



Synthesis and Biological Profiles of Some Benzimidazolyl-chalcones as Anti-leishmanial and Trypanocidal Agents

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Benzimidazole constitutes a starting point for the development of new antiprotozoal agents since this nucleus exists in several pharmacologically significant molecules, in particular possessing antifungal, antiviral, antibacterial, and antiparasitic properties.

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Objective: The present study aimed to identify a molecular hit likely to be developed as an anti-leishmanial and antitrypanosomal drug candidate.

Methods: Thus, 12 hybrids of chalcone or benzimidazolyl-arylpropenones were synthesized and screened *in vitro* for anti-leishmanial activity against promastigotes of *Leishmania donovani*. The microculture tetrazolium assay was used to determine their potential to inhibit 50% of a parasite growth (IC_{50}).

Results: Two compounds among 5-chlorobenzimidazole-chalcones (**4a** and **4c**), which exhibited potent activity ($IC_{50}<1 \mu M$) against *L. donovani* and one derivative (**4d**) poorly effective against *L. donovani* ($IC_{50}>50 \mu M$) were selected to check their trypanocidal activity. Lethal concentration (LC_{100}) values of these compounds were estimated by using observations on the viability of trypomastigotes of *Trypanosoma brucei brucei* in MEM medium with Earle's salts and L-glutamine. Seven of the tested compounds (**4a**, **4b**, **4c**, **4e**, **4g**, **4h**, and **4j**) showed particularly higher inhibitory activity than pentamidine ($IC_{50}= 7.6 \mu M$) against *L. donovani* promastigotes with IC_{50} values in a range from 0.5 to 1.8 μM . In addition, the 2'-chlorine derivative (**4c**), displayed potent anti-trypanosomal activity comparable to those of melarsoprol, used as reference drug.

Conclusion: These results support this series of benzimidazole holding arylpropenone group in position 2 as a starting point for future optimization to get novel agents active against leishmaniasis and trypanosomiasis.

Keywords: Benzimidazole; arylpropenone; SAR; anti-leishmanial; trypanocidal.

1. INTRODUCTION

Leishmaniasis and African trypanosomiasis are two of the world's most neglected parasitic infections. Among the three different forms of leishmaniasis characterized, visceral leishmaniasis caused by *Leishmania donovani* is the most severe form of the disease, which can be fatal when left untreated. This disease has been classified by the WHO as an emerging and uncontrolled disease [1]. Leishmaniasis is indeed endemic in some tropical areas of the world, causing about two million new cases yearly [2], 350 million people are at risk of infection with *Leishmania* spp and 30,000 deaths are attributed to leishmaniasis each year [3]. Drugs currently in use for the leishmaniasis treatment such as pentavalent antimonials (meglumine antimoniate, i.e. Glucantime®), bis-amidines (pentamidine), display moderate and severe liver, pancreas, kidney, or heart toxicities [4]. In addition, after a few weeks of treatment, there is a risk to develop clinical resistance. For these reasons, it becomes essential to discover new, potent, and more selective agents for treating visceral leishmaniasis [5, 6]. In the search for finding new anti-leishmanial drugs with different mechanisms of action with fewer side effects, both synthetic and natural origin compounds among which, chalcones present in *Glycyrhiza glabra* and *Piper aduncum*, are significant sources [7, 8]. In fact, chalcones continue to draw the interest of chemists and microbiologists due to their remarkable biological activities, including the anti-leishmanial activity [9].

In this perspective, several chalcone-like compounds in which benzene ring at position 1 or 3 of the prop-2-en-1-one functional group was replaced by a heterocycle moiety, have been reported to be potential agents against *Leishmania* in biological assays [10, 11].

In recent studies, structure-activity relationships revealed structural features of potent competitive *cruzain* and *rhodesain* inhibitors in the benzimidazole series. Micromolar activity against *Trypanosoma brucei brucei* has been achieved by 1-H-2-substituted benzimidazoles with a linker containing a carbonyl group [12].

Based on these observations, in this study, anti-leishmanial assays against *L. donovani* promastigotes of 2-arylpropenon-1H-benzimidazole hybrids have been performed, which have already displayed remarkable anthelmintics, antimalarials, and anticandidosis activities [13–15]. Furthermore, the molecules reported as potent anti-leishmanial agents have been selected to investigate their activities against *T. brucei*.

2. MATERIALS AND METHODS

2.1 Chemistry

For all compounds, the 1H NMR (300 MHz) and ^{13}C NMR (75 MHz) were spectra recorded at room temperature with a Brucker Avance 300. The tetramethylsilane (TMS) was used as internal standard and chemical shift values were

recorded in ppm on the δ scale. The description of the NMR spectra uses the following symbols: singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quadruplet (q); quintet (quint); multiplet (m). Mass spectra were recorded on a JEOL JMS DX300 spectrometer in ESI (electrospray ionization/quadrupole). All melting points (mp) were determined using a Kofler bench and are uncorrected. The thin layer chromatography (TLC) was carried out on silica plates Macherey-Nagel 60F254 (40– 63 μm) or alumina Macherey-Nagel ALOX N/UV254.

