

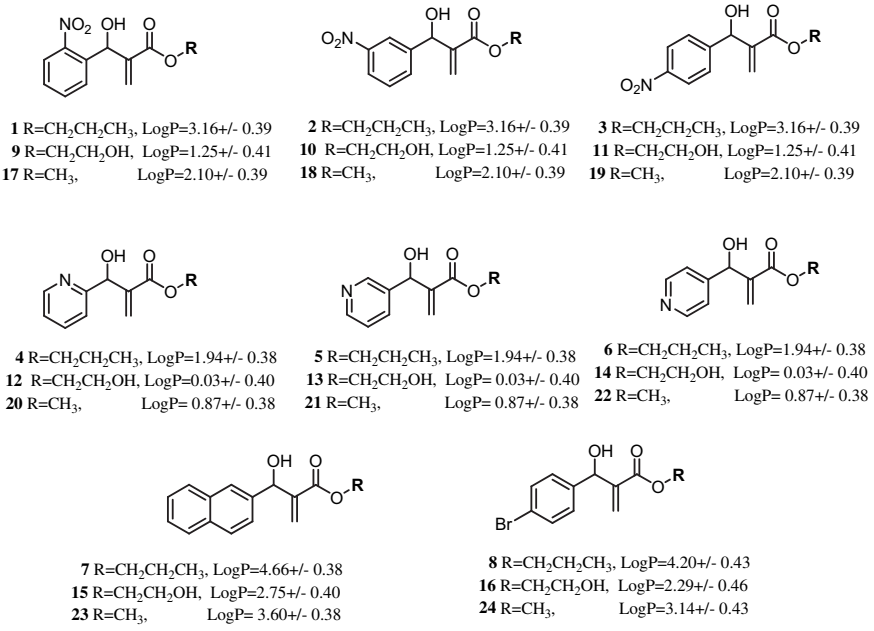
Our research group described in 2007, that some aromatic MBHA were very active compounds against the *L. amazonensis* (cutaneous and mucocutaneous infections) and some of them are safe compounds [28]. Following that work, we published the biological evaluation of aromatic MBHA against *L. chagasi* (visceral infections) parasites [29], and in 2010, we have shown that the MBHA 3-Hydroxy-2-methylene-3-(4-nitrophenyl) propanenitrile, a high anti-leishmania compound, is a highly active compound against epimastigote and trypomastigote form of *Trypanosoma cruzi*, the parasite that causes Chagas disease [30]. In the same year, we presented an improved synthesis for sixteen 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 new promising class of anti-parasitic drugs [31].

In our continuing search for bioactive substances [32–37] and in connection with our efforts toward the study of reactivity of MBHR [21,23,38–41], we have been trying to discover a lead compound against *in vitro* leishmaniasis, aiming to advance toward the *in vivo* studies. In this context we present here the design, synthesis, inhibitory activities on antipromastigote form of *L. amazonensis* (IC_{50}), results of cytotoxicity assays in macrophages (CC_{50}) and selectivity index (SI) of eight new aromatic MBHA **1**–**8** and their eight classic bioisosters **9**–**16** [42] (Fig. 1).

2. Results and discussion

2.1. Design of the MBHA *1*–*16*

This design was based on the anti-leishmania activities of **17**–**24** (Fig. 1) presented by us in previous paper [31]. We modified the lipophilicity [43] of **17**–**24** to design the MBHA **1**–**8**; and used the



1 R=$CH_2CH_2CH_3$, LogP=3.16+/- 0.39
9 R=CH_2CH_2OH, LogP=1.25+/- 0.41
17 R=CH_3, LogP=2.10+/- 0.39

2 R=$CH_2CH_2CH_3$, LogP=3.16+/- 0.39
10 R=CH_2CH_2OH, LogP=1.25+/- 0.41
18 R=CH_3, LogP=2.10+/- 0.39

3 R=$CH_2CH_2CH_3$, LogP=3.16+/- 0.39
11 R=CH_2CH_2OH, LogP=1.25+/- 0.41
19 R=CH_3, LogP=2.10+/- 0.39

4 R=$CH_2CH_2CH_3$, LogP=1.94+/- 0.38
12 R=CH_2CH_2OH, LogP=0.03+/- 0.40
20 R=CH_3, LogP= 0.87+/- 0.38

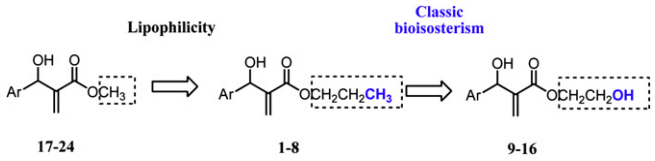
5 R=$CH_2CH_2CH_3$, LogP=1.94+/- 0.38
13 R=CH_2CH_2OH, LogP= 0.03+/- 0.40
21 R=CH_3, LogP= 0.87+/- 0.38

6 R=$CH_2CH_2CH_3$, LogP=1.94+/- 0.38
14 R=CH_2CH_2OH, LogP= 0.03+/- 0.40
22 R=CH_3, LogP= 0.87+/- 0.38

7 R=$CH_2CH_2CH_3$, LogP=4.66+/- 0.38
15 R=CH_2CH_2OH, LogP=2.75+/- 0.40
23 R=CH_3, LogP= 3.60+/- 0.38

8 R=$CH_2CH_2CH_3$, LogP=4.20+/- 0.43
16 R=CH_2CH_2OH, LogP=2.29+/- 0.46
24 R=CH_3, LogP=3.14+/- 0.43

Fig. 1. New MBHA **1**–**16** synthesized and biologically evaluated in this article. The MBHA **17**–**24**, recently described by us, that inspired our design through **1**–**16** from molecular modification. *In silico* LogP calculated from http://pharma-algorithms.com.



