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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, protease inhibition, and antileishmanial activity of new benzoxazoles derived from acetophenone or benzophenone and synthetic precursors

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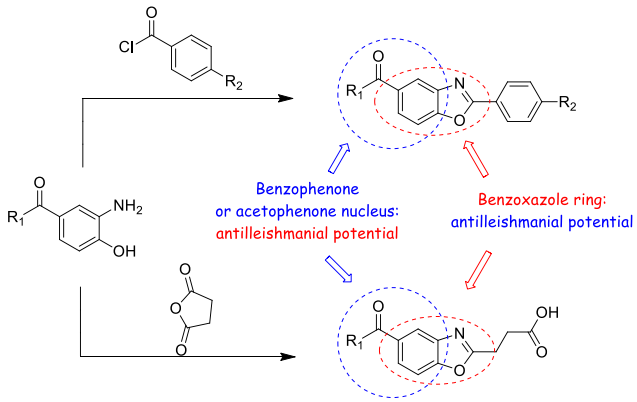
Abstract This work reports the synthesis, protease inhibition, and antileishmanial activity of ten benzoxazole derivatives, which were obtained in a three-step synthetic route from 4-hydroxy-acetophenone and 4-hydroxy-benzophenone. These benzoxazoles, the synthetic intermediates, and the starting ketones were evaluated for their inhibitory effect on the activity of cysteine (papain, rCPB2.8, and rCPB3.0) and serine (trypsin) proteases. All compounds showed significant values of IC_{50} against these enzymes (in the range of 0.0086–0.7612 μM for papain and 0.0075–0.5032 μM for trypsin), being more active than the standard inhibitors (1.7821 and 7.2318 μM, for E64 and TLCK, respectively). Following, all compounds were evaluated in vitro for their leishmanicidal activity against promastigote form of *Leishmania amazonensis*. The most active compounds were further evaluated against amastigote form and for its toxicity against murine macrophages. The benzoxazole **4d**, a benzophenone derivative, and the intermediate 4-hydroxy-3-nitroacetophenone **2b** showed significant antileishmanial activity ($IC_{50} = 90.3$ μM and $IC_{50} = 130.9$ μM, respectively) with selectivity indexes (5.22 and 18.09, respectively) compared to or better than those of two established leishmanicidal drugs, pentamidine (0.58) and amphotericin B (5.31).

Graphical Abstract



Keywords *Leishmania amazonensis* · Benzophenone · Acetophenone · Benzoxazoles · Proteases · Antiproteolytic activity

Introduction

Leishmaniosis is a group of infectious diseases caused by many protozoa of the genus *Leishmania*, which are transmitted to humans and small mammals by more than 30 different species of phlebotomine sandflies (Phillips and Stanley 2011). This disease can manifest as the less severe cutaneous form or as the lethal visceral form (Eschenlauer et al. 2009). According to the World Health Organization,

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there is an estimation of approximately 1.3 million cases of different clinical forms of leishmaniasis identified each year (WHO 2016). Throughout the Brazilian territory, many cases of both visceral and cutaneous leishmaniosis are documented each year (WHO 2015).

There are about 25 drugs used to treat the clinical complications associated to leishmaniosis, but few of them are classified as antileishmania drugs for human use and the most part is not administered orally (Singh and Sivakumar 2004). In some localities, the high consumption of these drugs has caused resistance to treatment, which leads to an urge need for the development of alternatives to treat these diseases (Ready 2014).

Intense investigations in host-parasite interaction and *Leishmania* virulence factors allowed the discovery of new targets for drug design. There are several enzymes that are essential for survival and proliferation of *Leishmania*, and among them the proteases are well studied, mainly cysteine, serine, aspartate, and metalloproteinases (Das et al. 2013). Studies consistently show that in vitro and in vivo inhibition of cysteine proteases leads to a significant loss of virulence of parasite (Lima et al. 2013), proving to be very promising targets in the development of antileishmanial drugs.

The mechanism of action is very similar for serine and cysteine proteases (Powers et al. 2002). Each enzyme has a specific amino acid residue in its active site, which is important for the catalytic action. For cysteine proteases, the mechanism of peptide hydrolysis of a substrate occurs by the nucleophilic attack of a thiol group belonging to a cysteine residue (Fricker 2010). However, serine proteases use the hydroxyl group of this amino acid as nucleophile for hydrolysis (Martins et al. 2009). After substrate accommodation, the sulfur or oxygen atoms of cysteine or serine, respectively, attack the carbonyl carbon of the substrate (Fricker 2010). A good protease inhibitor must mimic the natural substrate and possess sites which may react with the nucleophile of the corresponding amino acid residue. It is known that the pseudopeptide inhibitors containing heterocycles and α-ketoeterocycles are quite relevant, since they mimic this site from the initial attack (Steert et al. 2010).

Some reports on the bioactivities of natural benzophenones and benzophenone analogs (Ayres et al. 2007; Costa Júnior et al. 2013; Hay et al. 2008; Lenta et al. 2007; Pereira et al. 2010), and even synthetic and semi-synthetic benzophenones (Al-Kahraman et al. 2012; Maciel-Rezende et al. 2013; Román-Luque-Ortega et al. 2010) showed promising in vitro antileishmanial activity. In addition, these compounds were also quite active in inhibiting cysteine proteases that were specific of the genus *Leishmania* (Almeida et al. 2015). Moreover, compounds with the acetophenone scaffold have also demonstrated potential antileishmanial activity (Ansari et al. 2012).

Other studies indicated that some heterocycles could act as potent protease inhibitors and showed interesting antileishmanial activity (Loedige 2015; Sangshetti et al. 2015; Steert et al. 2010). Among them, benzoxazoles have been explored as bioactive agents in face of their wide biological potential as antibacterial, antiparasitic, anti-inflammatory, and anticancer agents (Radi et al. 2008). Tipparaju et al. (2008) pointed out the promising activities of natural and synthetic benzoxazoles against these parasites. In this work, we describe the synthesis of substances containing the benzoxazole ring fused to an acetophenone or benzophenone nucleus, their protease inhibitor potential and in vitro antileishmanial activity. Benzoxazoles derived from acetophenones or benzophenones can act as protease inhibitors since the carbonyl carbon can act as an acceptor electrophilic center and the benzoxazole nucleus can contribute to the fixation of these compounds in the active site of the enzymes (Martins et al. 2009; Steert et al. 2010).

