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Efficient synthesis of 16 aromatic Morita–Baylis–Hillman adducts: Biological evaluation on *Leishmania amazonensis* and *Leishmania chagasi*

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ABSTRACT

Sixteen aromatic Morita–Baylis–Hillman adducts (MBHA) **1**–**16** were efficiently synthesized in a one step Morita–Baylis–Hillman reaction (MBHR) involving commercial aldehydes with methyl acrylate or acrylonitrile (81–100% yields) without the formation of side products on DABCO catalysis and at low temperature (0 °C). The toxicities of these compounds were assessed against promastigote form of *Leishmania amazonensis* and *Leishmania chagasi*. The low synthetic cost of these MBHA, green synthetic protocols, easy one-step synthesis from commercially available and cheap reagents as well as the very good antileishmanial activity obtained for **14** and **16** (IC_{50} values of 6.88 μg mL^{-1} and 11.06 μg mL^{-1} respectively on *L. amazonensis*; 9.58 μg mL^{-1} and 14.34 μg mL^{-1} respectively on *L. chagasi*) indicates that these MBHA can be a novel and promising class of anti-parasitic compounds.

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1. Introduction

Leishmaniasis are a complex of diseases, 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 [1–4]. It causes around 70,000 deaths annually, as well as the occurrence of 2 million new cases, with an estimated 12 million people presently infected worldwide [1]. So far no vaccine approved for human use is available [5]. 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 [6]. However, these drugs are expensive, potentially toxic and require long-term treatment [7].

The scientific evaluation of medicinal plants has provided some alternatives drugs, [8–11] but the small quantities extracted from plants and/or the vast molecular complexity of these structures makes the mass production of these natural drugs impractical both through natural extraction and chemical synthesis. Thus, the discovery and identification of relatively simple drugs that can be prepared in a few steps and in good yields is a challenge that is being achieved by some research groups [12–16].

The Morita–Baylis–Hillman reaction (MBHR) is an important way for C–C bond formation [17]. It involves the coupling of alkenes containing electron-withdrawing groups (EWG) with aldehydes, ketones or imines, among others [18–22]. 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 [23–26]. An inconvenience associated with this reaction are the long reaction times. There are reports of reactions that, last up to 65 days [18]. Due to the synthetic utility of these MBHA, several protocols have been described to improve the reaction time and yields, such as the use of microwaves [27], ultrasound [28], high pressures [29], use of ionic liquids [30], and other protocol variations [18–22].

The bioactivity of some aromatic MBHA was first described in 1999 against *Plasmodium falciparum*, the etiologic agent of malaria [31,32]. In 2006, the molluscicidal activities of ten aromatic MBHA were described effectively against *Biomphalaria glabrata* (Say) snails, the intermediate host of schistosomiasis [33]. Also that year Kohn et al. showed *in vitro* antiproliferative effect of some MBHA derivatives on human tumor cell lines [34]. Kohn et al. also showed that aromatic MBHA derivatives had a better bioactivity when compared to aliphatic ones [34]. Subsequently a high *in vitro* leishmanicidal activity was also described for some MBHA, effective

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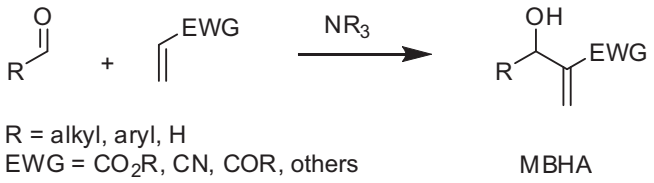


Scheme 1. The Morita–Baylis–Hillman reactions with, e.g. aldehydes.

against the *Leishmania amazonensis* [35] and *Leishmania chagasi* parasites [36]. Recently, we described that the MBRA 3-Hydroxy-2-methylene-3-(4-nitrophenyl propanenitrile) is a new highly active compound against epimastigote and trypomastigote form of *Trypanosoma cruzi*, the parasite that causes Chagas disease [37].

In connection with our continuum interest in organic synthesis of products with biological activity [38–42] our research group has been engaged in the development of several new conditions to produce MBHA in high yields from "one-pot reaction", [43–45] we present here a very efficient synthetic protocol for the preparation of the sixteen aromatic MBHA **1**–**16** (Fig. 1) in very high yields and subsequently *in vitro* antipromastigote biological evaluation on *L. amazonensis* (the primary cause of the tegumentary leishmaniasis) and *L .chagasi*. A relationship between the chemical structure and biological activity (structure–activity-relationship, SAR) is also being proposed in this article based on our previous theoretical conformational calculations [46], and on biological results obtained here.

2. Results and discussion

Due to higher reactivity of acrylonitrile versus methyl acrylate on MBHR, we have started our work with the synthesis of adducts **2**, **4**, **6**, **8**, **10**, **12**, **14** and **16** (Fig. 1). The rate and yields of MBHA preparations is very sensible to solvent structure [47,48]. Recently, we have described that ^{t}BuOH:water (6:4) was a good selective solvent mixture to prepare some MBHA [44]. In Table 1 we present our first results for **2**, **4**, **6**, **8**, **10**, **12**, **14** and **16** preparations using these conditions.