Scheme 2. Molecular modifications on the anti-leishmania MBHA **17**–**24** as strategy to idealize the new MBHA **1**–**16**.

classic bioisosterism strategy for molecular modification, to idealize the MBHA **9**–**16** [42] (Scheme 2 and Fig. 1).

The systematic change of the physicochemical properties of compounds in a series, *e.g.* lipophilicity, is a way of understanding the relationship between a drug molecule and drug action. Physicochemical properties help control the processes of drug absorption, distribution, metabolism, excretion and interaction of the drug with its molecular target. The distribution coefficient (Log*P*) is a measure of a drug's lipophilicity and an indication of its ability to cross cell membranes [43]. Drugs having values of *P* beyond 1 are classified as lipophilic, whereas those with partition coefficients smaller than 1 are indicative of hydrophilic drugs. These values can be *in silico* calculated or accurately determined by using an HPLC method [44]. The higher the value, more lipophilic is the drug. In order to gain further insight into the ability of MBHA **1**–**24** to cross cell membranes, Log*P* values were calculated *in silico* using the help of Pharma-algorithms (available from http://pharma-algorithms.com) and the results of these calculations are described in Fig. 1.

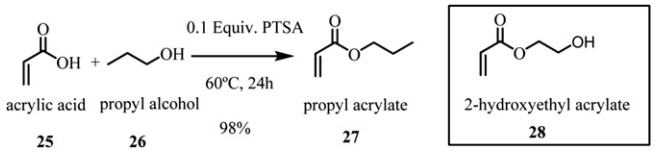
In fact, one of the most reliable methods in medicinal chemistry to improve *in vitro* activity is to incorporate properly positioned lipophilic groups. For example, addition of a single methyl group that can occupy a receptor 'pocket' improves binding by about 0.7 kcal/mol [45]. The Lipinski's rule of five [46] predicts that poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight (MWT) is greater than 500 and the calculated Log*P* (CLog*P*) is greater than 5 [47].

2.2. Chemistry

2.2.1. Synthesis of propyl acrylate (*27*)

We prepared the propyl acrylate (**27**, Scheme 3) from 1 Equiv. of acrylic acid and 3 Equiv. of propyl alcohol (no solvent was used) under catalytic quantity of *p*-toluenesulfonic acid (PTSA), at 60 °C for 24h, in high yield, as shown in Scheme 3. No antipolymerization substance was necessary to be added and we did observe no co-products in this easy and efficient synthetic protocol. Also, the 2-hydroxyethyl acrylate (**28**, Scheme 3) is a commercial compound.

The preparation of acrylates from different alcohols is an important chemical transformation for the synthesis of new materials, *e.g.* polymers. Most of these synthetic methodologies are described in patents. As far as we can tell, propyl acrylate (**27**) was prepared for the first time in 2005, using a microbiological method. This related invention described a method for preparing esters of acrylic acid and (C2-C8)-aliphatic alcohols involving hydrolysis of acrylonitrile to acrylic acid ammonium salt by using the strain of bacterium *Alcaligenes denitrificans* C-32 VKM 2243 D [48]. After



Scheme 3. Synthesis of propyl acrylate (**27**), and the commercial 2-hydroxyethyl acrylate (**28**).

Table 1
Experimental conditions to prepare the MBHA **1**–**16** (Fig. 2) and its yields. Several other conditions, changing solvent, temperature, reaction time and catalyst amount were also investigated.

Entry	MBHA	Aldehyde	Acrylate	Solvent	Reac. time	Yields(%)[a]
1	1	29	27	PrOH	3 days	68
2	2	30	27	PrOH	2 days	73
3	3	31	27	PrOH	3 days	97
4	4	32	27	CH_3CN	4 days	70
5	5	33	27	CH_3CN	7 days	80
6	6	34	27	CH_3CN	8 days	66
7	7	35	27	PrOH	6 days	60
8	8	36	27	PrOH	8 days	64
9	9	29	28	CH_3CN	24 h	71
10	10	30	28	CH_3CN	24h	58
11	11	31	28	CH_3CN	18 h	62
12	12	32	28	CH_3CN	28 h	94
13	13	33	28	CH_3CN	29 h	83
14	14	34	28	CH_3CN	18h	80
15	15	35	28	CH_2OHCH_2OH	6 days	67
16	16	36	28	CH_2OHCH_2OH	24 h	71

[a] yields obtained from the purified products; no co-product was detected; 100% of conversion based on the recovery of unreacted aldehyde (from CGMS analysis).

that, Hua et al. described in 2006 a synthesis of **27** with a 61% yield using $ZrOCl_2 \cdot 8H_2O$ at 50 °C for 24 h [49].