Material and methods

Chemistry

Reagents and solvents were purchased as reagent grade and were used without further purification. All melting point determinations were measured on an Aaker PFM-II apparatus and were not corrected. Infrared (IR) spectroscopy was performed on a Thermo Scientific Nicolet-iS50 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE DRX 300 MHz spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C spectra) or on a Bruker AVANCE DRX 400 MHz spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C spectra) using deuterated chloroform, methanol or dimethyl sulfoxide (DMSO). The results are presented as chemical shifts (δ) reported in parts per million (ppm) with reference to tetramethylsilane as internal standard. Coupling constants (J) were reported in Hertz (Hz) and the following abbreviations were used for the ^{1}H multiplicities: singlet (s), doublet (d), double doublet (dd), triplet (t), triple triplet (tt), and multiplet (m). The reactions were followed by thin-layer chromatography (TLC) on commercial silica gel 60 plates. Column chromatography purifications were performed over silica gel 60, 70–230 mesh (Merck). All the obtained substances are soluble in common organic solvents such as ethyl acetate, chloroform, methylene chloride, and methanol. High-resolution mass spectrometry (HRMS) were acquired using UltrOTOFq (Bruker Daltonics) ESI-qTOF mass spectrometer controlled by the software CLASS-VP 6.14 and the samples were solubilized in acetronitrilo. The *c*logP and pKa values were obtained from the ChemDraw Ultra 11.0 and Marvin Sketch 5.6.0.1, respectively.

The enzymes rCPB2.8 and rCPB3.0 were generously gifted by Dr. Luiz Juliano (Department of Biophysics, Federal University of São Paulo, Brazil), papain, trypsin, E-64 (1-[[(Ltrans-epoxysuccinyl)-L-leucyl]amino]-4-guanidinobutane), and fluorogenic substrate Z-FR-AMC (carbobenzyloxy-Phe-Arg-(7-amino-4-methylcoumarin) were commercially obtained from Sigma-Aldrich Sigma (St. Louis, USA). Substrate hydrolyzes were monitored in a F2500 Hitachi spectrofluorometer using $\lambda_{Ex} = 380$ nm and $\lambda_{Em} = 460$ nm as wavelengths and the enzymatic molar concentrations were estimated by titration according to kinetic parameters (Gontijo et al. 2015).

General procedure for the synthesis of 2a and 2b (Sudarma et al. 2014)

To a solution of 4-hydroxybenzophenone or 4-hydroxyacetophenone (1.26 mmol) in dichloromethane (15 mL), potassium hydrogen sulfate (6.82 mmol), sodium nitrate (7.32 mmol), and 0.875 g of wet silica 50% p/p was added. The slurry was was left under constant stirring at room temperature until the completion of the reaction, checked by TLC (hexane/ethyl acetate, 8:2v/v). Then the mixture was filtered through silica, the solid was washed with dichloromethane and the solvent removed under reduced pressure. The products were then purified by recrystallization.

*4-hydroxy-3-nitrobenzophenone (**2a**) (Cohen et al. 1984)*

90% yield, yellow solid after recrystallization from water/methanol (1:1v/v); M.p. 105–107 °C; clog$P = 3.12$; IR (ν_{max} cm^{-1}): 3296, 3069, 1660, 1609, 1525, 1319. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.28 (1H, d, H-5, $^3J = 8.7$ Hz), 7.49–7.55 (2H, m, H-3′, H-5′), 7.64 (1H, tt, H-4′, $^3J = 1.2$ Hz, $^4J = 7.5$ Hz), 7.74–7.78 (2H, m, H-2′, H-6′), 8.12 (1H, dd, H-6, $^4J = 2.1$ Hz, $^3J = 8.7$ Hz), 8.58 (1H, d, H-2, $^4J = 2.1$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 193.2 (C, C-7), 157.8 (C, C-4), 138.4 (CH, C-6, C, C-1′), 136.6 (C, C-3), 132.9 (CH, C-4′), 129.8 (C, C-1), 129.6 (CH, C-2′, CH, C-6′), 128.6 (CH, C-3′, CH, C-5′), 127.8 (CH, C-2), 120.3 (CH, C-5). pKa = 5.1.

*4-hydroxy-3-nitroacetophenone (**2b**) (Wang et al. 2010)*

65% yield, yellow solid after recrystallization from water/methanol (1:1v/v); M.p. 145–148 °C; clogP = 1.53; IR (ν_{max} cm^{-1}): 3269, 3106, 2927, 1676, 1616, 1525, 1334. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.62 (3H, s, H-8) 7.24 (1H, d, H-5, $^3J = 9.0$ Hz), 8.20 (1H, dd, H-6, $^3J = 9.0$ Hz, $^4J = 2.1$ Hz), 8.71 (1H, d, H-2, $^4J = 2.1$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 194.8 (C, C-7), 158.4 (C, C-4), 136.7 (C, C-3), 129.9 (CH, C-6), 126.3 (CH, C-1), 120.7 (CH, C-2), 115.7 (CH, C-5), 26.4 (CH_3, C-8). pKa = 5.4.

General procedure for the synthesis of 3a and 3b (Belamy and Ou 1984)

A mixture of **2a** or **2b** (1.03 mmol), $SnC1_2.2H_2O$ (5.14 mmol) and absolute ethanol (9 mL) was heated at 70 °C under stirring. When the reaction was over, observed by TLC (hexane/ethyl acetate, 8:2v/v), the clear solution was cooled to room temperature and then poured into ice. The pH was made slightly basic (pH 7–8) by addition of 10% aqueous sodium bicarbonate and then the mixture was extracted with ethyl acetate (3 × 30 mL). The organic phase was dried over magnesium sulfate, filtered and the solvent removed under reduced pressure. The obtained product was used immediately in the next step, without further purification.

*3-amino-4-hydroxy-benzophenone (**3a**) (Jadhav et al. 2017)*

97% yield, yellow oil; clogP = 2.34; IR (ν_{max} cm^{-1}): 3440, 3382, 3351, 3053, 1651, 1584, 1564, 1280, 1213. pKa = 9.0.

*3-amino-4-hydroxy-acetophenone (**3b**) (Blum et al. 2003)*

95% yield, brown oil; clogP = 0.75; IR (ν_{max} cm^{-1}): 3482, 3384, 3322, 3080, 1615, 1581, 1518, 1283, 1212. pKa = 9.3.