We notice in Table 1 that only the preparation of **2** was remarkably efficient (95%, isolated product, Entry 1). All the other products were prepared in moderate yields and in some cases it was observed one or two co-products formation (Entries 2–7, Table 1). Preparation of **16** was not possible using this methodology (Entry 8, Table 1). Since the preparation of **4** led to two side products

Table 1
Use of ^{t}BuOH:water (6:4) as mixture solvents, 1 Equiv. of DABCO on r.t. as experimental condition to prepare the MBHA **2**, **4**, **6**, **8**, **10**, **12**, **14** and **16**.

Entry	MBHA	Rate (h)	Yield (%)
1	2	1.5	95
2	4	2	70[b]
3	6	5	75[a]
4	8	24	70[a]
5	10	24	55[a]
6	12	24	75
7	14	5	58[b]
8	16	240	>10

[a] One co-product formation.
[b] Two co-products formation.

and 3-nitrobenzaldehyde is a very reactive and cheap reagent, we have decided to find an efficient experimental protocol to optimize the synthesis of **4** and then apply such protocol to synthesize MBHA **1–16**. The results are shown in Scheme 2 and Table 2.

Table 2 (Entry 3) points out a new and efficient methodology, through which MBHA **4** can be quantitatively prepared without solvents and at low temperatures. The use of ultrasound methodology did not improve reaction rate (see Entries 1 versus 2 and Entry 3 versus 4). In fact, use of the ultrasound decreased the yield at low temperatures (Entries 3 versus 4).

There are reports in literature that described increased efficiency of MBHR when the temperature is reduced from r.t. to 0 °C [49]. We can understand these experimental results based on the accepted mechanism proposed for the MBHR [50,51]. The highly organized transitions states involved on the slow steps of the currently accepted MBHR mechanisms, proposed by McQuade et al. [50] and Aggarwal et al. [51], and corroborated by Coelho et al. [52], suggests that the entropy of activation is a very important parameter in this reaction. In fact, the volume of activation of MBHR (-70 cm^3/mol) [53] is one of the highest in absolute values among known reactions [54]. Therefore, it is a reasonable assumption that the reduction of temperature from r.t. to 0 °C makes the entropic term ($-T{\cdot}\Delta S^{\pm} > 0$) less important, reducing Gibbs activation energy, thus improving MBHR rate in comparison with the rate of co-products formation and consequently increasing the selectivity of this reaction. The results for MBHA **1–16** synthesis using this very efficient methodology are shown in Table 3.

This methodology was highly efficient to prepare the all MBHA, except **15** (entry 16, Table 3). The addition of methanol as solvent, however, led to the quantitative preparation of **16** without side

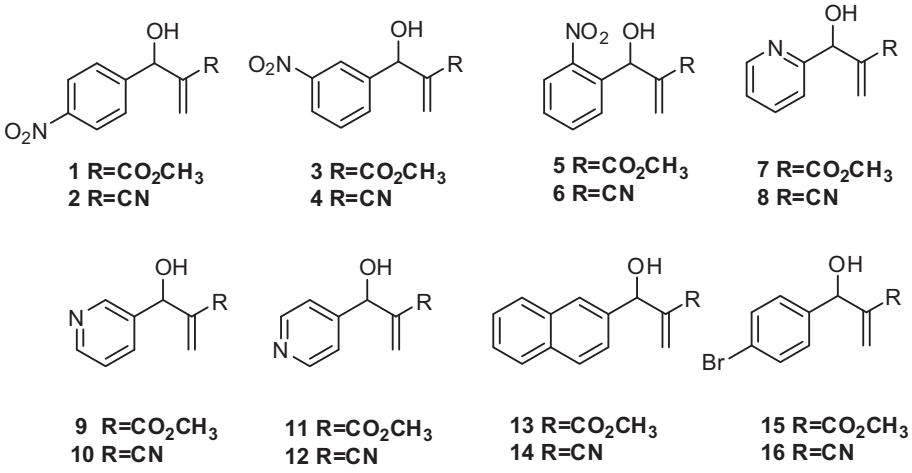
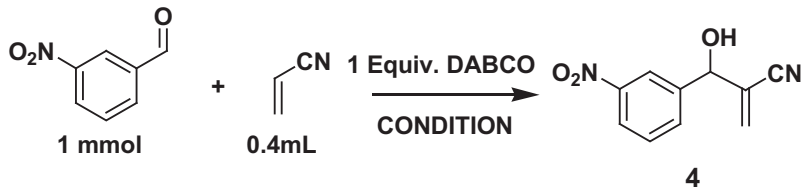


Fig. 1. Synthesized and biologically evaluated **1**–**16** MBHA.