2.2.2. Synthesis of MBHA *1*–*16*

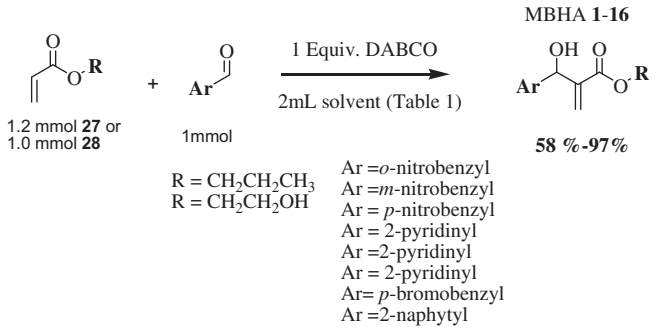
The Morita–Baylis–Hillman (MBH) reaction is an exquisite reaction [17]. In the two proposed reaction mechanisms, it is evident that small modifications on the experimental conditions, such as change of the aprotic solvent to a protic one, can change the reaction pathway and lead to the appearance and disappearance of side co-products. In independent works McQuade [50] and Aggarwal [51] presented a dualistic nature of the MBHR mechanism, which was supported by Coelho and co-workers through ESI-MS experiments [52].

In our work, several experimental conditions were investigated, changing solvents, temperature of reactions, reaction time and catalyst amount (0.1–1.0 Equiv.). However, we only present in Table 1 the best results to prepare the MBHA **1**–**16** (Scheme 4). The experimental details of the MBHRs between the aldehydes **29**–**36** (Fig. 2) and the acrylates **27** or **28** (Scheme 3) were presented in experimental procedures.

Finally, the characterization of the new MBHA **1**–**16** was established by FTIR, ^1H and ^{13}C NMR and HRMS. The purities of all compounds were also measured by gas chromatography (GC) (Experimental procedures).

2.3. Biology

Its important to notice that all new **1**–**16** MBHA synthesized here have *in silico* log*P* between +4.66 and +0.03, 1–2 H-bond



Scheme 4. Synthesis of the **1**–**16** Morita–Baylis–Hillman adducts (results in Table 1).

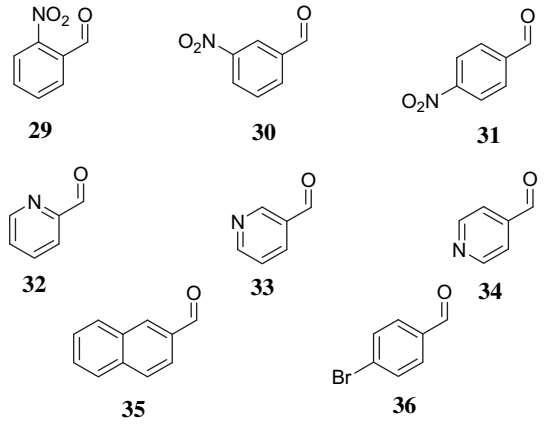


Fig. 2. The corresponding aldehydes **29**–**36**, used to prepare the MBHA **1**–**16**.

donors, 2–5 H-bond acceptors, the molecular weight between 193 and 300 g/mol, (Fig. 1). All of these are consistent with both the "Lipinski's rule of five" and the "Reynisson's rule", that consider respectively the drug-like chemical space and the known drug space (KDS) [53].

We can note in Table 2 that the two more toxic MBHA against *Leishmania amazonenesis* and safe in macrophages, are adducts **1** and **9** (Entry 1 and 9 of Table 2) more accurately evaluated due to their higher selectivity index (SI). It is noteworthy that, both of them (**1** and **9**) have the *o*-nitrophenyl moiety in these structures.

We could not be able to realize in this present study any evident relationship between the lipophilicity and bioactivity on MBHA **1**–**16**. In fact, both **1** (more lipophilic, Log*P* = 3.16, IC_{50} = 28.38 μM) and **9** (less lipophilic, Log*P* = 1.25, IC_{50} = 29.81 μM) adducts are more actives against *L. amazonensis* that the previously described MBHA **17** (Fig. 1, intermediate lipophilicity, log*P* = 2.10, IC_{50} = 62 μM) [31]. However, it is important to highlight that MBHA **9** (the least lipophilic between **1**, **9** and **17**) also present one more hydroxyl group, that increase one H-bond donor and H-bond acceptor into the structure. Of course, this fact surpasses a simple analysis of bioactivity based only on the log*P* values.

This fact could also reinforce our proposal that the *o*-nitrophenyl group is an important pharmacophore of this new class of anti-parasitic compounds [31]. We believe that the proximity between the NO_2 and the β carbonyl OH groups in **1** and **9** may alter its

Table 2
IC_{50} values (μg/mL and μM) in cell lines, CC_{50} values (μg/mL and μM) and selectivity index (SI).[a]

MBHA	IC_{50} (μg/mL)	IC_{50} (μM)	Cytotoxicity CC_{50} (μg/mL)	Cytotoxicity CC_{50} (μM)	Selectivity index[a]
1	7.52	28.38	35.77	134.98	4.75
2	19.14	72.23	30.15	113.77	1.57
3	13.79	52.04	32.20	121.50	2.33
4	42.89	194.07	158.10	715.38	3.68
5	46.59	210.81	84.76	383.53	1.81
6	32.84	148.60	60.17	272.26	1.83
7	15.52	54.48	40.35	149.44	2.59
8	22.26	74.70	37.28	125.10	1.67
9	5.48	20.52	29.81	111.64	5.43
10	17.24	64.57	18.42	68.99	1.06
11	14.16	53.03	12.53	46.93	0.88
12	59.39	266.32	166.92	748.52	2.81
13	72.20	323.77	93.25	418.16	1.29
14	85.11	381.65	102.21	458.34	1.20
15	14.29	52.54	32.10	118.01	2.24
16	17.02	56.73	47.26	157.43	2.77

[a] Selectivity index (SI) defined by the ratio CC_{50}/IC_{50}.