General procedure for the synthesis of 4a–h (Sener et al. 2000; Nakamura et al. 2013)

The appropriate acid chloride (1.02 mmol) was dissolved in ethyl ether (5 mL) and this solution was added dropwise during 30 min to a stirred ice-cooled mixture of the appropriate *o*-aminophenol **3a** or **3b** (1.02 mmol) and ethyl ether (5 mL). The mixture was then stirred at room temperature until the completion of the reaction, as showed by TLC (hexane/ethyl acetate, 6:4v/v). Following, the mixture was washed with water and extracted with ethyl acetate (3 × 30 mL). The organic phase was further washed with 10% aqueous sodium bicarbonate (3 × 25 mL), followed by water, and it was dried over anhydrous magnesium sulfate. After filtration and removal of the solvent under reduced pressure, the obtained product (1.02 mmol) and *p*-toluenesulfonic acid (3.06 mmol) were taken in toluene (25 mL) and this suspension was allowed to heating at 110 °C under stirring. When the reaction was complete, as recorded by TLC (hexane/ethyl acetate, 7:3v/v), the solvent was removed under reduced pressure and the crude product was purified by column chromatography.

*(2-phenyl-5-benzoxazolyl)phenyl methanone (**4a**) (So and Decaire 1998)*

71% yield, white solid after column chromatography (hexane/ethyl acetate, 95:05v/v); M.p. 182–193 °C; clogP = 4.84; IR (ν_{max} cm^{-1}): 3052, 1643, 1603, 1596, 1278, 1048. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.50 (2H, t, H-3a′ and H-7′, ^3J = 8.0 Hz), 7.53–7.58 (3H, m, H-11, H-12 and H-13), 7.61 (1H, tt, H-7a′, ^3J = 7.6 Hz, ^4J = 1.2 Hz), 7.69 (1H, d, H-7, ^3J = 8.4 Hz), 7.69 (1H, d, H-7, ^3J = 8.4 Hz), 7.84 (2H, dd, H-4′ and H-6′, ^3J = 8.2 Hz, ^4J = 1.2 Hz), 7.94 (1H, dd, H-6, ^3J = 8.4 Hz, ^4J = 1.6 Hz), 8.21 (1H, d, H4, ^4J = 1.6 Hz), 8.28 (2H, dd, H-10 and H-14, ^3J = 7.8 Hz, ^4J = 1.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.1 (C, C-8), 164.7 (C, C-2), 153.5 (C, C-7a), 142.1 (C, C-3a), 137.9 (C, C-5′), 134.7 (C, C-5), 132.6 (CH, C-7a′), 132.2 (C, C-9), 130.2 (CH, C-4′ and CH, C-6′), 129.2 (CH, C-11 and CH, C-13), 128.5 (CH, C-12), 127.9 (CH, C-3a′ and CH, C-7′), 127.8 (CH, C-10 and CH, C-14), 126.8 (CH, C-6), 122.9 (CH, C-4), 110.8 (CH, C-7). pKa = 14.7. HRMS-ESI: *m/z* calcd. for $C_{20}H_{14}NO_2^+$ $(M+H)^+$: 300.1019; found: 300.1020.

*[2-(4-nitrophenyl)-5-benzoxazolyl]phenyl methanone (**4b**)*

40% yield, yellow solid after column chromatography (hexane/ethyl acetate, 95:05v/v); M.p. 221–225 °C, with decomposition; clogP = 4.60; IR (ν_{max} cm^{-1}): 3108, 1654, 1596, 1554, 1512, 1340, 1281, 1049. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.51 (2H, t, H-3a′ and H-7′, ^3J = 7.2 Hz), 7.62 (1H, tt, H-7a′, ^3J = 7.2 Hz, ^4J = 1.5 Hz), 7.73 (1H, d, H-7, ^3J = 8.4 Hz), 7.83 (2H, dd, H-4′ and H-6, ^3J = 7.2 Hz, ^4J = 1.5 Hz), 7.99 (1H, dd, H-6, ^3J = 8.7 Hz, ^4J = 1.5 Hz), 8.24 (1H, d, H-4, ^4J = 1.2 Hz), 8.39 (2H, d, H-10 and H-14, ^3J = 9.0 Hz), 8.44 (2H, d, H-11 and H-13, ^3J = 9.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 195.7 (C, C-8), 162.2 (C, C-2), 153.6 (C, C-7a), 149.8 (C, C-12), 141.8 (C, C-3a), 137.6 (C, C-5′), 135.3 (C, C-9), 132.8 (C, C-5), 132.3 (CH, C-7a′), 130.2 (CH, C-10 and CH, C-14), 128.7 (CH, C4′, CH, C-6′, CH, C-11, and CH, C-13), 128.5 (CH, C-3a′ and CH, C-6′), 124.4 (CH, C-6), 123.4 (CH, C-4), 111.1 (CH, C-7). pKa = 14.9. HRMS-ESI: *m/z* calcd. for $C_{20}H_{13}N_2O_3^+$ $(M+H)^+$: 345.0870; found: 345.0870.

*[2-(4-chlorophenyl)-5-benzoxazolyl]phenyl methanone (**4c**)*

60% yield, white solid after column chromatography (hexane/ethyl acetate, 95:05v/v); M.p. 195–198 °C; clogP = 5.56; IR (ν_{max} cm^{-1}): 3067, 1657, 1617, 1596, 1293, 1048, 1016. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.48–7.54 (4H, m, H-11, H-13, H-3a′, H-7′), 7.61 (1H, tt, H-7a′, ^3J = 7.2 Hz, ^4J = 1.2 Hz), 7.66 (1H, dd, H-7, ^3J = 8.4 Hz, ^5J = 0.4 Hz), 7.82–7.84 (2H, m, H-4′ and H-6′), 7.94 (1H, dd, H-6, ^3J = 8.4 Hz, ^4J = 1.6 Hz), 8.18–8.22 (3H, m, H-10, H-14, H-4). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 195.9 (C, C-8), 163.7 (C, C-2), 153.5 (C, C-7a), 142.0 (C, C-3a), 138.5 (C, C-5′), 137.8 (C, C-5), 134.8 (C, C-12), 132.6 (CH, C-7a′), 130.2 (CH, C-4′ and CH, C-6′), 129.6 (CH, C-11 and CH, C-13), 129.2 (CH, C-10 and CH, C-14), 128.5 (C, C-9), 127.9 (CH, C-3a′ and CH, C-7′), 125.0 (CH, C-6), 122.9 (CH, C-4), 110.8 (CH, C-7). pKa = 14.7. HRMS-ESI: *m/z* calcd. for $C_{20}H_{12}ClNO_2$ $(M+Na)^+$: 356.0449; found: 356.0440; calcd. for $(M+H)^+$: 334.0634; found: 334.0619; calcd. for $(M+2+H)^+$: 336.0605; found: 336.0597.