Scheme 2. Experimental protocols in the optimization of the preparation of **4**.

Table 2
Use of low temperature and ultrasound as protocols to optimize the synthesis of **4** MBHA.

Entry	Condition	Rate (min.)	Yield (%)
1	r.t.	20	85[a]
2	Ultrasound, r.t.	70	90[b]
3	0 °C	20	100
4	Ultrasound, 0 °C	40	84[b]

[a] Two co-products formation.
[b] One co-product formation.

Table 3
Optimized protocols for the preparation of **1**–**16** MBHA. All reactions were performed at 0 °C.

Entry	MBHA	Rate	Yield (%)[a]
1	1	3 h	85
2	2	15 min	100
3	3	2 h	98
4	4	25 min	100
5	5	3 h	92
6	6	40 min	100
7	7	5 h	95
8	8	30 min	100
9	9	2 h	98
10	10	50 min	100
11	11	2 h	81
12	12	10 min	100
13	13	16 days	85
14	14	10 h	98
15	15	>20 days	0
16	15	3 days[b]	90
17	16	4 h	98

[a] Isolated products.
[b] Using 0.5 mL methanol/mmol as solvent.

product formation (Entry 17, Table 3). After a simple filtration the products were ready for biological assays which further reiterate this methodology easy handling when increasing the scale of the procedure.

The sixteen adducts evaluated by MTT colorimetric method showed a significant antileishmanial activity with IC_{50} values for *L. amazonensis* and *L. chagasi* ranged from 6.88 μg mL^{-1} to 141.30 μg mL^{-1} and 11.06 μg mL^{-1} to 138.99 μg mL^{-1}, respectively (Table 4). The inhibition of promastigote growth caused by MBHA was larger than that caused by Glucantime, the reference drug (IC_{50} > 8.0 mg/mL). We present in Table 4 the results of the *in vitro* toxicities of **1**–**16** on *L. amazonensis* and *L. chagasi*.

We can see in Table 4, that in general, nitrile-containing adducts (MBHA **2**, **4**, **8**, **10**, **12**, **14** and **16**) are much more active against *L. amazonensis* and *L. chagasi* than the compounds **1**, **3**, **7**, **9**, **11**, **13** and **15**, which contain carboxymethylester moiety in their structure.

In the other paper a six member intramolecular hydrogen bond (IHB) between the hydroxyl and ester carbonyl function (OH···O=C, 2.1 Å) was characterized by a QTAIM study into the 2-[Hydroxy(phenyl)methyl]propanoate MBHA [46], but no IHB involving 2-[Hydroxy(phenyl) methyl] acrylonitrile was observed

Table 4
IC_{50} to **1**–**16** MBHA from promastigote forms of *Leishmania amazonensis and Leishmania chagasi*.

MBHA	*L. amazonensis*		*L. chagasi*	
	μg mL^{-1}	μM	μg mL^{-1}	μM
1	46.53	196.16	27.85	117.41
2	26.19	128.27	18.86	92.37
3	107.12	451.58	64.67	262.63
4	48.65	238.27	23.24	113.82
5	11.88	50.08	19.52	82.29
6	12.66	62.00	16.66	81.59
7	141.30	731.37	138.99	724.59
8	48.50	302.80	37.49	234.06
9	86.65	448.50	67.66	350.57
10	62.75	391.77	36.62	228.88
11	94.47	488.98	95.50	494.81
12	40.15	250.95	32.08	200.50
13	20.23	83.50	23.08	95.37
14	6.88	32.88	11.06	52.92
15	23.19	85.54	33.03	122.33
16	9.58	40.23	14.34	60.50

due to the long distance between the OH and the nitrogen of the nitrile group (OH···NC, more than 4 Å, Fig. 2).

Based on these data we believe that the likely formation of the intramolecular hydrogen bond (IHB) between the hydroxyl and ester carbonyl function (OH···O=C) into **1**, **3**, **5**, **7**, **9**. **11**, **13** and **15** can decrease their interaction with enzyme active site hydrogen bond donor (HBD) and acceptor (HBA) groups. On the other hand, as the nitrogen atom of the nitrile group and the hydroxyl group in **2**, **4**, **6**, **8**, **10**, **12**, **14** and **16** cannot form IHB between each other, these groups efficiently bind with enzyme active site hydrogen bond donor (HBD) and acceptor (HBA) groups, which could explain their higher biological activity (Fig. 2).

