Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 99–102
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

High selective leishmanicidal activity of 3-hydroxy-2-methylene-3-(4-bromophenyl)propanenitrile and analogous compounds

R.O.M.A. de Souza [a], V.L.P. Pereira [a], M.F. Muzitano [b], C.A.B. Falcão [b], B. Rossi-Bergmann [b,*], E.B.A. Filho [c], M.L.A.A. Vasconcellos [c,*]

[a] *Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, Bloco H, CCS, Ilha do Fundão, Rio de Janeiro, RJ 21941-590, Brazil*
[b] *Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil*
[c] *Departamento de Química, Universidade Federal da Paraíba, Campus I, João Pessoa, PB 58059-900, Brazil*

Received 18 January 2006; received in revised form 6 July 2006; accepted 21 July 2006
Available online 28 September 2006

Abstract

Sixteen not new aromatic compounds were prepared by one-pot reaction i.e. through Baylis–Hillman reaction and were the first time evaluated against promastigote *Leishmania amazonensis* and infected mammalian cells. Most of the compounds were selectively more active against amastigotes than the reference drug sodium stibogluconate (Pentostam, $IC_{50} = 44.7$ μM). We found that 3-hydroxy-2-methylene-3-(4-bromophenyl)propanenitrile (**13**) was the most active ($IC_{50} = 12.5$ μM) and safer compound (0.0 (0.9); % macrophage LDH release), being the lead compound.

Keywords: Leishmanicide; *Leishmania amazonensis*; Baylis–Hillman adducts

1. Introduction

The leishmaniases are a complex of diseases caused by different species of the protozoan parasite *Leishmania* and a major public health problem in many developing countries where 350 million people live at risk of infection [1]. The parasite exists in two forms: the flagellate promastigote in the female phlebotomine sandfly vector, and the amastigote in the mammalian host. Amastigotes are obligate intracellular parasites of macrophages (and are rarely of other cell types), where they survive and multiply within the phagolysosome compartment. The disease has traditionally been classified in three different clinical forms, visceral (VL), cutaneous (CL) and mucocutaneous leishmaniasis (MCL), which have different immunopathologies and degrees of morbidity and mortality [2]. There is so far no vaccine approved for clinical use.

Toxicity and resistance to the pentavalent antimonials, which have been the mainstay of treatment of both visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) during the last 60 years are critical problems [3]. Although new drugs have become available in recent years, including lipid formulations of amphotericin B, the oral drug miltefosine for VL, and topical paromomycin for CL, these are not entirely satisfactory due to high cost, reported side effects or ineffectiveness [4,5]. Thus, the currently available chemotherapy is far from satisfactory, urging the search for new safe, affordable and effective drugs.

The general Baylis–Hillman reaction [6] affords adducts such as **1** (Fig. 1) from simple starting materials in a one-pot reaction. All the atoms from the substrate are present in the product (total atoms economy). This reaction was first reported in 1972 [7], and involves the coupling of alkenes containing electron withdrawing groups (EWG) with aldehydes, ketones or imines. Tertiary amines are normally employed as

* Corresponding authors. Tel.: +55 83 32167413; fax: +55 83 32167437 (M.L.A.A.V.).
E-mail addresses: bartira@biof.ufrj.br (B. Rossi-Bergmann), mlaav@quimica.ufpb.br (M.L.A.A. Vasconcellos).

0223-5234/$ - see front matter © 2006 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.07.013