*[2-(4-methoxyphenyl)-5-benzoxazolyl]phenyl methanone (**4d**)*

33% yield, white solid after column chromatography (hexane/ethyl acetate, 95:05v/v); M.p. 170–175 °C; clogP = 4.86; IR (ν_{max} cm^{-1}): 3067, 2922, 1645, 1608, 1498, 1292, 1030. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.90 (3H, s, H-15), 7.04 (2H, d, H-11 and H-13, ^3J = 9.2 Hz), 7.50 (2H, t, H-3a′ and H-7′, ^3J = 7.6 Hz), 7.59–7.62 (1H, m, H-7a′), 7.64 (1H, d, H-7, ^3J = 8.4 Hz), 7.83 (2H, d, H-4′ and H-6′, ^3J = 7,2 Hz), 7.90 (1H, dd, H-6, ^3J = 8.4 Hz, ^4J = 1.6 Hz), 8.16 (1H, d, H-4, ^4J = 1.2 Hz), 8.21 (2H, d, H-10 and H-14, ^3J = 8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.1 (C, C-8), 164. 8 (C, C-2), 162.9 (C, C-12), 153.5 (C, C-7a), 142.2 (C, C-3a), 137.9 (C, C-5′), 134.5 (C, C-5), 132.5 (C, C-7a′), 130.2 (CH, C-4′ and CH, C-6′), 129.8 (CH, C-3a′ and CH, C-7′), 128.5 (CH, C-6), 127.4 (C, C-9), 122.4 (CH, C-4), 119.2 (CH, C-10 and CH, C-14), 114.6 (CH, C-11 and CH, C-13), 110.5 (CH, C-7), 55.7 (CH_3, C-15). pKa = 14.7. HRMS-ESI: *m/z* calcd. for $C_{21}H_{15}NO_3(M+Na)^+$: 352.0944; found: 352.0944.

*1-(2-phenyl-5-benzoxazolyl)ethanone (**4e**) (Ge et al. 2012)*

21% yield, white solid after column chromatography (hexane/ethyl acetate, 95:05v/v); M.p. 160–165 °C; clogP = 3.24; IR (ν_{max} cm^{-1}): 3066, 2920, 1672, 1610, 1554, 1267, 1059. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.69 (3H, s, H-15), 7.52–7.58 (3H, m, H-11, H-12 and H-13), 7.63 (1H, d, H-7, ^3J = 8.4 Hz), 8.05 (1H, dd, H-6, ^3J = 8.4 Hz, ^4J = 1.6 Hz), 8.26 (2H, dd, H-10 and H-14, ^3J = 8.0 Hz, ^4J = 1.,2 Hz), 8.37 (1H, d, H-4, ^4J = 1.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.3 (C, C-8), 164.6 (C, C-2), 153.6 (C, C-7a), 142.2 (C, C-3a), 134.6 (C, C-5), 132.3 (C, C-9), 129.2 (CH, C-11 and CH, C-13), 127.9 (C, C-12), 126.7 (CH, C-10 and CH, C-14), 125.9 (CH, C-6), 121.1 (CH, C-4), 110.8 (CH, C-7), 26.9 (CH_3, C-15). pKa = 14.7. HRMS-ESI: *m/z* calcd. for $C_{15}H_{12}NO_2^+$ $(M+H)^+$: 238.0863; found: 238.0870.

*1-[2-(4-nitrophenyl)-5-benzoxazolyl)] ethanone (**4f**) (Botija et al. 1985)*

20% yield, as yellow solid after column chromatography (hexane/ethyl acetate 95:05); M.p. 244–248 °C with decomposition; clogP = 3.00; IR (ν_{max} cm^{-1}): 3104, 2921, 1683, 1597, 1556, 1516, 1343, 1263, 1058. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.71 (3H, s, H-15), 7.69 (1H, d, H-7, ^{3}J = 8.4 Hz), 8.12 (1H, dd, H-6, ^{3}J = 8.6 Hz, ^{4}J = 1.6 Hz), 8.39–8.46 (5H, m, H-10, H-11, H-13, H-14, H-4). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.9 (C, C-8), 162.2 (C, C-2), 153.9 (C, C-7a), 149.8 (C, C-12), 142.3 (C, C-3a), 135.1 (C, C-9), 132.3 (C, C-5), 128.8 (CH, C-10 and CH, C-14), 127.0 (CH, C-11 and CH, C-13), 124.4 (CH, C-6), 121.7 (CH, C-4), 111.2 (CH, C-7), 26.9 (CH_3, C-15). pKa = 14.7. HRMS-ESI: *m/z* calcd. for $C_{15}H_{11}N_2O_4^+$ $(M+H)^+$: 283.0713; found: 283.0721.

*1-[2-(4-chlorophenyl)-5-benzoxazolyl)] ethanone (**4g**) (Wynne et al. 2009)*

10% yield, white solid after column chromatography (hexane/ethyl acetate 95:05); M.p. 185–190 °C; clogP = 3.96; IR (ν_{max} cm^{-1}): 3066, 2921, 1671, 1621, 1606, 1262, 1043, 1008. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52 (2H, d, H-11 and H-13, ^{3}J = 8.4 Hz), 7.63 (1H, d, H-7, ^{3}J = 8.4 Hz), 8.06 (1H, dd, H-6, ^{3}J = 8.6 Hz, ^{4}J = 1.6 Hz), 8.19 (2H, d, H-10 and H-14, ^{3}J = 8.4 Hz), 8.36 (1H, d, H-4, ^{4}J = 1.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.1 (C, C-8), 163.7 (C, C-2), 153.8 (C, C-7a), 142.4 (C, C-3a), 138.5 (C, C-5), 134.7 (C, C-12), 129.6 (CH, C-11 and CH, C-13), 129.2 (CH, C-10 and CH, C-14), 126.2 (C, C-9), 125.2 (CH, C-6), 121.1 (CH, C-4), 110.8 (CH, C-7), 26.9 (CH_3, C-15). pKa = 14.7. HRMS-ESI: *m/z* calcd. for $C_{15}H_{11}ClNO_2^+$ $(M+H)^+$: 272.0473; found: 272.0475; calcd. for $(M+2+H)^+$: 274.0448; found: 274.0447.