Interestingly, we can see in Table 4 that the leishmanicidal activity is almost equivalent between compounds **5** and **6**. We have also characterized by QTAIM study a seven member IHB between the *o*-nitro group in the aromatic ring and hydroxyl (N=O···H–O) in MBHA **6** (Fig. 3) [46]. This IHB can inhibit the efficiency of hydroxyl group as HBD and the nitro group as HBA, approximating the leishmanicidal activities of those two molecules. It is important

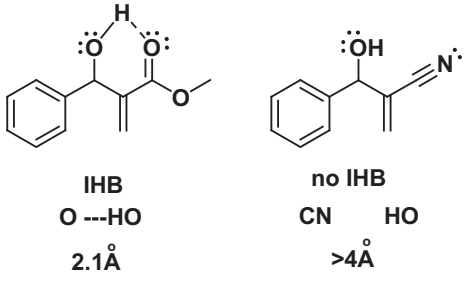
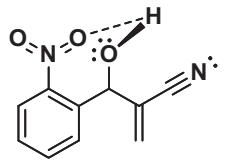


Fig. 2. 2-[hydroxy(phenyl)methyl]propanoate (drawing on the left, with the IHB formation) and 2-[hydroxy (phenyl) methyl]acrylonitrile (drawing on the right, no IHB formation).



6

Fig. 3. Seven members IHB ($NO_2\cdots HO$) in compound **6**.

to emphasize that the computational studies which characterized the IHBs were performed considering implicit aqueous environment. The study of the biological mechanism of action of these adducts will now be investigated.

3. Conclusion

In this article we have presented new protocols to synthesize aromatic MBHA **1**–**16** in one-step synthesis, under conditions suitable for scaling up and with high yields. All adducts were very active against *L. amazonenesis* and *L. chagasi*. The remarkable antileishmanial activity obtained for **14** and **16** (IC_{50} values of 6.88 μg mL^{-1} and 11.06 μg mL^{-1} respectively on *L. amazonensis*; 9.58 μg mL^{-1} and 14.34 μg mL^{-1} respectively on *L. chagasi*) as well as the easy one-step synthesis from cheap and commercially available aldehydes through a green chemistry procedure indicates that these MBHA can be a novel and promising class of anti-parasitic compounds.

4. Experimental

4.1. Chemistry

4.1.1. Materials and methods

Commercially available reagents were purchased from Aldrich and used without further purification. The compounds synthesized in this work are not new. The MBH adducts **1**–**16** were characterized using NMR by comparison with the compounds described in literature. ^1H and ^{13}C NMR spectra were obtained by using a Mercury Spectra AC 200 (200 MHz for ^1H and 50.3 MHz for ^{13}C) in $CDCl_3$ or Varian Spectra V NMR S-500 (100 MHz for ^{13}C). The Fourier Transform Infrared Spectroscopy spectra were obtained using a spectrophotometer IR-Prestige-21 (Shimadzu). 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. General protocols for the MBHA preparations

Reactions were carried out using the corresponding aldehydes (1 mmol), 0.5 mL of acrylonitrile or methyl acrylate and 1 mmol of DABCO at 0 °C for *x* min/h/day (Table 3). To synthesize MBHA **15** we used 0.5 mL/mmol of methanol as solvent (Entry 16, Table 3). After that, the reaction media was directly filtered through silica gel, using AcOEt:hexane (2:8) as solvent and the reaction products were concentrated under reduced pressure. The products were then ready for bioevaluation without the need of further purification.

4.1.2.1. 2-[Hydroxy(4-nitrophenyl)methyl]propanoate ***(1)*** *[41].* ^1H NMR ($CDCl_3$, 200 MHz): d = 8.16 (d, 2H, *J* = 8.8 Hz), 7.54 (d, 2H, *J* = 8.4 Hz), 6.36 (s, 1H), 5.86 (s, 1H), 5.60 (d, *J* = 5.8 Hz, 1H), 3.71 (s, 3H), 3.41 (d, *J* = 5.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 52.2(1C), 72.6(1C), 123.5(1C), 237.2(1C), 127.3(1C), 140.8(1C), 147.3(1C), 148.5(1C), 166.3(1C).

4.1.2.2. 2-[Hydroxy(4-nitrophenyl)methyl]acrylonitrile ***(2)*** *[36].* IR (KBr): 3447, 3115, 2228, 1599, 1520, 1348, 736 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.21(d, *J* = 8.8 Hz, 2H); 7.58 (d, *J* = 9.0 Hz, 2H); 6.07 (d, *J* = 0.8 Hz, 1H); 6.16 (d, *J* = 0.6 Hz, 1H); 5.42 (s, 1H); 3.23 (brs, 1H, CH*OH*). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 73.01; 116.62; 123.92(2C); 126.13; 127.34(2C); 130.51; 146.80; 147.82.

4.1.2.3. 2-[Hydroxy(3-nitrophenyl)methyl]propanoate ***(3)*** *[55].* ^1H NMR ($CDCl_3$, 200 MHz): δ 8.22 (t, *J* = 1.8 Hz, 1H); 8.08–8.13 (m, 1H); 7.69–7.73 (m, 1H); 7.50 (t, *J* = 8 Hz, 1H); 6.38 (s, 1H); 5.89 (s, 1H); 5.60 (d, *J* = 4.4 Hz, 1H); 3.70 (sl, 3H); 3.46 (d, *J* = 5.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 52.2(1C), 72.5(1C), 121.5(1C), 122.7(1C), 127.2(1C), 129.3(1C), 132.7(1C), 140.9(1C), 143.5(1C), 148.2(1C), 166.3(1C).