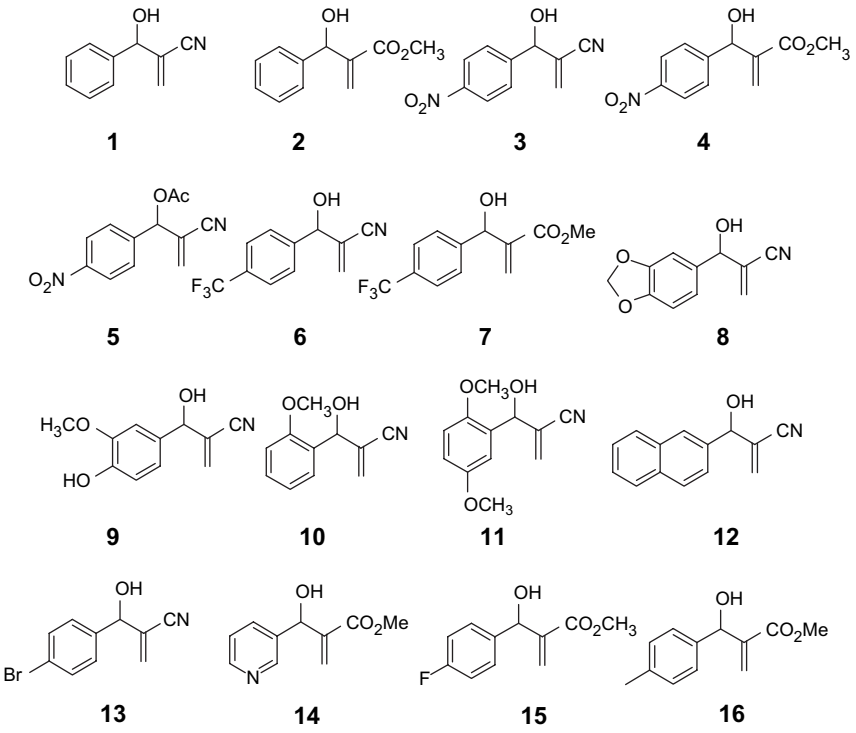
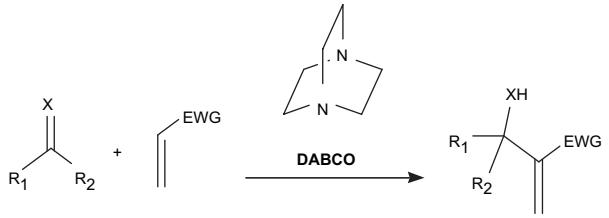


Fig. 1. The synthesized MBH adducts **1**–**16**.

catalyst and 1,4-diazabicyclo[2.2.2]octane (DABCO) is the usual choice (Scheme 1). These adducts have been extensively used as intermediates in organic synthesis for a variety of applications [6].

In our continuing search for bioactive substances [8,9] and in connection with our efforts towards the study of reactivity of Baylis–Hillman reaction (BHR) [10,11], we initiated an investigation on the bioactivities of the aromatic Baylis–Hillman adducts against parasites and vectors transmitting tropical diseases. First, we described an efficient synthetic protocol for the preparation of aromatic adducts that were shown to be active against malarial parasites [12,13]. Then we presented a new class of molluscicidals against the snail *Biomphalaria glabrata* that transmits schistosomiasis [14].

The purpose of this work is to evaluate the selective activity of the aromatic Baylis–Hillman adducts presented in Fig. 1 against *Leishmania amazonensis*, the main causal agent of diffuse CL, a rare clinical form very refractory to current chemotherapy. We also present here that 3-hydroxy-2-methylene-3-(4-bromophenyl)propanenitrile [15] (**13**) is a very active and promising new lead compound against *Leishmania*.



Scheme 1. The Baylis–Hillman reaction: R_1 = alkyl, aryl, heteroaryl; R2 = H, CO_2R, alkyl, X = O, NTs, NSO_2Ph; EWG = electron withdrawing group: COR, CHO, CN, CO_2R, others.

2. Chemistry

It is very important to point out that although all the compounds presented in Fig. 1 are not new, they can be efficiently synthesized in one step through the Baylis–Hillman reaction between the commercial precursor aldehydes and acrylonitrile or methylacrylate, in presence of 1,4-diazabicicle [2.2.2]octane (DABCO) as catalyst and DMSO as solvents. This protocol is easy to scale up. Some compounds were prepared as described previously by us or others [12,16–18]. The acetate derivative **5** was prepared through a classical acetylation of the precursor alcohol **3** [12] with acetic anhydride.

3. Experimental protocols

3.1. Preparation of the adducts

The reactions were carried out using dimethyl sulfoxide (DMSO, 1 ml), aldehyde (0.6 mmol) and acrylonitrile (0.6 mmol) at room temperature until all starting aldehyde was consumed, as indicated by TLC analysis using ethyl acetate/hexane (2:8 by volume). The mixtures were evaporated and filtered through silica gel. Purification of the products was done by silica gel column chromatography using a 30 cm × 1.5 cm column and 15 g of silica gel (230–400 mesh) with ethyl acetate/hexane (1:9 by volume) as eluent (50–95% purified yield). The adducts were characterized from ^{1}H NMR comparison of the compounds described in literature.