*1-[2-(4-methoxyphenyl)-5-benzoxazolyl)] ethanone (**4h**) (CAS Registry Number 1368597-94-7; source of registration: Chemical Catalog, supplier: Otava Chemicals)*

45% yield, white solid after column chromatography (dichloromethane/hexane 7:3); M.p. 160–165 °C; clogP = 3.26; IR (ν_{max} cm^{-1}): 3067, 2920, 1672, 1607, 1581, 1252, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.68 (3H, s, H-15), 3.90 (3H, s, H-16), 7.04 (2H, d, H-11 and H-13, ^{3}J = 8.8 Hz), 7.59 (1H, d, H-7, ^{3}J = 8.4 Hz), 8.01 (1H, dd, H-6, ^{3}J = 8.4 Hz, ^{4}J = 1.6 Hz), 8.20 (2H, d, H-10 and H-14, ^{3}J = 8.8 Hz), 8.32 (1H, d, H-4, ^{4}J = 1.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.3 (C, C-8), 164.8 (C, C-2), 162.9 (C, C-12), 153.8 (C, C-7a), 142.7 (C, C-3a), 134.4 (C, C-5), 129.8 (CH, C-6), 125.5 (C, C-9), 120.6 (CH, C-4), 119.2 (CH, C-10 and CH, C-14), 114.6 (CH, C-11 and CH, C-13), 110.5 (CH, C-7), 55.6 (CH_3, C-16), 26.9 (CH_3, C-15). pKa = 14.7. HRMS-ESI: *m/z* calcd. for $C_{16}H_{14}NO_3^+$ $(M+H)^+$: 268.0968; found: 268.0961.

General procedure for the synthesis of 4i–j (Temperini et al. 2010; Trujillo-Ferrara et al. 2004)

A mixture of succinic anhydride (1.12 mmol) in ethyl ether (5 mL) was added to a stirred solution of the *o*-aminophenol **3a** or **3b** (1.02 mmol) in ethyl ether (5 mL). The mixture was kept under stirring at room temperature until the completion of the reaction, as recorded by TLC (hexane/ethyl acetate, 6:4v/v). Then, the solvent was removed under reduced pressure and the product was purified by recrystallization from water/methanol (1:1v/v). Next, a solution of the obtained product (1.02 mmol) and acetic anhydride (1.02 mmol) in tetrahydrofuran (THF) (17 mL) was allowed to stir at 70 °C until the completion of the reaction, as shown by TLC (hexane/ethyl acetate, 6:4v/v). After removal of the solvent under reduced pressure, the pure product was obtained after recrystallization from isopropanol.

*3-(5-benzoyl-2-benzoxazolyl)propanoic acid (**4i**)*

48% yield, beige solid; M.p. 192–196 °C; clogP = 4.60; IR (ν_{max} cm^{-1}): 3483, 3066, 1761, 1712, 1651, 1597. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.76–2.91 (4H, m, H-9, H-10), 7.54–7.61 (3H, m, H-3a′, H-7′, H-7a′), 7.68–7.78 (3H, m, H-7, H-4′, H-6′), 7.79 (1H, d, H-4, ^{4}J = 2.1 Hz), 7.89 (1H, dd, H-6, ^{3}J = 8.4 Hz, ^{4}J = 2.1 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 193.9 (C, C-8), 176.1 (C, C-11), 167.5 (C, C-2), 149.2 (C, C-7a), 136.5 (C, C-3a), 134.4 (C, C-5'), 132.9 (C, C-5), 131.1 (CH, C-7a′), 130.9 (CH, C-4′, CH, C-6′), 129.5 (CH, C-3a′, CH, C-7′), 128.7 (CH, C-6), 125.0 (CH, C-4), 124.2 (CH, C-7), 28.6 (CH_2, C-9, CH_2, C-10). pKa = 4.1. HRMS-ESI: *m/z* calcd. for $C_{17}H_{14}NO_4^+$ $(M+H)^+$: 296.0917; found: 296.0915.

*3-(5-acetyl-2-benzoxazolyl)propanoic acid (**4j**)*

15% yield, yellow solid; M.p. 196–200 °C; clogP = 3.01; IR (ν_{max} cm^{-1}): 3073, 2955, 1759, 1700, 1682, 1601. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.58 (3H, s, H-12), 2.92 (4H, s, H-9, H-10), 7.46 (1H, d, H-7, ^{3}J = 8.4 Hz), 7.87 (1H, d, H-4, ^{4}J = 2.1 Hz), 8.03 (1H, dd, H-6, ^{4}J = 2.1 Hz, ^{3}J = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 195.8 (C, C-8), 175.1 (C, C-11), 167.3 (C, C-2), 149.5 (C, C-7a), 135.1 (C, C-3a), 130.1 (C, C-5), 129.4 (CH, C-6), 124.3 (CH, C-4), 124.2 (CH, C-7), 28.2 (CH_2, C-9, CH_2, C-10), 26.6 (CH_3, C-12). pKa = 4.1. HRMS-ESI: *m/z* calcd. for $C_{12}H_{12}NO_4^+$ $(M+H)^+$: 234.0761; found: 234.0763.

Biological assays

Antiproteolytic activity

Compounds **1a**, **2b**, and **4d** were tested for their inhibitory potential on papain, trypsin, rCPB2.8, and rCPB3.0 by spectrofluorometric measurements. Enzyme inhibition power data were stated as compound concentrations giving 50% decrease in enzyme activity (IC_{50} values), which had been calculated by time-course dose–response curves using inhibitor at different concentrations and the data analyzed in the Grafit 5.0 software using the Eq. 1.

$$y = \frac{\text{Range}}{1 + \left(\frac{x}{IC_{50}}\right)^{s}} \quad (1)$$

where Range is the fitted uninhibited value, y is the enzyme activity, x is the inhibitor concentration, and s is a slope factor. The equation assumes that y falls with increasing x. Inhibition reversibility assay enzymes were carried out in 100 mM sodium acetate buffer, pH 5.5, 20% glycerol (to stabilize the enzymes), 5 mM EDTA, 5 mM dithiothreitol as cysteine protease papain and cruzain activator, and the enzyme solutions pre-activated by 5 min, at 37 °C. The trypsin assays was carried out in 100 mM Trizma base HCl buffer, pH 7.5, 10% glycerol (to stabilize the enzyme). The substrate Z-FR-AMC was used as fluorogenic probe and the hydrolysis of the substrate was followed in a spectrofluorometer Hitachi F2500 in the wavelength $\lambda_{Ex} = 360$ nm (excitation) and $\lambda_{Em} = 480$ nm (emission) as reference inhibitors were used E-64 for papain, rCPB2.8 and rCPB3.0 and TLCK for trypsin (Gontijo et al. 2015).