4.1.2.4. 2-[Hydroxy(3-nitrophenyl)methyl]acrylonitrile ***(4)*** *[36].* IR (KBr): 3345, 3105, 2239, 1583, 1520, 1348 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.24 (dd, *J* = 1.8/1.6 Hz, 1H); 8.18 (ddd, *J* = 8.0/1.0/1.2 Hz, 1H); 7.57 (t, *J* = 8.0 Hz, 1H); 7.75 (ddd, *J* = 7.8/1.6 Hz, 1H); 6.09 (d, *J* = 0.8 Hz, 1H); 6.20 (d, *J* = 1.6 Hz, 1H); 5.43 (s, 1H); 3.02 (brs, 1H, CHOH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 72.66(1C), 116.31(1C), 121.14(1C), 123.35(1C), 125.01(1C), 129.73(1C), 131.52(1C), 132.53(1C), 141.21(1C), 147.98(1C).

4.1.2.5. 2-[Hydroxy(2-nitrophenyl)methyl]propanoate ***(5)*** *[41].* ^1H NMR ($CDCl_3$, 200 MHz): δ 7.92(dd, *J* = 8.2/1.2 Hz, 1H); 7.73 (dd, *J* = 7.8/1.8 Hz, 1H); 7.62 (ddd, *J* = 7.8/1.2/1.0 Hz, 1H); 7.43 (ddd, *J* = 8.0/1.6/1.6 Hz, 1H); 6.38 (s, 1H); 5.89 (d, 1H); 5.60(d, *J* = 4.4 Hz, 1 Hz); 3.70 (s, 3H); 3.60 (d, *J* = 5.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.20(1C), 67.56(1C), 124.55(1C), 126.48(1C), 128.67(2C), 133.47(1C), 136.05(1C), 140.65(1C), 148.32(1C), 166.39(1C).

4.1.2.6. 2-[Hydroxy(2-nitrophenyl)methyl]acrylonitrile ***(6)*** *[36].* IR (KBr): 3345, 2228, 1348, 1609, 1520 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.01(dd, *J* = 8.0/1.4 Hz, 1H); 7.84 (dd, *J* = 6.0/1.8 Hz, 1H); 7.72 (ddd, *J* = 8.0/1.8/1.4 Hz, 1H); 7.52 (ddd, *J* = 8.0/1.6/1.4 Hz, 1H); 6.12 (d, *J* = 1.4 Hz, 1H); 6.09 (d, *J* = 1.2 Hz, 1H); 5.98 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 69.13(1C), 116.51(1C), 124.30(1C), 125.07(1C), 129.11(1C), 129.71(1C), 132.03(1C), 134.16(1C), 134.30(1C), 147.93(1C).

4.1.2.7. 2-[Hydroxy(pyridin-2-yl)methyl]propanoate ***(7)*** *[41].* ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 8.51 (d, *J* = 4.4 Hz, 1H); 7.66 (ddd, *J* = 7.8/1.6 Hz, 1H); 7.4 (d, *J* = 8.0 Hz, 1H); 7.21 (m, 1H); 6.34(sl, 1H); 5.96 (sl, 1H); 5.61(sl, 1H); 3.71 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 51.8(1C), 72.0(1C), 121.2(1C), 122.6(1C), 126.8(1C), 136.8(1C), 141.5(1C), 148.1(1C), 159.4(1C), 166.4(1C).

4.1.2.8. 2-[Hydroxy(pyridin-2-yl)methyl]acrylonitrile ***(8)*** *[36].* IR (Film) 3200, 2225, 1600 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.56 (ddd, *J* = 8.0/1.4 Hz, 1H); 7.75 (ddd, *J* = 7.8/7.6/1.6 Hz, 2H); 7.37 (d, *J* = 7.8 Hz, 1H); 7.29 (ddd, *J* = 0.8/1.0/1.2 Hz, 1H); 5.28 (s, 1H); 6.21(s, 1H); 6.05(s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 72.89(1C), 116.69(1C), 121.19(1C), 123.67(1C), 125.82(1C), 130.86(1C), 137.43, 148.47(1C), 156.09(1C).

4.1.2.9. 2-[Hydroxy(pyridin-3-yl)methyl]propanoate ***(9)*** *[41].* ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm):8.48(d, *J* = 2.0 Hz, 1H); 8.39(dd, *J* = 5.0/1.5 Hz, 1H); 7.74 (ddd, *J* = 8.0/2.0/2.0 Hz, 1H); 7.28(m, 1H); 6.40(d, *J* = 0.6, 1H); 6.01(sl, 1H); 5,60(sl, 1H); 3,71(s, 3H).^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 52.0(1C), 70.5(1C), 123.4(1C), 126.1(1C), 134.7(1C), 137.6(1C), 141.6(1C), 148.2(1C), 148.4(1C), 166.2(1C).