The revelation of products was performed under a UV light source (254 nm). The solvents and reagents including benzaldehydes were purchased from Acros Organics (France) or Aldrich (France). Commercial reagents were used without further purification. No attempt was made to optimize the yields. The purification of the final product was made by chromatography on silica gel or recrystallized in an appropriate solvent. Pentamidine standard antiparasitic powder was purchased from SIGMA Chemical Co. (USA).

2.1.1 Synthesis of 5'-substituted-2-acetylbenzimidazoles

As described in our previous work [14, 15], the access to 5-substituted-2-acetylbenzimidazole required the prior synthesis of 2-(α -hydroxy) ethyl benzimidazoles. In this perspective, O-phenylenediamine (**1a-c**) (0.042 mol) suitably chosen, with lactic acid (2) (0.060 mol) in presence of 4N hydrochloric acid (50 mL) were refluxed on the water for 45 min. After completion of the reaction (monitored by TLC), the reaction mixture was cooled and neutralized with ammonia solution to get a solid which is separated through filters and recrystallized from absolute ethanol to afford **2a-c** in pure form (**Scheme 1**). Then, this crude material was used for the synthesis of 2-acetyl benzimidazoles (**3a-c**).

To a solution of 2-hydroxyethylbenzimidazole (**2a-c**) (0.022 mol) in acetic acid (30 mL) was wisely dropped to the aqueous solution of potassium dichromate (0.011 mol) and aqueous H_2SO_4 (25%, 8 mL) with constant stirring then heated at reflux for 45 minutes. After completion of the reaction, the mixture neutralized with NH_3 solution (1:1) gave a precipitate which was filtered off, washed with water and dried, recrystallized from ethyl acetate to afford 2-acetylbenzimidazole (**3a-c**) in pure form (**Scheme 1**).

1-(5-chloro-1H-benzo[d]imidazol-2-yl)-1-ethanone (**3a**)

Yellow solid, yield 75%, m.p.: 185–187°C. ^1H NMR (DMSO-d6) δ : 13.40 (s, 1H, N-H), 7.72 (s, 1H, H4), 7.43 (d, $J=7.4$ Hz, 1H, H7), 7.21 (m, $J=7.2$ Hz, 1H, H6), 2.68 (s, 3H, CH_3). ^{13}C NMR (DMSO-d6, δ ppm): 191.2 (C=O), 161.2 (C-Cl), 149.3 (C=N), 145.0 (Car), 138.8 (Car), 122.4 (Car), 117.1 (Car), 116.0 (Car), 25.9 (CH_3). MS m/z for $\text{C}_9\text{H}_7\text{ClN}_2\text{O}$ calc. 195 [M+H]⁺, found 195.02.

1-(5-Fluoro-1H-benzo[d]imidazol-2-yl)-1-ethanone (**3b**)

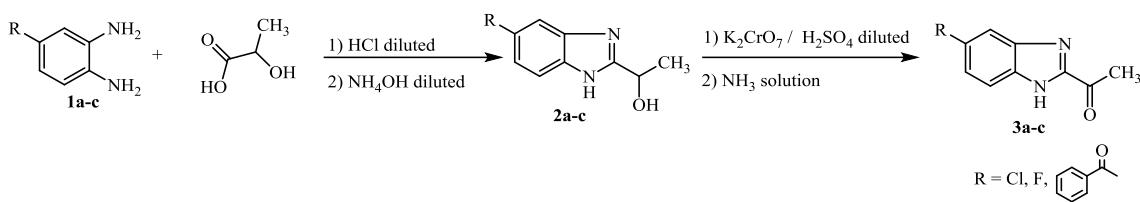
Yellow solid, Yield = 73%, ^1H NMR (DMSO-d6, δ ppm): 13.40 (s, 1H, N-H); 7.71 (s, 1H, H4); 7.43 (d, $J=7.4$ Hz, 1H, H7); 7.23 (m, $J=7.2$ Hz, 1H, H6); 2.68 (s, 3H, CH_3). ^{13}C NMR (DMSO-d6, δ ppm): 191.1 (C = O), 161.3 (C-F), 149.3 (C=N), 145.2 (Car), 138.3 (Car), 122.4 (Car), 117.4 (Car), 116.9 (Car), 25.9 (CH_3). MS m/z for $\text{C}_9\text{H}_7\text{FN}_2\text{O}$ calc. 179 [M+H]⁺, found 179.05.

1-(5-Benzoyl-1H-benzo[d]imidazol-2-yl)-1-ethanone (**3c**)