Leishmanicidal activity against promastigotes

Promastigotes of *L. amazonensis* (MHOM/BR/71973/M2269) were grown on 24-well plates in Schneider's Drosophila medium (Sigma, USA) supplemented with 10.0% (v/v) heat-inactivated fetal bovine serum and 1.0% penicillin (10,000 UI/mL)/streptomycin (10.0 mg/mL) (Sigma, USA). Cells were harvested in the log phase, suspended in fresh medium, counted in Neubauer's chamber and adjusted to a concentration of 1×10^6 cells/mL, using 24-wells plates. The compounds **1a**, **1b**, **2a**, **2b**, **4a–j** were added to promastigote cultures (1×10^6 cells/mL) in the range of 0.10–160.00 µg/mL, solubilized in DMSO (0.6% v/v in all wells) and incubated at 25 °C. After 72 h of incubation, the surviving parasites were counted in a Neubauer's chamber and compared with controls, with just DMSO in concentration of 0.6% v/v, for the determination of 50.0% inhibitory growth concentration (IC_{50}). All tests were performed in triplicate on three different times using pentamidine (Sigma) and amphotericin B (Sigma) as the reference drugs.

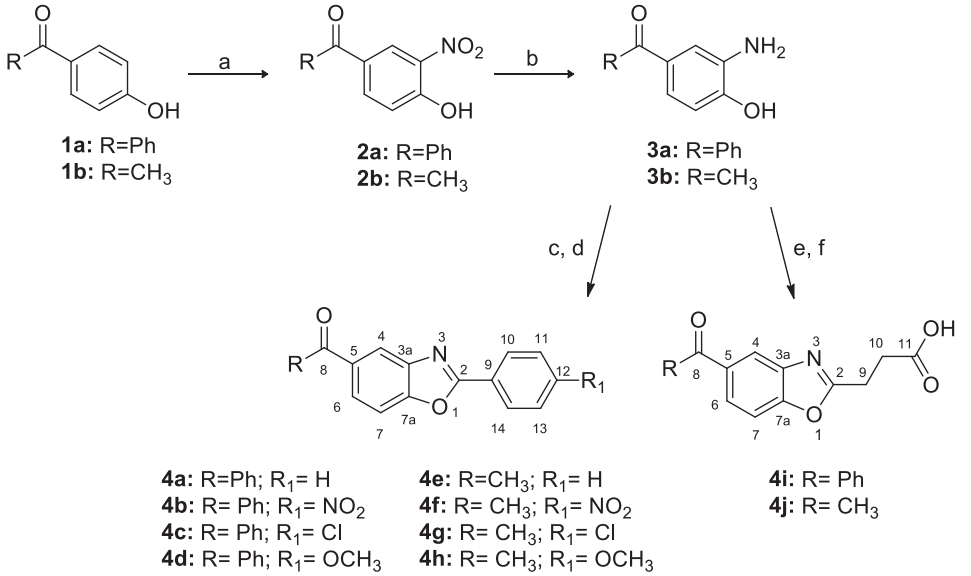
Leishmanicidal activity against amastigotes

Murine peritoneal macrophages were maintained in RPMI 1640 medium (Sigma, USA) supplemented with 10.0% heat-inactivated fetal bovine serum at 37 °C in 5.0% CO_2 incubator. Cells were cultured in 24-well plates chamber on the glass slides of 13 mm (Nunc, USA) in a 8×10^5 cells density per well and infected with late log-phase promastigotes at a ratio of 10:1 (parasite/macrophage) and incubated at 37 °C in 5.0% CO_2 incubator for 24 h. Non-phagocytosed promastigotes were removed by washing, and compounds **1a**, **2a**, **2b**, and **4d** solubilized in DMSO (from 0.10 to 40.00 µg/mL) were administered at the concentration of 0.6% v/v. After 72 h, chamber slides were fixed in absolute methanol, stained with 10.0% Giemsa and examined on an optical light microscope in oil immersion. The percentage of infected cells per well was calculated taking account at least 200 macrophages. The ratio of inhibition (IC_{50} value) was calculated in comparative to the control only with DMSO. All assays were performed in triplicate on three different times using pentamidine (Sigma) and amphotericin B (Sigma) as the reference drugs.

Cytotoxicity evaluation

A suspension of 8×10^5 murine peritoneal macrophages, in RPMI 1640 medium, supplemented with 10.0% heat-inactivated fetal bovine serum and 1.0% penicillin (10000 UI/ml)/streptomycin (10 mg/mL) were added to each well in 96-well plates. The plates were incubated in a 5.0% CO_2 air mixture at 37 °C to adhesion of the cells. After 24 h, the non-adherent cells were removed by washing with the RPMI 1640 medium. Then, several concentrations of compounds and reference drugs ranging from 3.91 to 500.00 µg/mL in DMSO at the final concentration of 0.6% (v/v) were added to the wells containing the cells and the plates were incubated for more 48 h. The non-adherent cells were removed by washing with the RPMI 1640 medium. Afterwards, the 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) was solubilized in phosphate-buffered saline (5.0 µg/mL) as solvent. Ten microliter of this solution was added to RPMI 1640 medium in a final volume of 200.0 µL per well and incubated for 4 h (Mosmann 1983). Then, the medium was removed and 100.0 µL of DMSO was added to each well and homogenized for 15 min. Next, the absorbance of each individual well was calculated at 570 nm according to the following

Scheme 1 Synthesis of the benzoxazoles derived from benzophenone or acetophenone



Reagents and conditions: a) $NaNO_2$, $KHSO_4$, CH_2Cl_2, wet silica, 50% p/p., r.t., 12 h, 65-90%; b) $SnCl_2.2H_2O$, ethanol, 70 °C, 3 h, 95-97%; c) Acyl chlorides, ethyl ether, r.t., 2 h, quantitative; d) TsOH, toluene, 110°C, 4 h, 10-71%; e) Succinic anhydride, ethyl ether, r.t., 12 h, quantitative;
f) Ac_2O, THF, 70 °C, 5 days, 15-48%.

formula (OD represents optical density) using the Equation:

$$\text{Inhibition} = \left(\frac{O_{\text{Dcontrol}} - O_{\text{Dcompounds}}}{O_{\text{Dcontrol}} \times 100}\right)$$

Each experiment was performed in triplicate on three different occasions, and the percentage of viable cells was calculated taking into account the cell culture control (medium + cells + DMSO 0.6% v/v). The 50.0% cytotoxicity concentrations (CC_{50}) were determined and the selectivity index (SI) established by the ratio between the values of CC_{50} and IC_{50} for promastigote and amastigote forms.

Results and discussion

Chemistry

The benzoxazoles derived from acetophenone and benzophenone were designed considering that the ketone carbonyl would be the electrophilic group responsible for the nucleophilic attack by the amino acid residues of the proteases, whereas the heterocycle core could assist increasing this effect.