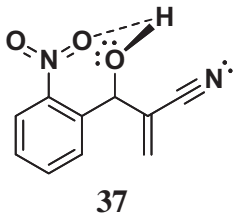


Fig. 3. Seven members IHB (NO_2···HO) in compound 37.

conformations. This proposal is reasonable, based on our previous results [54] where it was characterized by QTAIM theory a seven member Intramolecular Hydrogen Bond (IHB) between the *o*-nitro group in the aromatic ring and the hydroxyl group (N=O···H–O) in MBHA **37** (Fig. 3). It is important to emphasize that this computational study which characterized the IHBs were performed considering *in silico* aqueous environment [55].

Evidently this conformational modification caused by the nitro group change the interaction between the molecule and its biological site of action. It is well established that the redox system TR/Trypanothione is vital for parasite survival within the host cell and a good target for chemotherapy anti-leishmania [56,57]. Also, this conformational effect can also modify the reduction potential of these compounds changing its biological activities [55]. Even if, up to now, the correct biological mechanism of action of these MBHA is not known, it is reasonable to assume that both the conformation induced by the *o*-nitro group and the redox potentials of these compounds are key for their activities. The study of the biological mechanism of action of these MBH adducts will now be investigated.

3. Conclusion

In this article we presented the synthesis, the physical characterization and the biological activities (IC_{50} and CC_{50}) of sixteen new aromatic MBHA **1**–**16**. All new adducts were very active against *L. amazonensis* (Table 2). The similar structures previously described MBHA **17**–**24** [31] were, in most studied cases here, less bioactive against promastigote form of *L. amazonensis* than the new **1**–**16** MBHA. Curiously, both the more lipophilic MBHA **1**–**8** (idealized from change of calculated Log*P*) and the MBHA **9**–**16** (idealized from the classic bioisosterism strategy) are, in most cases presented here, more bioactives than **17**–**24** [31]. A possible conclusion for the lack of a direct relationship between lipid solubility and biological activity in the **1**–**24** MBHA could be the presence of different numbers of H-bond donor and H-bond acceptor in **9**–**16**. The most of the selectivity indexes (SI) were greater than 1. The one-step efficient synthesis and the good selectivity indexes obtained indicates that **1**–**16** can be a new promising class of anti-parasitic compounds. Therefore, we assume that the preparations of each compound were racemates. One stereoisomer could be much more active than the other, as is often the case with biologically active molecules. In the future, we intend for determine *in vivo* the relative activities of the stereoisomers of the most active racemate mixtures [58].

4. Experimental procedures

4.1. Chemistry

4.1.1. General methods

Commercially available reagents were purchased from Aldrich and used without further purification. The compounds Propyl

acrylate **27** synthesized in this work are not new and it was characterized using ^{1}H NMR and ^{13}C NMR by comparison with the compounds described in literature [44,45]. The new MBHA **1**–**16** were characterized using ^{1}H NMR and ^{13}C NMR spectra, obtained by using a Mercury Spectra AC 200 (200 MHz for ^{1}H and 50.3 MHz for ^{13}C) in $CDCl_3$ or Varian Spectra VNMR S-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) using TMS as an internal standard. The Fourier Transform Infrared Spectroscopy (FTIR) spectra were obtained using a spectrophotometer IR-Prestige-21 (Shimadzu). The CG were recorded on an GC:AGILENT-6890/HP-MSD5973 instrument under electron impact (EI) at 70 e.V., injection temperature = 280 °C, injection mode = split, total flow = 45 mL/min, column initial flow = 1.0 mL/min, O.T.P. = rate 20.00 °C/min (40–280 °C). HRMS spectra were obtained on a GCT Premier (TOF-MS), using the column: HP 5-MS. TLC was done by using the flexible plates for TLC silica gel Kieselgel 60 (Whatman) and spots were visualized with short wavelength UV light 254 nm.

*4.1.2. Synthesis of propyl acrylate **27** [44,45]*

The reaction was carried out using 1.0 mmol of acrylic acid **25**, 3.0 mmols of propyl alcohol **26** and 0.1 Equiv. *p*-Toluenesulfonic acid (PTSA) under heating at 60 °C for 1 day. The reaction was allowed to reach room temperature, diluted with CH_2Cl_2 (15 mL) and washed with a cold solution of 10% NaOH (2 × 8 mL) and then with a cold solution of saturated NaCl (1 × 8 mL). The organic phase was dried with $MgSO_4$, filtered and distilled at 70 °C, 98% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 0.96 (t, 4H, *J* = 7.4 Hz); 1.69 (sex, 3H); 4.11 (t, 2H, *J* = 6.6 Hz); 5.81 (dd, 1H, *J* = 10,4/1.8 Hz); 6.12 (dd, 1H, *J* = 17.4/10.4 Hz); 6.40 (dd, 1H, *J* = 17.4/1.8 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 10.39, 21.96, 66.12, 128.59, 130.48, 166.36.