4.1.2.10. 2-[Hydroxy(pyridin-3-yl)methyl]acrylonitrile ***(10)*** *[36].* ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.41 (m, 2H); 7.79 (ddd, J = 7.8/1.8/1.6 Hz, 1H); 7.33 (dd, J = 8.0 Hz, 1H); 6.05 (d, J = 1.0 Hz, 1H); 6.17 (d, J = 1.2, 1H); 5.33 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 71.51(1C), 116.69(1C), 124.09(1C), 125.99(1C), 130.39(1C), 135.04(1C), 136.13(1C), 147.40(1C), 148.94(1C).

4.1.2.11. 2-[Hydroxy(pyridin-4-yl)methyl]propanoate ***(11)*** *[56].* ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 8.47 (dd, J = 4.6/1.6 Hz, 2H); 7.30 (dd, J = 4.6/1.6 Hz, 2H); 5.89 (s, 1H); 5.51 (s, 1H); 3.70 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 52.09(1C), 71.86(1C), 121.49(1C), 127.07(1C), 140.92(1C), 149.32(1C), 151.02(1C), 166.29(1C).

4.1.2.12. 2-[Hydroxy(pyridin-4-yl)methyl]acrylonitrile ***(12)*** *[36].* ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.50 (d, J = 6 Hz, 2H); 7.36 (d, J = 5.8 Hz, 2H); 5.31(s, 1H); 6.16 (d, J = 0.6 Hz, 1H); 6.06 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 72.48(1C), 116.50(1C), 121.42(2C), 125.67(1C), 130.63(1C), 149.14(1C), 149.58(2C).

4.1.2.13. 2-[Hydroxy(naphth-2-yl)methyl]propanoate ***(13)*** *[41,57].* ^{1}H NMR (200 MHz, $CDCl_3$) δ = 7.80 (m, 4H), 7.45 (m, 3H), 6.36 (s, 1H), 5.87 (s, 1H), 5.71 (s, 1H), 3.69 (s, 3H), 3.26 (bs, 1H); ^{13}C NMR (50.0 MHz, $CDCl_3$) δ = 166.5 (C), 141.6 (C), 138.3 (C), 132.9 (C), 132.73 (C), 127.97 (CH), 127.90 (CH), 127.4 (CH), 126.1 (CH_2), 125.9 (CH), 125.8 (CH), 125.3 (CH), 124.3 (CH), 73.0 (CH), 51.7 (CH_3).

4.1.2.14. 2-[Hydroxy(naphth-2-yl)methyl]acrylonitrile ***(14)*** *[41].* ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.42–7.89 (m, 7H). 6.15 (d, J = 1.4 Hz, 2H); 6.06 (d, J = 1.6 Hz, 2H); 5.46(d, J = 3.8 Hz, 1H); 2.50(d, J = 3.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 137.8 (C), 134.9 (C), 134.5 (C), 131.6 (CH_2), 130.4 (CH), 129.6 (CH), 129.18 (CH), 129.16 (CH), 128.0 (CH), 127.5 (CH), 127.4 (CH), 125.2 (CH), 118.4 (C), 75.9 (CH).

4.1.2.15. 2-[Hydroxy(4-bromophenyl)methyl]propanoate ***(15)*** *[55].* ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.38 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.4 Hz, 2H), 6.26 (s, 1H), 5.78 (s, 1H), 5.41 (s, 1H), 3.63 (s, 3H), 3.39 (bs, 1H); ^{13}C NMR (101.0 MHz, $CDCl_3$) δ = 166.4 (C), 141.5 (C), 140.3 (C), 131.3 (CH), 128.3 (CH), 126.1 (CH_2), 121.6 (C), 72.3 (CH), 51.9 (CH_3).

4.1.2.16. 2-[Hydroxy(4-bromophenyl)methyl]acrylonitrile (16) [36]. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.54 (dd, J = 6.0/2.0 Hz, 2H); 7.28 (dd, J = 6.0/1.8 Hz, 2H); 6.11 (d, J = 1.6 Hz, 1H); 6.04 (d, J = = 1.0 Hz, 1H); 5.27 (s, 1H); 2.71(s, CHOH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 73.29(1C); 116.66(1C); 122.74(1C); 125.77(1C); 128.10(2C); 130.34(1C); 131.89(2C); 138.12(1C).

4.2. Biology

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 [58] Promastigote forms of *L. amazonensis* (IFLA/BR/67/PH8) and *L. chagasi* (MCAN/BR/99/JP15) 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 SDS at 10% for 16 h and the absorbance was measured by spectrophotometry at a 570 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.