Benzoxazoles **4a–j** were synthesized following the steps shown in Scheme 1. Briefly, this synthesis was initiated by selective *ortho* aromatic nitration of 4-hydroxybenzophenone or 4-hydroxyacetophenone with potassium hydrogen sulphate and sodium nitrate (Sudarma et al. 2014), followed by reduction by $SnCl_2.2H_2O$ to obtain the *o*-aminophenols **3a** and **3b** (Belamy and Ou 1984). Then, the benzoxazole derivatives were prepared by reaction of **3a** or **3b** with the appropriate acyl chlorides (Nakamura et al. 2013) or succinic anhydride (Temperini et al. 2010), followed by an intramolecular cyclization of the amide intermediates in toluene and catalytic *p*-toluenesulfonic (**4a–h**) or under heating in a THF/water mixture (**4i–j**). This afforded the benzoxazoles in good yields and high purity degree after column chromatography or recrystallization. All the compounds were characterized by spectroscopic methods (IR, ^{1}H, and ^{13}C NMR and HRMS).

The analysis of NMR spectra of the proposed compounds confirmed the success in obtaining the designed compounds. In the ^{1}H NMR spectra, in addition to the the signals of benzophenone and acetophenone core, it was possible to note the signals concerning to all substitution patterns of the desired benzoxazole derivatives. In ^{13}C NMR spectra, in addition to the signals related to the different side chains of these compounds it could be seen the signal of the azomethine carbon in the 162–167 ppm range which confirms the ring closure. Other findings, as the maintenance of the ketonic carbonyl (signals in 196.1–195.7 ppm range for **4a–d**, 197.3–196.9 ppm range for **4e–h**, 193.9 ppm for **4i** and 195.8 ppm for **4j**) and the presence of three hydrogenated aromatic carbons (signals in 7.64–8.34 ppm range) corroborate the obtaining of final products.

Biological activity

The benzoxazoles **4a–j**, 4-hydroxybenzophenone **1a**, 4-hydroxyacetophenone **1b**, the nitro compounds **2a** and **2b**

and the reference protease inhibitors TLCK (*N*-α-tosyl-L-lysinyl-chloromethylketone) and E64 (*trans*-epoxysuccinyl-L-leucylamido(4-guanidino)butane) were next assayed to determine their inhibitory potential against serine (trypsin) and cysteine (papain, rCPB2.8, and rCPB3.0) proteases (Table 1). As can be observed, all the compounds were very active on both enzymes, but especially on papain. It is noteworthy that all of them were more active (IC_{50} in the range of 0.0086–0.7612 μM for papain, and 0.0075–0.5032 μM for trypsin) than the standard papain (E64—IC_{50} 1.7821 μM) or trypsin (TLCK—IC_{50} 7.2318 μM) reference inhibitors. The benzophenone benzoxazoles were the most potent inhibitors compounds against trypsin with IC_{50} values in the range of 0.0075–0.089 μM (except derivative **4a** with an IC_{50} value of 0.3355 μM). The acetophenone benzoxazoles **4e–h** were much less active against trypsin with IC_{50} values from 0.2865–0.523 μM and this may be related to the absence of bulky phenyl group which might be involved in favorable enzyme interactions, responsible for the lower inhibition values seen in benzophenone series. Considering papain, it was possible to notice that both benzophenone and acetophenone derivatives showed interesting inhibitory activities, but those carrying eletron withdrawing groups (Cl or NO_2) were the most active (**4b**, IC_{50} 0.0086 μM; **4c**, IC_{50} 0.0495 μM; **4f**, IC_{50} 0.0266 μM, and **4g**, IC_{50} 0.0121 μM).

Proteases have an essential role in the hydrolysis of peptide bonds in parasites, such as trypanosomatids, leading to peptides of variable sizes or even free amino acids. These enzymes are also involved in several adaptation mechanisms for the survival of parasite, as the regulation in its immune response, invasion and damage of tissues, differentiation and dissemination of parasite and uptake of essential nutrients (Coombs and Mottram 1997; Sajid and McKerrow 2002; Aparicio et al. 2004). Proteases are also key enzymes in the metabolism of proteins, or biologically active peptides (Mótyán et al. 2013). Martins et al. (2009) described three natural polyprenylated benzophenone derivatives, which have shown potential inhibitory effect on proteases. There is also another report about benzophenone derivatives with inhibitory activity of cysteine (papain, rCPB2.8, and rCPB3.0) and serine (trypsin) proteases (Almeida et al. 2015). Besides, it was observed that different ketonic heterocycles show cysteine protease inhibitors rCPB2.8 (Steert et al. 2010), wherein the authors comment on the participation of heterocyclic framework in providing greater inhibitor–enzyme interactions.

In face of these significant results in enzyme inhibition, the most active compounds against papain (**2a**, **4b**, **4g**), as well as their precursors (**1a** and **1b**) were selected for evaluation against two specific strains of *L. (L) mexicana* cysteine proteases, rCPB2.8, and rCPB3.0. According to Pereira et al. (2011), cysteine proteases are expressed at high levels in parasites of the genus *Leishmania* and rCPB2.8 and rCPB3.0 are similar to papain. They represent specific cysteine proteases from different species of *Leishmania* sp (Lanfranco et al 2008). Once again, it was possible to note excellent results for all compounds evaluated

Table 1 Quantitative in vitro inhibitory effect of synthetic compounds on cysteine protease papain and rCPB2.8 and rCPB3.0 enzymes of *L. (L) mexicana* and serine protease trypsin

Compounds	IC_{50} values (μM)[a]			
	Trypsin	Papain	rCPB2.8	rCPB3.0
1a	0.5235 ± 0.1502	0.4428 ± 0.0294	0.0115 ± 0.0018	0.0146 ± 0.0004
1b	0.2865 ± 0.0038	0.0214 ± 0.0029	0.0518 ± 0.0012	0.0126 ± 0.0006
2a	0.3788 ± 0.0525	0.0176 ± 0.0062	0.0131 ± 0.0013	0.0227 ± 0.0007
2b	0.5032 ± 0.0641	0.4456 ± 0.0432	–	–
4[a]	0.3355 ± 0.0489	0.1059 ± 0.0038	–	–
4b	0.0077 ± 0.0013	0.0086 ± 0.0003	0.0103 ± 0.0032	0.0291 ± 0.0016
4c	0.0075 ± 0.0018	0.0495 ± 0.0036	–	–
4d	0.0895 ± 0.0003	0.7612 ± 0.0554	–	–
4e	0.4547 ± 0.0196	0.0479 ± 0.0079	–	–
4f	0.3929 ± 0.0304	0.0266 ± 0.0022	–	–
4g	0.4400 ± 0.0354	0.0121 ± 0.0080	0.0351 ± 0.0092	0.0075 ± 0.0015
4h	0.4017 ± 0.0159	0.0659 ± 0.0023	–	–
4i	0.3866 ± 0.0156	0.030 ± 0.0072	–	–
4j	0.4054 ± 0.0422	0.0212 ± 0.0009	–	–
TLCK[b]	7.2318 ± 0.7185	–	–	–
E64[b]	–	1.7821 ± 0.1356	1.7821 ± 0.1356	1.7821 ± 0.1356