*4.1.3. General synthesis of the MBHA **1**–**16***

The reactions were carried out using 2 mL of solvent (Table 1), 1.0 mmol of corresponding aldehydes, 1.2 mmols of acrylate **27** or 1.0 mmols of acrylate **28** (Table 1) at room temperature until all starting aldehyde was consumed or until the reaction parked, as indicated by TLC analysis using ethyl acetate/hexane (7:3 by volume). The mixtures were evaporated and filtered through silica gel. Purification of the product was done by chromatography column using 15 g of silica gel (230–400 mesh) with ethyl acetate/hexane (3:7 by volume) as eluent.

*4.1.3.1. 2-[Hydroxy(2-nitrophenyl)propyl]propanoate (**1**).* 68% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 0.85 (t, 3H, *J* = 7.2 Hz, CH_3), 1.59 (sex, 2H, CH_2); 3.15 (sl, 1H, OH); 4.05 (t, 2H, *J* = 6.6 Hz, CH_2); 5.73 (s, 1H, CH–OH); 6.16 (s, 1H, CH_2=C); 6.37 (s, 1H, CH_2=C); 7.45 (ddd, 1H, *J* = 7.6/7.2/1.6 Hz, Ar); 7.63 (ddd, 1H, *J* = 7.6/7.2/1.2 Hz, Ar); 7.73 (dd, 1H, *J* = 7.8/1.6Hz, Ar); 7.93 (dd, 1H, *J* = 8.0/1.2 Hz, Ar). ^{13}C NMR ($CDCl_3$, 50 MHz): 9.84, 21.35, 66.29, 67.23, 124.14, 125.88, 128.25, 128.43, 133.06, 135.78, 140.38, 147.91, 165.55; IR (KBr, cm^{-1}): 3452, 1712, 1527, 1352; GC: RT = 14.97 min (100%); HRMS - Mass calculated: 265.0950, Found: 265.0950; $C_{13}H_{15}NO_5$.

*4.1.3.2. 2-[Hydroxy(3-nitrophenyl)propyl]propanoate (**2**).* 73% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 0.89 (t, 3H, *J* = 7.4 Hz, CH_3), 1.64 (sex, 2H, CH_2), 2.89 (sl, 1H, OH), 4.07 (t, 2H, *J* = 6.8 Hz, CH_2), 5.62 (s, 1H, CH–OH), 5.90 (s, 1H, CH_2=C), 6.40 (s, 1H, CH_2=C), 7.51 (t, 1H, *J* = 8.0 Hz, Ar), 7.73 (d, 1H, *J* = 7.8 Hz, Ar), 8.13 (ddd, 1H, *J* = 8.2/2.2/1.0 Hz, Ar), 8.24 (sl, 1H, Ar). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 9.89, 21.37, 66.40, 72.16, 121.12, 122.28, 126.55, 128.90, 132.25, 140.73, 143.30, 147.82, 165.55; IR (KBr, cm^{-1}): 3473, 1710, 1531, 1350; GC: RT = 15.68 min (100%); HRMS - Mass calculated: 265.0950, Found: 265.0950; $C_{13}H_{15}NO_5$.

*4.1.3.3. 2-[Hydroxy(4-nitrophenyl)propyl]propanoate (**3**).* 97% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 0.90 (t, 3H, *J* = 7.2 Hz, CH_3), 1.64 (sex, 2H, CH_2), 2.85 (sl, 1H, OH), 4.08 (t, 2H, *J* = 6.8 Hz, CH_2), 5.62 (s, 1H, CH–OH); 5.86 (s, 1H, CH_2=C), 6.39 (s, 1H, CH_2=C), 7.56 (d, 2H, *J* = 8.4 Hz, Ar), 8.19 (d, 2H, *J* = 8.8 Hz, Ar). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 9.90, 21.38, 66.42, 72.33, 123.162 (2C), 126.61, 126.89 (2C), 140.71, 146.95, 148.30, 165.57; IR (KBr, cm^{-1}): 3481, 1712, 1523, 1350; GC: RT = 15.84 min (100%); HRMS -Mass calculated: 265.0950, Found: 265.0950; $C_{13}H_{15}NO_5$.

*4.1.3.4. 2-[Hydroxy(pyridin-2-yl)propyl]propanoate (**4**).* 70% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 0.84 (t, 3H, *J* = 7.4 Hz, CH_3), 1.58 (sex, 2H, CH_2), 4.03 (t, 2H, *J* = 6.6 Hz, CH_2), 4.18 (sl, 1H, OH), 5.58 (s, 1H, CH–OH), 5.93 (s, 1H, CH_2=C), 6.34 (s, 1H, CH_2=C), 7.17 (t, 1H, *J* = 6.2 Hz, Ar), 7.38 (d, 1H, *J* = 7.8 Hz, Ar), 7.64 (ddd, 1H, *J* = 7.6/7.6/1.4 Hz, Ar), 8.49 (d, 1H, *J* = 4.8 Hz, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz) δ:10.30, 21.76, 66.34, 72.17, 121.17, 122.57, 126.73, 136.95, 141.76, 148.08, 159.49, 166.08; IR (KBr, cm^{-1}): 3151, 1712; GC: RT = 13.32 min (100%); HRMS - Mass calculated: 221.1052, Found: 221.1052; $C_{12}H_{15}NO_3$.