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References

[1] J. Guillon, I. Forfar, M. Mamani-Matsuda, V. Desplat, M. Saliège, D. Thiolat, S. Massip, A. Tabourier, J.-M. Léger, B. Dufaure, G. Haumont, C. Jarry, D. Mossalayi, Bioorg. Med. Chem. 15 (2007) 194.
[2] B.L. Herwaldt, Lancet 354 (1999) 1191.
[3] S.L. Croft, G.H. Coombs, Trends Parasitol. 19 (2003) 502.
[4] P. Desjeux, Comp. Immunol. Microbiol. Infect. Diseases 27 (2004) 305.
[5] R.N. Coler, S.G. Reed, Trends Parasitol. 21 (2005) 244.
[6] J. Mishra, A. Saxena, S. Singh, Curr. Med. Chem. 14 (2007) 1153.
[7] S. Raht, A. Trivellin, T.R. Imbrunito, D.M. Tomazela, M.N. Jesus, P.C. Marzal, H.F.A. Junior, A.G. Tempone, Quim. Nova 26 (2003) 550.
[8] M. Chen, S.B. Christensen, T.G. Theander, A. Kharazmi, Antimicrob. Agents Chemother. 38 (1994) 1339.
[9] E.C. Torres-Santos, D.L. Moreira, M.A.C. Kaplan, M.N. Meirelles, B. Rossi-Bergmann, Antimicrob. Agents Chemother. 43 (1999) 1234.
[10] M.F. Muzitano, E.A. Cruz, A.P. Almeida, S.A.G. Da-Silva, C.R. Kaiser, C. Guette, B. Rossi-Bergmann, S.S. Costa, Planta Med. 72 (2006) 81.
[11] S. Martínez-Luis, G. Della-Togna, P.D. Coley, T.A. Kursar, W.H. Gerwick, L.J. Cubilla-Rios, Nat. Prod. 71 (2008) 2011.
[12] M. Aveniente, E.F. Pinto, L.S. Santos, B. Rossi-Bergmann, L.E.S. Barata, Bioorg. Med. Chem. 15 (2007) 7337.
[13] K.S. Charret, R.F. Rodrigues, A.M.R. Bernardino, A.O. Gomes, A.V. Carvalho, M.M. Canto-Cavalheiro, L. Leon, V.F. Amaral, Am. J. Trop. Med. Hyg. 80 (2009) 568.
[14] J.C. Aponte, D. Castillo, Y. Estevez, G. Gonzalez, J. Arevalo, G.B. Hammonda, M. Sauvain, Biorg. Med. Chem. Lett. 20 (2010) 100.
[15] P.F. Torrence, X. Fan, X. Zhanga, P.M. Loiseau, Biorg. Med. Chem. Lett. 16 (2006) 5047.
[16] S.B. Ferreira, M.S. Costa, N. Boechat, R.J.S. Bezerra, M.S. Genestra, M.M. Canto-Cavalheiro, W.B. Kover, V.F. Ferreira, Eur. J. Med. Chem. 4 (2007) 21388.
[17] A.B. Baylis, M.E.D. Hillman, Chem. Abstr. 77 (1972) 34174q.
[18] D. Basavaiah, A.J. Rao, T. Satyanarayana, Chem. Rev. 103 (2003) 811.
[19] V. Singh, S. Batra, Tetrahedron 64 (2008) 4511.
[20] A. Dondoni, A. Massi, Angew. Chim., Int. Ed. 47 (2008) 4638.
[21] S.E. Denmark, G.L. Beutner, Angew. Chem., Int. Ed. 47 (2008) 1560.
[22] D. Basavaiah, A.J. Rao, R. Reddy, J. Chem. Soc. Rev. 36 (2007) 1581.
[23] D. Basavaiah, R.S. Hyma, N. Kumaragurubaran, Tetrahedron 56 (2000) 5905.
[24] F. Coelho, W.P. Almeida, Quim. Nova 23 (2000) 98.
[25] P.T. Kaye, M.A. Musa, Synth. Commun. 33 (2003) 1755.
[26] A. Patra, S. Batra, A.P. Bhaduri, A. Khanna, R. Chander, M. Dikshit, Biosci. Med. Chem. 11 (2003) 2269.
[27] K. Kundu, S.B. Mukherjee, N. Balu, R. Padmakumar, S.V. Bhat, Synlett (1994) 444.
[28] F. Coelho, W.P. Almeida, D. Veronese, C.R. Mateus, L.E.C. Silva, R.C. Rossi, G.P. Silveira, C.H. Pavam, Tetrahedron 58 (2002) 7437.
[29] J.S. Hill, N.S. Isaacs, J. Chem. Res. Synop. (1988) 330.
[30] R.O.M.A. De Souza, P.H. Fregadoli, K.M. Gonçalves, L.C. Sequeira, V.L.P. Pereira, L.C. Filho, P.M. Esteves, M.L.A.A. Vasconcellos, O.A.C. Antunes, Lett. Org. Chem. 3 (2006) 186.
[31] M.K. Kundu, N. Sundar, S.K. Kumar, S.V. Bhat, S. Biswas, N. Valecha, Bior. Med. Chem. Lett. 9 (1999) 731.
[32] P. Narender, U. Srinivas, B. Gangadasu, S. Biswas, R.V. Jayathirtha, Bioor. Med. Chem. Lett. 15 (2005) 5378.
[33] M.L.A.A. Vasconcellos, T.M.S. Silva, C.A. Camara, R.M. Martins, K.M. Lacerda, H.M. Lopes, V.L.P. Pereira, R.O.M.A. de Souza, L.T.C. Crespo, Pest. Manag. Sci 62 (2006) 288.
[34] L.K. Kohn, C.H. Pavam, D. Veronense, F. Coelho, J.E. De Carvalho, W.P. Almeida, Eur. J. Med. Chem. 41 (2006) 738.
[35] R.O.M.A. de Souza, V.L.P. Pereira, M.F. Muzitano, B. Rossi-Bergmann, E.B.A. Filho, M.L.A.A. Vasconcellos, Eur. J. Med. Chem. 42 (2007) 99.
[36] T.C. Barbosa, C.G. Lima Junior, F.P.L. Silva, H.M. Lopes, L.R.F. Figueiredo, S.C.O. Souza, G.N. Batista, T.G. Silva, T.M. Silva, M.R. Oliveira, M.L.A.A. Vasconcellos, Eur. J. Med. Chem. 44 (2009) 1726.
[37] J.M. Sandes, A.R. Borges, C.G.L. Junior, F.P.L. Silva, G.A.U. Carvalho, G.B. Rocha, M.L.A.A. Vasconcellos, R.C.B.Q. Figueiredo, Bioorg. Chem. 38 (2010) 190.
[38] L.S.M. Miranda, M.L.A.A. Vasconcellos, Synthesis (2004) 1767.
[39] L.S.M. Miranda, B.G. Marinho, S.G. Leitão, E.M. Matheus, P.D. Fernandes, M.L.A.A. Vasconcellos, Bioorg. Med. Chem. Lett 14 (2004) 1573.
[40] L.S.M. Miranda, B.A. Meireles, J.S. Costa, V.L.P. Pereira, M.L.A.A. Vasconcellos, Synlett (2005) 869.