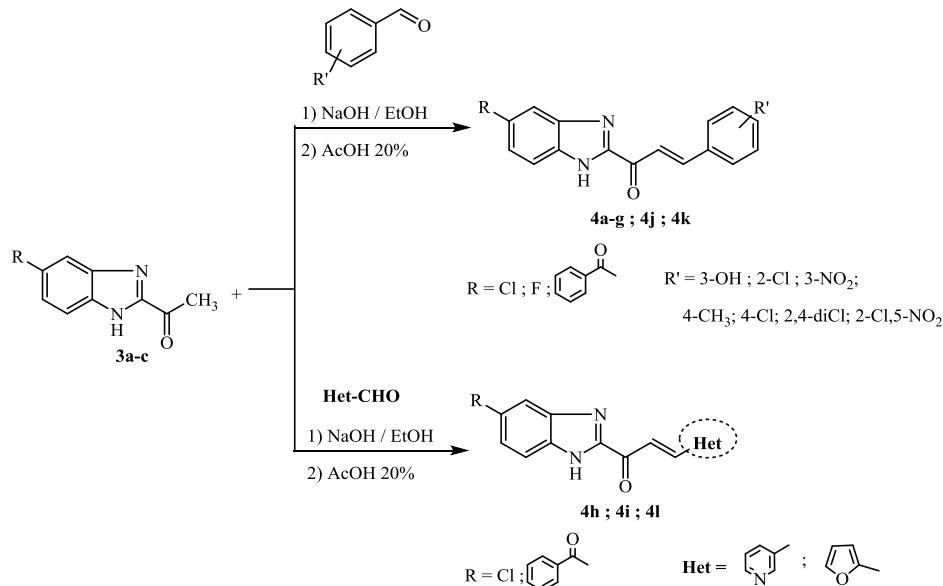
Yellow solid, Yield = 68%; ^1H NMR (DMSO-d6, δ ppm): 13.50 (s, 1H, N-H); 8.04 (s, 1H, H4); 7.77 - 7.68 (m, 5H, Har); 7.59 (d, $J=7.4$ Hz, 1H, H7); 7.55 (m, 1H, Har); 2.72 (s, 3H, CH_3). ^{13}C NMR (DMSO-d6, δ ppm): 195.5 (C=O), 191.4 (C=O), 151.1 (C=N), 134.2 (Car), 132.4 (Car), 131.3 (Car), 129.5 (Car), 129.1 (Car), 129.1 (2 Car), 128.6 (2 Car), 124.9 (Car), 122.6 (Car), 113.8 (Car), 26.2 (CH_3). MS: for $\text{C}_{16}\text{H}_{12}\text{N}_2\text{O}_2$ calc. 265 [M+H]⁺, Found 265.09.

2.1.2 General method of preparation of the benzimidazolyl-chalcones

To a mixture of an ethanolic solution of sodium hydroxide (75 mmol of sodium hydroxide in 40 mL of ethanol) and 2-acetyl benzimidazole (**3a-c**) (0.01 mol), was added an aromatic benzaldehyde properly chosen (0.011 mol). The mixture was constantly stirred at room temperature for 3 to 5 h. After completion of the reaction which monitored by TLC, the neutralization of the cooling medium was carried out with a solution of acetic acid to 30% to get a solid which is filtered, dried, and recrystallized from water/Ethanol (1:1) to get benzimidazolyl-chalcones or 1-(1H-benzimidazol-2-yl)-3-(substituted phenyl) prop-2-en-1-ones (**4a-l**) in pure form (**Scheme 2**).



Scheme 1. Synthesis of 2-acetyl benzimidazoles (3a-c)



Scheme 2. Synthesis of benzimidazolyl-chalcones (4a-l)

1- (5-chloro-1H-benzimidazol-2-yl)phenylprop-2-en-1-one (4a)

Pale yellow solid, m.p.: 227–239°C, yield 78%. ¹H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, NH); 8.10 (s, 1H, H4); 7.97 (d, 1H, J = 16 Hz, CH=CH); 7.85 (m, 2H, Ph-2,6); 7.76 (m, 1H, H7) ; 7.46-7.51 (m, 3H, Ph-3,4,5); 7.25 (m, 1H, H6); 6.77 (d, 1H, J = 16 Hz, CH=CH). ¹³C NMR (75MHz, DMSO-d6) δ: 181.2 (C=O), 153.7 (CH=CH), 144.0 (C2), 143.8 (C3a), 142.6 (C7a), 141.3 (Ph-1), 139.9 (C5), 128.8 (Ph-3,5), 124.6 (Ph-2,6), 122.7 (Ph-4), 122.5 (C6), 121.7 (CH=CH), 117.0 (C7). MS: for C₁₆H₁₁ClN₂O₂ calc. 299.0467 [M+H]⁺, found 299.0513.

1-(5-chloro-1H-benzo[d]imidazol-2-yl)-3-(3-hydroxyphenyl)prop-2-en-1-one (4b)

Yellow solid, m.p.: 212–214 °C, yield 66%. ¹H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 8.03 (d, 1H, J = 8.4 Hz, OH-Ph-3); 8.15 (s, 1H, H4); 7.75 (d, 1H, J = 16 Hz, CH=CH); 7.51 - 7.45 (m, 1H, H7); 7.32 - 7.25 (m, 2H, Ph-5,6); 7.24 - 7.18 (m, 2H, H6, Ph-2); 6.86 - 6.81 (m, 1H, Ph-4); 6.80 (d, 1H, J = 16 Hz, CH=CH). ¹³C NMR

(75MHz, DMSO-d6) δ: 180.6 (C=O), 161.2 (OH-Ph-3), 158.0 (CH=CH), 152.6 (C2), 143.8 (C3a), 141.7 (C7a), 139.3 (Ph-1), 135.2 (Ph-5), 130.1 (C5), 124.2 (C6), 122.7 (Ph-6), 122.5 (CH=CH), 121.6 (Ph-2), 118.2 (C7), 117.0 (C4), 116.7 (Ph-4). MS: for C₁₆H₁₁ClN₂O₂ calc. 299.0467 [M+H]⁺, found 299.0513.