*4.1.3.5. 2-[Hydroxy(pyridin-3-yl)propyl]propanoate (**5**).* 80% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 0.84 (t, 3H, *J* = 7.4 Hz, CH_3), 1.58 (sex, 2H, CH_2), 4.01 (t, 2H, *J* = 6.8 Hz, CH_2), 4.54 (sl, 1H, OH), 5.55 (s, 1H, CH–OH), 5.93 (s, 1H, CH_2=C), 6.36 (s, 1H, CH_2=C), 7.22 (dd, 1H, *J* = 7.8/4.8 Hz, Ar), 7.69 (d, 1H, *J* = 8.2 Hz, Ar), 8.37 (d, 1H, *J* = 4.6 Hz, Ar), 8.46 (sl, 1H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 9.98, 21.45, 66.26, 70.41, 123.11, 125.72, 134.30, 137.22, 141.37, 147.93, 148.21, 165.58; IR (KBr, cm^{-1}): 3191, 1714; GC: RT = 13.76 min (100%); HRMS - Mass calculated: 221.1052, Found: 221.1052; $C_{12}H_{15}NO_3$.

*4.1.3.6. 2-[Hydroxy(pyridin-4-yl)propyl]propanoate (**6**).* 66% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 0.84 (t, 3H, *J* = 7.4 Hz, CH_3), 1.58 (sex, 2H, CH_2), 4.02 (t, 2H, *J* = 6.6 Hz, CH_2), 4.73 (sl, 1H, OH), 5.51 (s, 1H, CH–OH), 5.91 (s, 1H, CH_2=C), 6.35 (s, 1H, CH_2=C), 7.29 (d, 2H, *J* = 6 Hz, Ar), 8.40 (d, 2H, *J* = 5.6 Hz, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 11.74, 23.22, 68.09, 73.09, 123.12 (2C), 128.07, 142.84, 150.57 (2C), 153.04, 167.31; IR (KBr, cm^{-1}): 3.124, 1708; GC: RT = 13.81 min (100%); HRMS - Mass calculated: 221.1052, Found: 221.1052; $C_{12}H_{15}NO_3$.

*4.1.3.7. 2-[Hydroxy(naphth-2-yl)propyl]propanoate (**7**).* 60% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.90 (t, 3H, *J* = 7.5 Hz, CH_3), 1.65 (sex, 2H, CH_2), 3.23 (d, 1H, *J* = 5.5 Hz, OH), 4.09 (t, 2H, *J* = 7.0 Hz, CH_2), 5.75 (d, 1H, *J* = 5.5 Hz, CH–OH), 5.88 (s, 1H, CH_2=C), 6.41 (s, 1H, CH_2=C), 7.49 (m, 3H, Ar), 7.86 (m, 4H, Ar); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 10.59, 22.10, 66.81, 73.70, 124.84, 125.75, 126.26, 126.37 (2C), 127.88, 128.33, 128.41, 133.26, 133.48, 138.93, 142.37, 166.69; IR (KBr, cm^{-1}): 3446, 1714; GC: RT = 16.51 min (100%); HRMS - Mass calculated: 270.1256, Found: 270.1256; $C_{17}H_{18}O_3$.

*4.1.3.8. 2-[Hydroxy(4-bromophenyl)propyl]propanoate (**8**).* 64% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.92 (t, 3H, *J* = 7.5 Hz, CH_3), 1.66 (sex, 2H, CH_2), 3.16 (d, 1H, *J* = 6 Hz, OH), 4.10 (t, 2H, *J* = 7.0 Hz, CH_2), 5.52 (d, 1H, *J* = 5.5 Hz, CH–OH), 5.83 (s, 1H, CH_2=C), 6.36 (s, 1H CH_2=C), 7.27 (d, 2H, *J* = 8.5 Hz, Ar), 7.48 (d, 2H, *J* = 8.5 Hz, Ar); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 10.58, 22.10, 66.89, 73.12, 121.95, 126.43, 128.54 (2C), 131.74 (2C), 140.65, 142.01, 166.48; IR (KBr, cm^{-1}): 3446, 1712; GC: RT = 18.90 min (100%); HRMS - Mass calculated: 298.0204, Found: 298.0204; $C_{13}H_{15}BrO_3$.

*4.1.3.9. 2-[Hydroxy(2-nitrophenyl)hydroxyethyl]propanoate (**9**).* 71% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.48 (sl, 2H, OH),

3.79 (t, 2H, $J = 4.6$ Hz, CH_2), 4.25 (t, 2H, $J = 4.6$ Hz, CH_2), 5.57 (s, 1H, CH–OH), 6.24 (s, 1H, CH_2=C), 6.34 (s, 1H, CH_2=C), 7.45 (ddd, 1H, $J = 8.2/7.3/1.4$ Hz, Ar), 7.64 (ddd, 1H, $J = 7.6/7.4/1.2$ Hz, Ar), 7.79 (d, 1H, $J = 7.8$ Hz, Ar), 7.94 (dd, 1H, $J = 8.0/1.2$ Hz, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 60.65, 66.53, 67.11, 124.58, 126.92, 128.64, 128.85, 133.59, 136.49, 141.38, 147.82, 166.12; IR (KBr, cm^{-1}): 3390, 1714, 1525, 1350. GC: RT = 11.76 min (100%); HRMS - Mass calculated: 267.0743, Found: 267.0743; $C_{12}H_{13}NO_6$.