[a] Each IC_{50} value represents the mean ± standard deviation of triplicate determined by the software Grafit 5.0

[b] The TLCK and E64 substances were used as reference inhibitor for the corresponding proteases were assayed

for inhibition of both enzymes (Table 1), with interesting IC_{50} values found in the range 0.0103–0.0518 μM against rCPB2.8 and 0.0075–0.0291 μM against rCPB3.0. All compounds were about 34-folds more active than the standard inhibitor of cysteine proteases E64 (IC_{50} 1.7821 μM).

Thus, it could be confirmed that all evaluated compounds exhibited excellent results on inhibition of cysteine proteases papain, rCPB2.8 and rCPB3.0. As stated by Lima and co-workers (2013), there are consistent studies showing that a significant inhibition of cysteine protease can lead to an expressive in vitro antileishmanial activity. Accordingly, compounds **1a**, **1b**, **2a**, **2b**, and **4a–j** were chosen for in vitro antileishmanial evaluation against promastigote and amastigote forms of *L. (L) amazonensis*. Unfortunately, derivatives **4b**, **4c**, and **4f** were not soluble in the culture medium, so they could not be tested. The IC_{50} values of the other compounds, pentamidine and amphotericin B are listed in Table 2. The cytotoxic effects against murine peritoneal macrophages (expressed as CC_{50} values) of the most active compounds were also checked and were used to determine their SI.

Compounds **2a**, **2b**, **4d**, and the benzophenone **1a** were active against promastigote forms with IC_{50} values of 158.4, 130.9, 90.3, and 190.9 μM, respectively (Table 2). Benzoxazole **4d**, which bears a methoxy group in the benzoxazole ring, was twice as active than benzophenone **1a**. In fact, it was the only benzoxazole which showed significant activity against promastigote form. Curiously, those nitro or chloro-substituted benzoxazoles that were very active in enzymatic tests did not have good activity in these tests. By the other hand, the acetophenone analog **4h** showed no expressive activity and this may be, again, related to the benzophenone nucleus in **4d**. Although a SI greater than 10 is required to ensure good safety for a drug (Pires et al 2013), the two drugs used as reference did not reach this value, which may explain the adverse effects related to them. Although less active than amphotericin B and pentamidine, the benzoxazole **4d** showed a SI (5.22) comparable to that of amphotericin B (5.31) and nine-fold greater than that of pentamidine (0.58). The two nitro derivatives **2a** and **2b** were also active at 158.4 and 130.9 μM, respectively, against promastigotes forms, and for these two compounds the methyl group of acetophenone nucleus contributed to a better activity than the phenyl group in benzophenone analog. Furthermore, derivative **2b** showed a SI of 18, considered ideal according to Pires et al (2013).

The more active compounds against promastigote forms were then evaluated against amastigote intracellular forms. Although benzoxazole **4d** showed to be the most active against the promastigote forms, it was less active on amastigote forms and this low intracellular activity may be related to its large molecular size, which may have restricted its diffusion across the parasite cell membrane. Nitro derivative **2b** was more potent against amastigote forms (IC_{50} = 98.26 μM) compared to its action against promastigote forms (IC_{50} = 130.9 μM) and we postulate that this difference in intracellular activity may be related to the formation

Table 2 Antipromastigote, antiamastigote, and cytotoxicity activities for benzoxazoles and their precursors

Compounds	Promastigotes[a] IC_{50}, in μM/(SI)	Amastigotes[a] IC_{50}, in μM/(SI)	Macrophages[b] CC_{50}, in μM
1a	190.9 ± 2.5/(2.85)	19.93 ± 2.02/(27.49)	547.9 ± 17.5
1b	> 1176.5	nd	nd
2a	158.4 ± 77.4	> 164.61	nd
2b	130.9 ± 0.55/(18.09)	98.26 ± 1.10/(24.1)	2368.3 ± 317.8
4a	> 535.1	nd	nd
4b	nd	nd	nd
4c	nd	nd	nd
4d	90.3 ± 2.7/(5.22)	> 121.45	471.1 ± 64.5
4e	> 675.1	nd	nd
4f	nd	nd	nd
4g	> 589.3	nd	nd
4h	> 599.3	nd	nd
4i	> 542.4	nd	nd
4j	> 686.7	nd	nd
Pentamidine	19.47 ± 0.08/(0.58)	18.82 (0.6)	11.23 ± 1.3
Amphotericin B	5.09 ± 0.39/(5.31)	6.15 (4.4)	27 ± 2.1

[a] Each IC_{50} value represents the mean ± standard deviation of triplicate determined by the software Grafit 5.0

[b] Cytotoxicity concentration for 50% of macrophages

nd not determined, *SI* selectivity index

of a more active metabolite after its penetration in the infected cell. The SI value of **2b** was 24, indicating again the high selective toxicity of this derivative for the parasite. The commercial benzophenone **1a** was active against promastigote forms at 190.9 μM and was also more active against amastigote forms with IC_{50} value comparable to that of pentamidine (19.93 μM), which can also indicate a possible intracellular metabolism leading to a more active substance. The selectivity index of **1a** was higher than 27, showing a low toxicity for this compound.

Conclusion

Ten benzoxazole derivatives, five of which are new compounds (**4b**, **4c**, **4d**, **4i**, **4j**), were obtained of from a three-step synthetic route from acetophenone and benzophenone under mild conditions. These compounds and some of their precursors showed good results when evaluated as inhibitors of papain, rCPB2.8, and rCPB3.0 proteases. Benzophenone benzoxazoles which bear electron withdrawing groups were the best inhibitors of proteases. However, their potential against *L. amazonensis* promastigote forms were not significant, wherein benzoxazole **4d** was the most active with IC_{50} of 90.3 μM and SI value 9-fold greater than that of pentamidine. In the in vitro evaluation with amastigote forms of *L. amazonensis* compounds **1a**, **2b** and, again, **4d**, exhibited satisfactory activities and very low cytotoxicity when compared to standards drugs. In view of the results, we assume that these compounds are good candidates to be exploited in further studies of chemical modifications in order to improve their potential and check in vivo activity as leishmanicidal drug candidates.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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