1-(5-chloro-1H-benzo[d]imidazol-2-yl)-3-(2-chlorophenyl)prop-2-en-1-one (4c)

Yellow solid, m.p.: 118–120°C, yield 78%. ¹H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 8.12 (s, 1H, H4); 8.08 (d, 1H, J = 16 Hz, CH=CH); 7.97 - 7.92 (m, 1H, Ph-3); 7.79 – 7.81 (m, 1H, H7); 7.75 - 7.72 (m, 2H, Ph-5,6); 7.69 - 7.66 (m, 1H, H6); 7.55 - 7.51 (m, 1H, Ph-4) ; 7.50 - 7.42 (d, 1H, J = 16 Hz, CH=CH). ¹³C NMR (75MHz, DMSO-d6) δ: 180.1 (C=O), 153.7 (Ph-3), 145.3 (CH=CH), 143.8 (C2), 141.7 (C3a), 141.1 (C7a), 139.3 (Ph-1), 135.2 (Ph-5), 130.7 (C5), 130.1 (C6), 129.2 (CH=CH), 122.7 (Cl-Ph-2), 122.5 (Ph-6), 121.6 (C7), 120.8 (C4), 117.0 (Ph-4). MS: for C₁₆H₁₀Cl₂N₂O calc. 317.0169 [M+H]⁺, found 317.0257.

1- (5-chloro-1H-benzimidazol-2-yl) -3-pyridin-3-ylprop-2-en-1-one (4d**)**

Yellow solid, m.p.: 187–189 °C, yield 61%. ¹H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 9.07 (s, 1H, Pyr-2); 8.96 (m, 1H, Pyr-4); 8.10 (s, 1H, H4); 8.08 - 8.05 (m, 2H, Pyr-6 and CH=CH); 7.51 - 7.48 (m, 1H, H7); 7.32 - 7.27 (m, 1H, Pyr5); 7.14 - 7.10 (m, 1H, H7); 7.85 - 7.82 (m, 1H, H6); 7.70 (d, 1H, J = 16 Hz, CH=CH). ¹³C NMR (75MHz, DMSO-d6) δ: 181.0 (C=O), 154.5 (Pyr-4), 151.1 (Pyr-2), 150.2 (C2), 145.0 (C3a), 143.8 (CH=CH), 141.7 (C7a), 139.2 (Ph-6), 133.0 (Pyr-1), 127.6 (C5), 124.2 (CH=CH), 122.6 (C6), 121.7 (Pyr-5), 120.8 (C7), 117.5 (C4). MS: for C₁₅H₁₀ClN₃O calc. 284.0484 [M+H]⁺, found 284.0588.

1-(5-chloro-1H-benzo[d]imidazol-2-yl)-3-(3-nitrophenyl)prop-2-en-1-one (4e**)**

Yellow solid, m.p.: 212–214 °C, yield 66%. ¹H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 8.15 (s, 1H, H4); 8.11 (s, 1H, Ph-2); 7.98 - 7.95 (m, 1H, Ph-4); 7.76 - 7.72 (m, 1H, Ph-6); 7.57 (d, 1H, J = 16 Hz, CH=CH); 7.49 - 7.45 (m, 2H, Ph-5 and H7); 7.32 - 7.25 (m, 1H, H6); 6.83 (d, 1H, J = 16 Hz, CH=CH). ¹³C NMR (75MHz, DMSO-d6) δ: 185.1 (C=O), 151.8 (NO₂-Ph-3), 148.7 (CH=CH), 142.6 (C2), 138.8 (C3a), 135.3 (Ph-1), 133.7 (C7a), 132.7 (Ph-6), 132.2 (Ph-5), 130.2 (C5), 126.6 (C6), 125.7 (Ph-4), 123.9 (Ph-2), 122.5 (CH=CH), 118.2 (C7), 117.3 (C4). MS: for C₁₆H₁₀ClN₃O₃ calc. 328.6654 [M+H]⁺, found 328.6604.

1-(5-chloro-1H-benzo[d]imidazol-2-yl)-3-(p-tolyl)prop-2-en-1-one (4f**)**

Green solid, m.p.:> 260 °C, yield 76%. ¹H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 8.12 (s, 1H, H4); 7.62 (d, 1H, J = 16 Hz, CH=CH); 7.55 - 7.47 (m, 2H, Ph-2,6); 7.44 - 7.41 (m, 1H, H7); 7.39 - 7.33 (m, 2H, Ph-3,5); 7.23 - 7.20 (m, 1H, H6); 6.82 (d, 1H, J = 16 Hz, CH=CH); 2.51 (s, 3H, CH₃-Ph-4). ¹³C NMR (75MHz, DMSO-d6) δ: 182.4 (C=O), 144.0 (CH=CH), 142.5 (C2), 141.7 (C3a), 141.1 (Ph-4), 139.6 (C7a), 133.2 (Ph-1), 130.8 (C5), 130.1 (Ph-5), 129.9 (Ph-3), 129.3 (Ph-2), 129.2 (Ph-6), 123.7 (C-6), 121.5 (CH=CH), 117.2 (C7), 116.3 (C4), 25.7 (CH₃-Ph-4). MS: for C₁₇H₁₃ClN₂O calc. 297.0668 [M+H]⁺, found 297.0755.