3.2. Instrumentation and measurements

The antileishmanial activity of the synthesized adducts was fluorimetrically determined *in vitro* against both promastigote and amastigote forms of *L. amazonensis* (Josefa strain) transfected with the green fluorescence protein (GFP), as previously described [19]. Briefly, promastigotes were plated in triplicates at 10^5 parasites/well with 50 μM of drugs in medium DMEM containing 5% serum and 1% hybri-max DMSO. After 72 h at 27 °C, the fluorescence intensity was measured using a plate-reader fluorometer [20]. For antiamastigote activity, 10^6 mouse peritoneal macrophages were infected with 5×10^6 fluorescent promastigotes for 4 h at 37 °C, washed and cultured for a further 72 h with the test compounds in 1% DMSO. A relatively long incubation period was used to allow for various cycles of parasite replication. Controls were 1% DMSO alone or Pentostam (Welcome). The fluorescence intensity of the cell monolayers was measured as indicative of parasite growth [21]. Maximum and minimum inhibitory activities were fluorescence units of uninfected macrophages and infected cells without drugs, respectively. The IC_{50} values were calculated by linear regression analysis.

Nitric oxide (NO) production was determined in the supernatants of macrophages cultures that were incubated for 48 h in the presence of 50 μM of the drugs. Interferon-gamma (IFN-γ, 10 U/ml, BD Biosciences) was used as a positive control. The NO by-product nitrite was measured using a modification of the Griess method [22].

For cytotoxicity, drugs were tested at 50 μM on uninfected adherent mouse peritoneal macrophages for 48 h at 37 °C. The release of the cytoplasmic enzyme lactate dehydrogenase (LDH) into the culture medium was measured using an assay kit (Doles Reagentes, Brazil). Maximum and minimum release values were in cells cultured with 2% Triton X-100 or 1% DMSO, respectively.

4. Results and discussion

The antileishmanial activity (promastigote and amastigote) and the cytotoxicity results obtained in the compounds **1**–**16** are presented in Table 1.

In a general evaluation of the biological activities, the Baylis–Hillman compounds **1**, **3**, **4**, **6**, **7**, **9**, **11**, **12** and **13** were more active against intracellular amastigotes than against free promastigotes, suggesting drug metabolization by macrophages, as occurs with the pentavalent antimonial Pentostam [20]. The higher activity is unlikely due to activation of nitric oxide production by macrophages, a main leishmanicidal mechanism of these cells, as none of the adducts induced significant nitrite production in the culture medium, and in fact some even inhibited the spontaneous NO production (negative values, Table 2).

Compounds **1**, **3**, **7**, **12** and **13** were either very active (**3**, **7**, **12** and **13**) and/or very safe to macrophages (**1** and **13**), so their antiamastigote IC_{50} values were determined. The antiamastigote activity of those compounds were superior to Pentostam ($IC_{50} = 44.7$ μM), especially compounds **3**, **12** and **13** that displayed IC_{50} of 7.9 μM, 11.2 μM and 12.5 μM, respectively (Table 3), whereas the most selectively active compound was **13**. The steep antiamastigote dose–response curve of compound **7** (see activities in Tables 1 and 3) may be due to its concomitant toxicity to macrophages, shared by the other trifluoromethyl compound **6**.

The preliminary analysis of the structure activity relationship (SAR) of the aromatic **1**–**16** compounds (Fig. 1) revealed that the presence of a high electron-attractor group such as NO_2 in the aromatic moiety significantly increased the antiamastigote activity but also rendered them more toxic to macrophages (**1** and **2** versus **3** and **4**, Table 1). The OH group acetylation in **3**

Table 1
Antileishmanial activity and cytotoxicity against macrophages of compounds **1**–**16** and reference drug Pentostam (50 μM)