[41] R.O.M.A. de Souza, B.A. Meireles, L.S. Sequeira, M.L.A.A. Vasconcellos, Synthesis (2004) 1595.
[42] U.F.L. Filho, S. Pinheiro, M.L.A.A. Vasconcellos, P.R.R. Costa, Tetrahedron Asymmetry 5 (1994) 1219.
[43] R.O.M.A. de Souza, A.L.F. Souza, T.L. Fernandez, A.C. Silva, V.L.P. Pereira, P.M. Esteves, M.L.A.A. Vasconcellos, O.A.C. Antunes, Lett. Org. Chem. 5 (2008) 379.
[44] R.O.M.A. de Souza, V.L.P. Pereira, P.M. Esteves, M.L.A.A. Vasconcellos, Tetrahedron Lett 49 (2008) 5902.
[45] R.O.M.A. de Souza, M.L.A.A. Vasconcellos, Catal. Commun. 5 (2004) 21.
[46] E.B.A. Filho, E. Ventura, S.A. do Monte, B.G. Oliveira, C.G. Lima Junior, G. B Rocha, M.L.A.A. Vasconcellos, Chem. Phys. Lett. 449 (2007) 336.
[47] V.K. Aggarwal, S.Y. Fulford, G.C. Lloyd-Jones, Angew. Chem., Int. Ed. 44 (2005) 1706.
[48] J. Cai, Z. Zhou, G. Zhao, C. Tang, Org. Lett. 26 (2002) 4723.
[49] S. Rafel, J.W. Leahy, J. Org. Chem. 62 (1997) 1521.
[50] D.T. Mcquade, K.E. Price, S.J. Broadwater, B.J. Walker, J. Org. Chem. 70 (2005) 3980.
[51] R. Robiette, V.K. Aggarwal, J.N. Harvey, J. Am. Chem. Soc. 129 (2007) 15513.
[52] G.W. Amarante, H.M.S. Milagre, B.G. Vaz, B.R.F. Vilachã, M.N. Ebelin, F. Coelho, J. Org. Chem. 74 (2009) 3031.
[53] J.S. Hill, N.S. Isaacs, Tetrahedron Lett. 27 (1986) 5007.
[54] K.R. Brower, J. Am. Chem. Soc. 85 (1963) 1401.
[55] M. Shi, Li. C-Q, J.-K. Jiang, Tetrahedron 59 (2003) 1181.
[56] V.K. Aggarwal, I. Emme, S.Y. Fulford, J. Org. Chem. 68 (2003) 692.
[57] R.O.M.A. de Souza, M.L.A.A. Vasconcellos, Synth. Commun. 33 (2003) 1383.
[58] T. Mosmann, J. Immunol. Methods 16 (1983) 55.