1-(5-chloro-1H-benzo[d]imidazol-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one (4g**)**

Yellow solid, m.p.: 242–244 °C, yield 83%. ¹H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H);

8.12 (s, 1H, H4); 7.88 (d, 1H, J = 16 Hz, CH=CH); 7.77-7.65 (m, 2H, Ph-2,6); 7.59 - 7.53 (m, 2H, Ph-3,5); 7.48 -7.43 (m, 1H, H7); 7.25 - 7.20 (m, 1H, H6); 6.85 (d, 1H, J = 16 Hz, CH=CH). ¹³C NMR (75MHz, DMSO-d6) δ: 182.5 (C=O), 145.0 (CH=CH), 142.2 (C2), 141.7 (C3a), 139.3 (C7a), 140.1 (Ph-4), 139.5 (Ph-1), 130.8 (C5), 130.3 (Ph-2), 130.2 (Ph-6), 129.7 (Ph-3), 129.5 (Ph-5), 124.5 (C-6), 121.7 (CH=CH), 116.9 (C7), 116.1 (C4). MS: for C₁₆H₁₀Cl₂N₂O calc. 317.0234 [M+H]⁺, found 317.0288.

1-(5-chloro-1H-benzo[d]imidazol-2-yl)-3-(2,4-dichlorophenyl)prop-2-en-1-one (4h**)**

Yellow solid, m.p. 262–264°C, yield 56%. ¹H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 8.12 (s, 1H, H4); 7.77 (d, 1H, J = 16 Hz, CH=CH); 7.71 - 7.65 (m, 2H, Ph-3,6); 7.59 - 7.53 (m, 1H, H7); 7.46 - 7.41 (m, 1H, Ph-5); 7.21 - 7.18 (m, 1H, H6); 6.78 (d, 1H, J = 16 Hz, CH=CH). ¹³C NMR (75MHz, DMSO-d6) δ: 181.5 (C=O), 144.1 (CH=CH), 142.4 (C2), 141.8 (C3a), 140.9 (C7a), 140.1 (Ph-6), 139.5 (Ph-1), 133.3 (Ph-2), 131.8 (C5), 130.5 (Ph-5), 130.2 (Ph-3), 129.8 (Ph-4), 125.3 (C-6), 121.9 (CH=CH), 117.3 (C7), 115.9 (C4). MS: for C₁₆H₉Cl₃N₂O calc. 350.9782 [M+H]⁺, found 350.9801.

1- (5-chloro-1H-benzo [d] imidazol-2-yl) -3-(furan-2-yl) prop-2-én-1-one (4i**)**

Brown solid, m.p.: 237–239 °C, yield 61%. ¹H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 8.32 (s, 1H, H4); 8.17- 8.14 (m, 1H, Fur-5); 7.80 - 7.78 (m, 1H, Fur-3); 7.65 (d, 1H, J = 16 Hz, CH=CH); 7.48 – 7.43 (1H, m, H7); 7.10 – 7.07 (m, 1H, H6); 6.82 – 6.79 (m, 1H, Fur-4); 6.72 (d, 1H, J = 16 Hz, CH=CH). ¹³C NMR (75MHz, DMSO-d6) δ: 183.0 (C=O), 151.2 (Fur-1); 144.5 (Fur-5), 141.2 (C2), 140.8 (C3a), 138.7 (C7a); 128.6 (C5), 126.8 (CH=CH), 123.6 (C6), 122.2 (CH=CH), 117.5 (C7), 116.8 (C4); 113.7 (Fur-3), 112.6 (Fur-4). MS: for C₁₄H₉ClN₂O₂calc. 273.0823 [M+H]⁺, found 273.0724.

3-(2-chlorophenyl)-1-(5-fluoro-1H-benzo[d]imidazol-2-yl)prop-2-en-1-one(4j**)**

Yellow solid, m.p.: 218–220 °C, yield 58%. ¹H NMR (300MHz, DMSO-d6) δ: 13.50 (s, 1H, N-H); 8.02 (d, 1H, J = 16 Hz, CH=CH); 7.59 – 7.51 (m, 2H, H7 and Ph-3); 7.49 – 7.43 (s, 1H, H4); 7.22 – 7.18 (m, 2H, Ph-5,6); 7.02 – 6.97 (m, 1H, Ph-4); 6.89 -6.86 (m, 1H, H6); 6.59 (d, 1H, J = 16 Hz, CH=CH). ¹³C NMR (75MHz, DMSO-d6) δ: 183.1 (C=O), 157.8 (F-C5), 146.2 (CH=CH), 140.7

(C2), 130.1 (C3a), 153.7 (Cl-Ph-3), 145.3 (CH=CH), 141.7 (C3a), 133.9 (C7a), 133.3 (Ph-2), 132.7 (Ph-1), 129.5 (Ph-3), 128.7 (Ph-4), 127.0 (Ph-6), 126.6 (Ph-5), 120.3 (CH=CH), 117.6 (C7), 112.8 (C6), 103.5 (C4). MS: for $C_{16}H_{10}ClFN_2O$ calc. 301.0467 [M+H]⁺, found 301.0537.