*4.1.3.10. 2-[Hydroxy(3-nitrophenyl)hydroxyethyl]propanoate (**10**).* 58% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 2.79 (sl, 1H, OH), 3.78 (t, 2H, $J = 4.8$ Hz, CH_2), 4.09 (sl, 1H, OH), 4.22 (t, 2H, 3.0 Hz, CH_2), 5.65 (s, 1H, CH–OH), 5.87 (s, 1H, CH_2=C), 6.42 (s, 1H, CH_2=C), 7.50 (t, 1H, $J = 8.0$ Hz, Ar), 7.70 (d, 1H, 7.8 Hz, Ar), 8.10 (ddd, 1H, $J = 8.2/2.0/1.0$ Hz, Ar), 8.22 (sl, 1H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 60.59, 66.43, 71.94, 121.56, 122.72, 127.73, 129.36, 132.78, 141.30, 143.61, 148.17, 165.97; IR (KBr, cm^{-1}): 3355, 3326, 1708, 1529, 1350; GC: RT = 16.84 min (100%); HRMS - Mass calculated: 267.0743, Found: 267.0743; $C_{12}H_{13}NO_6$.

*4.1.3.11. 2-[Hydroxy(4-nitrophenyl)hydroxyethyl]propanoate (**11**).* 62% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 2.76 (sl, 2H, OH), 3.81 (t, 2H, $J = 4.6$ Hz, CH_2), 4.26 (t, 2H, $J = 4.4$ Hz, CH_2), 5.66 (s, 1H, CH–OH), 5.86 (s,1H, CH_2=C), 6.44 (s, 1H, CH_2=C), 7.57 (d, 2H, $J = 8.6$ Hz, Ar), 8.19 (d, 2H, $J = 8.8$ Hz, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 60.75, 66.51, 72.40, 123.61 (2C), 127.34 (2C), 127.83, 141.09, 147.39, 148.53, 165.99; IR (KBr, cm^{-1}): 3529, 3371, 1699, 1519, 1350; GC: RT = 17.04 min (100%); HRMS - Mass calculated: 267.0743, Found: 249.0637 [M − 18]; $C_{12}H_{11}NO_5$ [M − 18].

*4.1.3.12. 2-[Hydroxy(pyridin-2-yl)hydroxyethyl]propanoate (**12**).* 94% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.62 (t, 2H, $J = 4.4$ Hz, CH_2), 4.09 (m, 2H, CH_2), 5.57 (s, 1H, CH–OH), 5.85 (s, 1H, CH_2=C), 6.29 (s, 1H, CH_2=C), 7.11 (m, 1H, Ar), 7.35 (d, 1H, $J = 8$ HZ, Ar), 7.60 (ddd, 1H, $J = 7.8/7.7/1.6$ Hz, Ar), 8.34 (d, 1H, $J = 5$ Hz, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 59.99, 66.15, 72.08, 121.03, 122.54, 126.86, 137.03, 141.71, 147.71, 159.81, 165.66; IR (KBr, cm^{-1}): 3344, 1716; GC: RT = 14.26 min (100%); HRMS - Mass calculated: 223.0845, Found: 205.0739 [M − 18]; $C_{11}H_{13}NO_4$ [M − 18].

*4.1.3.13. 2-[Hydroxy(pyridin-3-yl)hydroxyethyl]propanoate (**13**).* 83% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.66 (t, 2H, $J = 4.8$ Hz, CH_2), 4.10 (t, 2H, $J = 4.8$ Hz, CH_2), 4.37 (sl, 2H, OH), 5.52 (s, 1H, CH–OH), 5.87 (s, 1H, CH_2=C), 6.31 (s, 1H, CH_2=C), 7.18 (m, 1H, Ar), 7.65 (m, 1H, Ar), 8.27 (m, 1H, Ar), 8.39 (sl, 1H, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 59.97, 66.20, 69.80, 123.36, 126.28, 134.78, 137.69, 141.64, 147.75, 147.91, 165.58; IR (KBr, cm^{-1}): 3336, 1714; GC: RT = 18.81 min (100%); HRMS - Mass calculated: 223.0845, Found: 223.0845; $C_{11}H_{13}NO_4$.

*4.1.3.14. 2-[Hydroxy(pyridin-4-yl)hydroxyethyl]propanoate (**14**).* 80% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.27 (sl, 1H, OH), 3.76 (sl, 1H, OH), 3.85 (t, 2H, $J = 5$ Hz, CH_2), 4.30 (t, 2H, $J = 5$ Hz, CH_2), 5.56 (s, 1H, CH–OH), 5.89 (s, 1H, CH_2=C), 6.46 (s, 1H, CH_2=C), 7.35 (d, 2H, $J = 6.5$ Hz, Ar), 8.61 (d, 2H, $J = 6.0$ Hz, Ar); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 59.98, 66.21, 73.09, 123.11 (2C), 128.06, 142.84, 150.56 (2C), 153.05, 167.31; IR (KBr, cm^{-1}): 3309, 1724; GC: RT = 14.73 min (100%); HRMS - Mass calculated: 223.0845, Found: 224.0921 [M + 1]; $C_{11}H_{13}NO_4$.