Compounds	% Amastigote inhibition	% Promastigote inhibition	% Macrophage LDH release
1	48.1 (±2.9)	42.2 (±0.9)	0.0 (±3.7)
2	14.4 (±5.5)	37.6 (±3.4)	0.0 (±0.9)
3	93.9 (±7.1)	47.0 (±7.6)	41.7 (±0.0)
4	80.6 (±1.4)	67.3 (±4.8)	41.6 (±3.4)
5	21.5 (±8.2)	59.3 (±0.6)	0.0 (±5.6)
6	86.0 (±0.9)	62.8 (±1.2)	36.3 (±3.5)
7	89.0 (±0.0)	67.6 (±5.8)	36.2 (±4.3)
8	9.1 (±3.2)	25.5 (±0.5)	0.0 (±7.8)
9	19.7 (±0.9)	6.6 (±1.8)	0.0 (±2.1)
10	14.7 (±3.6)	30.0 (±7.1)	6.1 (±1.8)
11	35.3 (±2.9)	22.3 (±9.9)	0.0 (±0.4)
12	95.6 (±8.3)	24.0 (±8.5)	26.6 (±0.0)
13	84.3 (±0.1)	17.5 (±3.9)	0.0 (0.9)
14	8.9 (±0.69)	39.6 (±2.3)	13.2 (±4.7)
15	33.6 (±2.2)	32.4 (±0.9)	0.0 (±2.5)
16	28.7 (±6.6)	32.4 (±4.0)	0.0 (±1.8)
Pentostam	35.3 (±2.7)	0.0 (±0.0)	13.2 (±0.5)

Values are means of triplicate samples; standard deviations are given in parentheses.

Table 2
Production of nitric oxide by macrophages

Compounds	Nitrite (μM)
1	−1.6 (±0.5)
2	−2.1 (±0.9)
3	5.0 (±0.7)
4	−3.7 (±0.4)
5	0.0 (±0.0)
6	−4.0 (±0.4)
7	−3.5 (±0.1)
8	−2.1 (±0.7)
9	−2.3 (±1.3)
10	0.4 (±0.1)
11	−4.9 (±0.2)
12	−7.1 (±0.5)
13	2.5 (±0.1)
14	0.1 (±0.9)
15	−4.8 (±0.4)
16	−3.7 (±0.8)
IFN-γ	29.3 (±1.3)
Pentostam	0.0 (±0.3)

Values are means of triplicate samples; standard deviations are given in parentheses.

Table 3
Antiamastigote IC_{50} of selected compounds

Compounds	IC_{50} (μM)
1	49.3 (±1.2)
3	7.9 (±0.5)
7	42.8 (±1.2)
12	11.2 (±0.9)
13	12.5 (±1.0)
Pentostam	47.7 (±0.5)

lowers its cytotoxic effects (**3** versus **5**, Table 1). Oxygenation of aromatic ring also seems to decrease the biological activities (e.g. **8**–**11**). Substitution of the nitrile group with the carboxymethyl group, in some cases does not alter the biological activities (e.g. **3** versus **4** and **6** versus **7**). On the other hand, note that the compound **1** is more active than **2**. The effect of the aromatic ring of carboxymethylated compounds seems to be important as **2**, **14**, **15** and **16** are practically devoid of activity as compared with the active **4** and **7** compounds.

A molecular modeling using DTF theory and the B3LYP 6-31+G* level, suggests that there is a great conformational topology difference between **1** and **2**. We observed in **2** an intra-molecular hydrogen bond between the oxygen of carboxy group and the OH moiety on the most stable conformation. On the other hand, we did not observe hydrogen bond between OH and the nitrogen of nitrile group of **1** in its most stable conformation. We suppose that this 3D different conformation can also be related to the very different leishmanicidal and toxicological activities of these compounds.

Compound **13** was found to be most selectively active against amastigotes, inhibiting 84.3% of parasite growth concomitant with 0% release of LDH by macrophages at 50 μM. This was likely due to the presence of the bromine atom, not found in the less active **1**. That was less toxic to macrophages than the naphthyl group in compound **12** (26.6% LDH release). Bromine seems to be a key antileishmanial component, as this halogen is also associated with enhanced activity of similar chalcones [19] The IC_{50} of the most promising drugs in Table 1, confirms the higher potency in relation to Pentostam (Table 3).

5. Conclusion

We present in this short communication a very important selective leishmanicidal activity of the aromatic Baylis–Hillman adducts. The compounds discussed in this paper are not new but they could be prepared by one-pot reaction by a very simple and efficient synthesis, essential for subsequent studies for scale up. The higher potency and lesser toxicity than the reference drug Pentostam (the most used drug in the developing countries) are enough to classify **1**, **3**, **7**, **12** and **13** in the new potential antileishmanial drugs. We also detach here the brominated adduct **13** as a lead compound. Our present study thus indicates **13** as a new alternative to the conventional chemotherapeutic agent on *L. amazonenesis*. This new class of leishmanicide drugs is very promising for our further studies on the other different species of the protozoan parasite *Leishmania* (e.g. *Leishmania chagasi*, *Leishmania donovani*), an endemic disease on Brazil.