1-(5-benzoyl-1H-benzo[d]imidazol-2-yl)-3-(pyridin-3-yl)prop-2-en-1-one (**4k**)

Light-yellow solid, m.p.: 185–187 °C, yield 56%. ¹H NMR (300MHz, DMSO-d6) δ: 14.0 (s, 1H, N-H); 9.02 -9.00 (m, 1H, Pyr-2); 8.91 – 8.89 (m, 1H, Pyr-4); 8.36 -8.34 (m, 1H, Pyr-6); 8.03 -8.01 (m, 1H, H7); 7.97 (s, 1H, H4); 7.91-7.89 (m, 2H, Ph-2',6'); 7.88 - 7.85 (d, 1H, J = 16 Hz, CH=CH); 7.79 - 7.73 (m, 3H, Ph-3',4',5'). 7.72 – 7.68 (m, 2H, H6 and Pyr-5); 7.64 (d, 1H, J = 16 Hz, CH=CH). ¹³C NMR (75MHz, DMSO-d6) δ: 195.4 (C=O), 180.7 (C=O), 154.0 (Pyr-4), 151.1 (Pyr-2); 150.8 (C7a), 144.9 (C2), 137.6 (CH=CH), 135.4 (C3a), 133.0 (Pyr-6); 132.4 (C-5); 131.4 (Ph-4'), 131.2 (Pyr-1), 129.5 (Ph-2'); 129.1 (Ph-6'); 129.0 (Ph-3'); 127.1 (Ph-5'); 126.2 (CH=CH); 124.2 (C6); 122.5 (Pyr-5); 121.7 (C4), 120.9 (C7). MS: for $C_{22}H_{15}N_3O_2$ calc. 354.1197 [M+H]⁺, found 354.1210.

1-(5-benzoyl-1H-benzo[d]imidazol-2-yl)-3-(2-chloro-5-nitrophenyl)prop-2-en-1-one (**4l**)

Yellow solid, m.p.: 208–210 °C, yield 78%. ¹H NMR (300MHz, DMSO-d6) δ: 14.0 (s, 1H, N-H); 8.54 (s, 1H, H4); 8.20 – 8.17 (m, 2H, H7 and Ph-4); 8.04 -8.01 (s, 1H, Ph-6); 7.96 -7.94 (m, 2H, Ph-2',6'); 7.88-7.85 (d, 1H, J = 16 Hz, CH=CH); 7.79 -7.77 (m, 1H, Ph-3); 7.76-7.73 (m, 3H, Ph-3',4',5'); 7.72 -7.68 (1H, m, H6); 7.64 (d, 1H, J = 16 Hz, CH=CH). ¹³C NMR (75MHz, DMSO-d6) δ: 195.6 (C=O), 180.3 (C=O), 151.1 (Ph-5), 146.7 (CH=CH), 144.7 (C7a), 137.6 (C2), 135.1 (Ph-2), 133.0 (C3a), 132.4 (Ph-1), 131.4 (C5), 131.3 (C6), 130.7 (Ph-4'), 129.9 (Ph-3), 129.6 (Ph-2',6'), 129.0 (Ph-3',5'), 127.0 (Ph-4), 126.3 (C-6), 122.5 (Ph-6), 121.7 (CH=CH), 121.2 (C4), 119.7 (C7). MS: for $C_{23}H_{14}ClN_3O_4$ calc. 432.1023 [M+H]⁺, found 432.0712.

2.2 Biological Study

2.2.1 *In vitro* antileishmanial activity

The antileishmanial activity of the synthesized compounds was evaluated *in vitro* against the *L. donovani* LV9 (MHOM/ET/1967/L82) strain, according to a method based on the tetrazolium-

dye, allowing the measurement of the mitochondrial deshydrogenase activity, and therefore the determination of an IC₅₀ value, corresponding to the 50% reduction of the mitochondrial activity, as described by Abada et al. [16]. Promastigotes were cultivated in HEPES (25 mM)-buffered RPMI 1640 medium enriched with 10% fetal calf serum (FCS) and 40 mg / mL gentamicin at 27°C in a dark environment.

The screening was performed in flat-bottomed 96-well plastic tissue-culture plates maintained at 27 °C. The tested compounds were dissolved in dimethylsulfoxide and diluted with RPMI to lower the final solvent concentration to less than 2% (v / v) and obtain different concentrations up to 0.08 mg / L. Each concentration was screened in triplicate. The promastigotes forms from an exponential growth phase culture (approximately 72 hours after cultivation) were captured. In each well, 195µL of the suspension of promastigote stage parasites corresponding to a density of 2,105 were used, and then incubated in an oven at 27° C for 1 h before adding drug. After this incubation, each tested compound was added at a rate of 5 µL per well for 4 hours. Three wells are used for each concentration, and the tests are performed three times. The evaluation of anti-leishmanial activity, expressed as an IC₅₀, was carried out by two methods. The first method is a direct reading under an optical microscope and by comparison with negative controls. This qualitative test evaluates the vitality of the leishmanias in each well based on three criteria: (1) the number of parasites present in each well compared to the controls, (2) the mobility of the parasites and (3) the shape of the cells. The second method or Microculture Tetrazolium Assay (MTA) is a quantitative colorimetric test based on the use of tetrazolium salts. The viability of the promastigotes was assessed by the Tetrazolium-dye (MTT) colorimetric method. The results are expressed as the effective concentrations inhibiting parasite growth by 50% (IC₅₀) after a 3-day incubation period. The pentamidine was used as reference compound.