*4.1.3.15. 2-[Hydroxy(naphth-2-yl)hydroxyethyl]propanoate (**15**).* 67% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.90 (sl, 1H, OH), 3.20 (sl, 1H, OH), 3.82 (t, 2H, $J = 4.5$ Hz, CH_2), 4.26 (t, 2H, $J = 4.5$ Hz, CH_2), 5.78 (s, 1H, CH–OH), 5.94 (s, 1H, CH2 = C), 6.47 (s, 1H, CH_2=C), 7.51 (m, 3H, Ar), 7.87 (m, 4H, Ar); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 60.98, 66.46, 73.30, 124.54, 125.56, 126.21, 126.34, 126.98, 127.72, 128.10, 128.34, 133.07, 133.25, 138.63, 141.92, 166.41; IR (KBr, cm^{-1}): 3394, 1712; GC: RT = 17.68 min (100%); HRMS - Mass calculated: 272.1049, Found: 272.1049; $C_{16}H_{16}O_4$.

*4.1.3.16. 2-[Hydroxy(4-bromophenyl) hydroxyethyl]propanoate (**16**).* 71% yield; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.79 (sl, 1H, OH), 3.04 (sl, 1H, OH), 3.82 (t, 2H, $J = 4.5$ Hz, CH_2), 4.28 (t, 2H, $J = 4.5$ Hz, CH_2), 5.55 (s, 1H, CH–OH), 5.87 (s, 1H, CH_2=C), 6.42 (s, 1H, CH_2=C), 7.28 (d, 2H, $J = 8.5$ Hz, Ar), 7.48 (d, 2H, $J = 8.5$ Hz, Ar); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 61.22, 66.71, 72.96, 122.11, 127.21, 128.52 (2C), 131.85 (2C), 140.49, 141.77, 166.43; IR (KBr, cm^{-1}): 3390, 1712; GC: RT = 15.91 min (100%); HRMS - Mass calculated: 299.9997, Found: 299.9997; $C_{12}H_{13}BrO_4$.

4.2. Biological evaluation

4.2.1. Antipromastigotes activities

The promastigotes viability was determined by the ability of living cells to reduce the yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) to purple formazan [59]. Promastigote forms of *L. amazonensis* (IFLA/BR/67/PH8) in the log phase of growth were incubated at 25 °C in 96-well cellular culture plates (TPP, Switzerland) with 1×10^5 cells/well in 100 μL Schneider's Drosophila medium supplemented with 20% of FBS in the presence or absence of different concentrations of **1**–**16** MBHA. The growth of promastigote forms was evaluated simultaneously in the presence of Glucantime®, as reference drug. After a 72 h incubation, 10 μL of a 5 mg mL^{-1} MTT solution was added to it. After 4 h of incubation at 25 °C the formed product formazan was dissolved in 50 μL of Sodium Dodecyl Sulfate (SDS) at 10% for 24 h and the absorbance was measured by spectrophotometry at a 545 nm wavelength. The percentage viability was calculated from the ratio of OD readings in wells with compounds versus wells without compounds × 100. The concentration which inhibits 50% of growth (IC_{50}) was determined by regression analysis using the SPSS 8.0 software for Windows. All experiments were done at least three times and each experiment was performed in triplicate [31].

4.2.2. Cytotoxicity in murine macrophages

The animals were maintained at constant room temperature (21 + 2 °C) and on a 12/12 h light–dark cycle, with free access to food pellets and water. Initially, the animals were elicited by peritoneal injection of 1 mL of sterile 4% thioglycollate solution (Gibco) and after five days, the mice were euthanized by cervical dislocation and cells were recovered by peritoneal lavage using 10 ml of cold PBS containing 3% fetal bovine serum (FBS). Cells were immediately stored on ice. The suspension was centrifuged at 2500 rpm for 10 min and the pellet resuspended in 1 ml of RPMI 1640 (Cultilab, Brazil) supplemented with 10% FBS and 50 μg/ml of streptomycin and penicillin 1000 U/ml (RPMI complete). Aliquots of the suspension were used to quantify the number of viable cell using the trypan blue method at a final concentration of 0.4% in order to discriminate between live and dead cells. The quantification was done in Neubauer hemocytometer chamber, and the number of cells in suspension was adjusted to 4×10^6 cells/mL and was then incubated in 100 μL of complete RPMI in 96-well plates, in the absence and presence of different concentrations of the MBHA **1**–**16**. After 24 h of incubation in an incubator at 5% CO_2, was inoculated 10 μL of 3-(4,5-dimethyl-2-thiazole)-2,5-diphenyl-tetrazolium bromide (MTT) at a concentration of 5 mg/mL and, after 4 h, was added to 50 μl of SDS at a concentration of 10 mM. After 24 h we became the reading of the plates in a microplate reader using the absorbance wavelength of 545 nm. We calculated the concentration that caused a 50% reduction in cell viability [CC_{50}] using SPSS version 8.0 for Windows.

Tests of cytotoxicities were performed in murine macrophages as a model eukaryotic cell of great importance in the cycle of the disease. The macrophages were collected from swiss female mices (3 months of age), weighing 28–35 g and this animals were obtained from the vivarium Thomas George of Laboratory of Pharmaceutical Technology, Federal University of Paraíba. The sacrifice of experimental animals followed international recommendations (AVMA, 2007). All the experimental protocols were previously approved by the Animal Research Ethical Committee (CEPA) at the Federal University of Paraíba (process number 208/2007).

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