Acknowledgments

This work was supported by grants from FAPESQ (Fundação de Amparo à Pesquisa do Estado da Paraíba) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) in Brazil.

References

[1] World Health Organization, Division of Control of Tropical Diseases, <http://www.who.int/tdr/diseases/leish/diseaseinfo.Htm/> (accessed on January 2006).
[2] B.L. Herwaldt, Lancet 354 (1999) 1191–1199.
[3] S.L. Croft, S. Sundar, A.H. Fairlamb, Clin. Microbiol. Rev. 19 (2006) 111–126.
[4] S.L. Croft, G.H. Coombs, Trends Parasitol. 19 (2003) 502–508.
[5] J.A. Valderrama, C. Zamorano, M.F. González, E. Prinab, A. Fournet, Bioorg. Med. Chem. 13 (2005) 4153–4159.
[6] D. Basavaiah, J. Rao, T. Satyanarayana, Chem. Rev. 103 (2003) 811–891.
[7] A.B. Baylis, M.E.D. Hillman, German Patent 2155113, 1972. Chem. Abstr. 77 (1972) 34174q.
[8] 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) 1573–1575.
[9] L.S.M. Miranda, B.A. Meireles, J.S. Costa, V.L.P. Pereira, M.L.A.A. Vasconcellos, Synlett (2005) 869–871.
[10] R.O.M.A. de Souza, M.L.A.A. Vasconcellos, Synth. Commun. 33 (2003) 1383–1399.
[11] R.O.M.A. de Souza, M.L.A.A. Vasconcellos, Catal. Commun. 5 (2004) 21–24.
[12] R.O.M.A. de Souza, B.A. Meireles, L.S. Sequeira, M.L.A.A. Vasconcellos, Synthesis (2004) 1595–1600 (in this article we also described the synthesis and the spectroscopic data of the compounds **1**–**4**, **9**–**12** and **14**).
[13] M.K. Kundu, N. Sundar, S.K. Kumar, S.V. Bhat, S.V.N. Biswas, Bioorg. Med. Chem. Lett. 9 (1999) 731–736.
[14] 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, de R.O.M.A. de Souza, L.T.C. Crespo, Pest. Manage. Sci. 62 (2006) 288–296.
[15] For the spectroscopic data of **13** see: L.R. Reddy, K.R. Rao Org. Prep. Proced. Int. 32 (2000) 185–188.
[16] For the syntheses and spectroscopic data of **6** see: I. Beltaief, S. Hbaieb, R. Besbes, H. Amri, M. Villieras, J. Villieras Synthesis (1998) 1765–1768.
[17] For the syntheses and spectroscopic data of **7**, **15** and **16** compounds, see: J. Cai, Z. Zhou, G. Zhao, C. Tang Org. Lett. 4 (2002) 4723–4725.
[18] For the synthesis and spectroscopic data of **8** see: F. Coelho, W.P. Almeida, D. Veronese, C.R. Mateus, E.C. Silva Lopes, R.C. Rossi, G.P.C. Silveira, C.H. Pavam Tetrahedron 58 (2002) 7437–7447.
[19] P. Boeck, C.B. Falcão, P.V. Leal, V. Cechinel-Filho, E.C. Torres-Santos, R.A. Yunes, B. Rossi-Bergmann, Bioorg. Med. Chem. 16 (2006) 1538–1545.
[20] C. Demicheli, R. Ochoa, J.B.B. Silva, C.A.B. Falcão, B. Rossi-Bergmann, A.L. Melo, R.D. Sinisterra, F. Frezard, Antimicrob. Agents Chemother. 48 (2004) 100–103.
[21] W.L. Roberts, J.D. Berman, P.M. Rainey, Antimicrob. Agents Chemother. 39 (1995) 1234–1239.
[22] S.A. Da-Silva, S.S. Costa, B. Rossi-Bergmann, Parasitology 118 (1999) 575–582.