2.2.2 *In vitro* trypanocidal activity

The tests were carried out at the Antiparasitic Chemotherapy Laboratory of the Faculty of Pharmacy of Châtenay-Malabry UMR CNRS 8076 BioCIS, using the GVR 35 strain (Glasgow Veterinary Research) of *Trypanosoma brucei brucei*, frozen in liquid nitrogen. The parasites were adequately diluted using culture medium to obtain 200,000 trypanosomes per mL. The

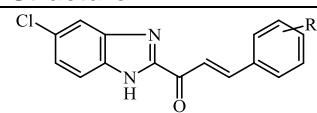
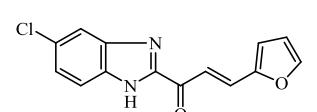
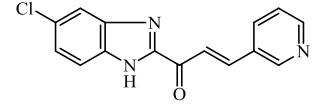
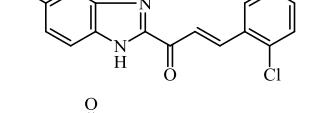
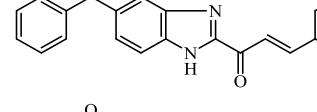
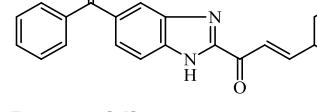
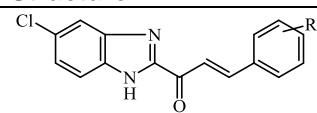
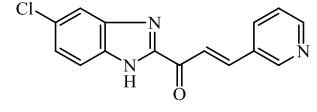
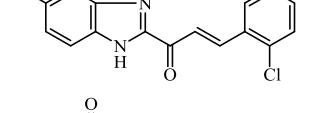
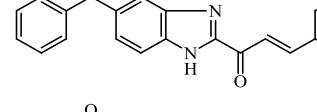
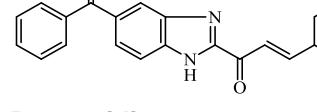
circulating forms of the parasite were cultured *in vitro* without loss of their infectivity, for 24 hours at 37°C in an air atmosphere containing 5% CO₂. The culture medium was composed of β-mercaptoethanol (0.2 mM): 1.4 μL; hypoxanthine: 1.36 mg; thymidine: 0.387 mg; sodium pyruvate: 22 mg; Hepes: 650 mg; glucose: 100 mg; NaHCO₃: 220 mg; de complemented horse serum: 15 mL; gentamicin: 5 mg; "MEM (Minimum Essential Medium) non essential I amino acids": 1 mL; "MEM with Earle's salts" and L-glutamine: qsp 100 mL. The parasites were distributed in a 96-well plate of 200 μL at a rate of 2,105 per mL. Then, 5 μL of the appropriate dilution of the compounds in DMSO are added, each concentration being tested in triplicate. The control wells only received DMSO (5 μL, ie 2.5%). After 24 hours of incubation, the viability of the trypanosomes was estimated by direct observation using an optical

microscope. Experiments were performed in triplicate and repeated three times. The test results were expressed as a lethal concentration killing all the parasites in the wells after 24 hours (LC₁₀₀, Lethal Concentration) after microscope observation. Melarsoprol was used as reference drug.

3. RESULTS AND DISCUSSION

The antileishmanial activities of the benzimidazolyl-chalcones were assessed by evaluating their inhibition activity against promastigote forms of *Leishmania donovani* strains to determine their effective concentration (IC₅₀) values. The results of the antileishmanial screening of the twelve derivatives synthesized and antileishmanial drug pentamidine, used as a standard for comparison purpose is presented in Table 1.

Table 1. Antileishmanial activities of 4a-4l compounds and reference drug against *Leishmania donovani*

Structure	Compounds	R	IC ₅₀ (μM) ± SD
	4a	H	0.53± 0.05
	4b	3-OH	0.50± 0.05
	4c	2-Cl	0.47± 0.04
	4d	3-NO ₂	74.45± 7.44
	4e	4-CH ₃	1.04± 0.10
	4f	4-Cl	24.59± 2.46
	4g	2,4-diCl	1.79± 0.18
	4h		1.14± 0.11
	4i		13.79± 1.38
	4j		1.33± 0.13
	4k		100.80± 10.08
	4l		43.07± 4.30
Pentamidine			7.60± 0.76

*Mean ± SD= Mean values ± Standard deviation of means of three experiments

A structure-activity relationship was observed for all twelve compounds (**4a–4l**) where the nature of the C-5 group of benzimidazole as well as the presence of a heterocycle, or hydroxyl groups, or an electron-withdrawing group in the arylpropenone functional group of chalcones may affect the antileishmanial activity. It appears that all of the compounds exhibit antileishmanial activities. Indeed, the first derivative **4a** showed excellent inhibitory potential ($IC_{50} = 0.53 \mu M$) when compared with a standard drug (pentamidine) having an IC_{50} value of $7.6 \mu M$. A SAR analysis indicates that first, hydroxyl group in compound **4b** increases the activity ($IC_{50} = 0.5 \mu M$). This finding agrees with studies reported by Aponte et al. [10] and by Brezan et al. [17] in which the leishmanicidal activity of chalcones and coumarins was found to be enhanced by the presence of polar hydroxyl groups.

Secondly, the position of halogen atoms on the phenylpropenone impacted diversely the activity. For instance, the 2'-chloro derivative **4c** had a higher activity ($IC_{50} = 0.47 \mu M$), while the 4'-chloro derivative **4f** had a lower activity ($IC_{50} = 24.59 \mu M$). In addition, introducing two chlorine atoms on phenyl ring (**4g**) didn't improve the activity as much as expected. Based on their activities, the chlorine derivatives of the series can be classified as follows 2-Chloro >2,4-dichloro > 4-Chloro, with IC_{50} of 0.47; 1.79 and $24.59 \mu M$, respectively. However, 3-nitro analogue **4d** was found to be the least active among the 5-chlorobenzimidazole series ($IC_{50} = 74.45 \mu M$). Thus, this observation suggests that the presence of an electron-withdrawing group such as nitro does not allow efficient binding with the target on *Leishmania donovani*.

Likewise, the replacement of the benzene nucleus of phenylpropenone chain with its pyridine-type isostere (**4l**) or its furyl-type isostere (**4h**) didn't improve their activities. These heterocyclic derivatives of compound **4a**, demonstrated moderate anti-leishmanial activities with IC_{50} of 13.79 and $1.14 \mu M$, respectively. Moreover, changing chlorine atom of **4c** ($IC_{50} = 0.47 \mu M$) to fluorine (compound **4j**) decreased the activity ($IC_{50} = 1.33 \mu M$). However, this anti-leishmanial activity of **4j** remains greater than that of pentamidine ($IC_{50} = 7.6 \mu M$). Ultimately, changing chlorine group at position 5 of **4l** to benzoyl (**4i**) led to total loss of the activity ($IC_{50} = 100.8 \mu M$). Similarly, the concomitant presence of a nitro group at 5' position and chlorine at 2' position of the phenyl ring of **4k** compound didn't ameliorate the

activity. In this series, seven of the tested compounds (**4a–c**, **4e,4g**, **4h**, **4j**) exhibited remarkable inhibitory activity with IC_{50} ranging from 0.5 to $1.8 \mu M$. These compounds were at least 4-fold more active against *L. donovani* strain than pentamidine ($7.6 \mu M$).

In order to study the trypanocidal properties of our series of benzimidazole-chalcones on bloodstream form *T. brucei*, we selected two compounds among the 5-chlorobenzimidazole-chalcones (**4a** and **4c**) which exhibited potent activity ($IC_{50} < 1 \mu M$) against *L. donovani* and one derivative (**4d**) less effective against *L. donovani* ($IC_{50} > 50 \mu M$).

The trypanocidal activities of the selected 5-chlorobenzimidazole-phenylpropenones, expressed in lethal concentration (LC_{100}) are shown in **Table 2**.

Table 2. Trypanocidal activity against *Trypanosoma brucei brucei* of selected compounds

Compounds	R	$LC_{100} (\mu M)$
4a	H	2
4c	2-Cl	1
4d	3-NO ₂	50
Melarsoprol		0.5

As regards the antitrypanosomal activity, compound with substituted halogens at the benzene nucleus (**4c**) showed significant activity among all three tested compounds. The nitro electron-withdrawing group of the phenyl group (**4d**), resulted in a weak trypanocidal activity.

Interestingly, results of trypanocidal activity showed that selected compounds previously active against *L. donovani* (**4a** and **4c**) are also active on trypanosomes. Likewise, compound **4d** was found to be moderately active against *Leishmania donovani* as well as on *Trypanosoma brucei brucei*, with a LC_{100} value of $50 \mu M$. Based on these considerations, the data suggest that in the 5-chlorobenzimidazole-chalcone series, compounds effective against *L. donovani* are likely effective against *T. brucei*. In addition, those which have shown modest activity against *L. donovani* will show little inhibition on the trypanosome. It is worth highlighting that compound **4c** ($LC_{100} = 1 \mu M$) was found to be the most active of the tested compounds. However, the standard drug, melarsoprol, was slightly more active against *Trypanosoma b. b* ($LC_{100} = 0.5 \mu M$).

4. CONCLUSION

We have reported the synthesis of 2-arylpropenone-benzimidazole hybrids and their antiprotozoal screening. Interestingly, seven of the tested compounds displayed strong inhibitory activity against *Leishmania donovani* promastigotes. General investigation of SAR established that chalcone hybrids of 5-chlorobenzimidazole furnished in general highly active compounds against *L. donovani* ($IC_{50} < 25\mu M$). Accordingly, hybrids **4c**, **4b**, and **4a** with respective IC_{50} values of 0.47, 0.5, and 0.53 μM , are potential candidates for antileishmanial drug development based on their excellent activity showed against Leishmania parasites compared to the reference drug pentamidine. In addition, the lead compound for trypanocidal activity was the 2-chlorine derivative (**4c**) of these benzimidazole-chalcones. Although **4c** demonstrated promising efficacy with micromolar activity against the two parasites tested (*L. donovani* and *T. brucei*), comparable or better than the reference, further studies on a large number of compounds are needed to validate the *in vitro* activity against *Trypanosoma brucei* obtained here. Overall, the tests performed demonstrated that benzimidazole-chalcones are antiparasitic candidates with significant activity against *L. donovani* and *T. brucei*. Thus, the results of the present work suggest halogenated C-5 benzimidazoles carrying the arylpropenone functional group in C-2 as a new pharmacophore in the search for new drugs against the human pathogenic protozoa responsible for leishmaniasis and trypanosomiasis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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