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In vitro activity of new *N*-benzyl-1*H*-benzimidazol-2-amine derivatives against cutaneous, mucocutaneous and visceral *Leishmania* species

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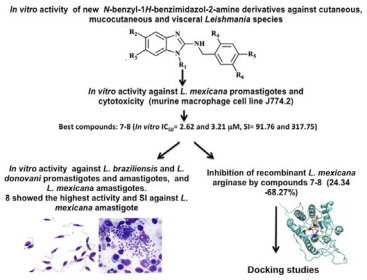
HIGHLIGHTS

- A series of 28 *N*-benzyl-1*H*-benzimidazol-2-amine derivatives were synthesized.
- Compounds **7** and **8** were very active against *L. mexicana* promastigotes and amastigotes.
- Compound **8** had high selectivity index against *L. mexicana* and *L. braziliensis*.
- Compound **8** inhibited 68.27% the activity of recombinant *Leishmania mexicana* Arginase.
- Compound **8** is a good scaffold for the development of new antileishmanial agents.

GRAPHICAL ABSTRACT



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ABSTRACT

The identification of specific therapeutic targets and the development of new drugs against leishmaniasis are urgently needed, since chemotherapy currently available for its treatment has several problems including many adverse side effects. In an effort to develop new antileishmanial drugs, in the present study a series of 28 *N*-benzyl-1*H*-benzimidazol-2-amine derivatives was synthesized and evaluated *in vitro* against *Leishmania mexicana* promastigotes. Compounds **7** and **8** with the highest antileishmanial activity (micromolar) and lower cytotoxicity than miltefosine and amphotericin B were selected to evaluate their activity against *L. braziliensis* 9and *L. donovani,* species causative of mucocutaneous and visceral leishmaniasis, respectively. Compound **7** showed significantly higher activity against *L. braziliensis* promastigotes than compound **8** and slightly lower than miltefosine. Compounds **7** and **8** had IC_{50} values in the micromolar range against the amastigote of *L. mexicana and L. braziliensis.*

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Selectivity Index
Arginase

However, both compounds did not show better activity against *L. donovani* than miltefosine. Compound **8** showed the highest SI against both parasite stages of *L. mexicana*. In addition, compound **8** inhibited 68.27% the activity of recombinant *L. mexicana* arginase (LmARG), a therapeutic target for the treatment of leishmaniasis. Docking studies were also performed in order to establish the possible mechanism of action by which this compound exerts its inhibitory effect. Compound **8** shows promising potential for the development of more potent antileishmanial benzimidazole derivatives.

1. Introduction

Leishmaniasis is a disease caused by several species of the genus *Leishmania,* having a wide range of hosts, including man. It is considered a neglected tropical disease (NTDs), affecting mainly developing countries. The World Health Organization (WHO) estimates that approximately 350 million people are living in areas characterized by active transmission of *Leishmania,* with 14 million people directly affected by the disease. There are three main types of leishmaniasis: cutaneous (CL), the most common form of the disease, causes ulcers, leading to disfigurement, permanent scars and in some cases disability; mucocutaneous (MCL), the most destructive form of the disease, causes partial or total mutilation of mucous membranes in the nose, mouth and throat; and visceral kala–azar (VL), the most severe form of the disease, fatal if left untreated. It is estimated that there are 300,000 cases of VL and more than 20,000 to 40,000 annual deaths from this form of the disease; in the case of CL, it has been reported over one million cases around the world in the last 5 years (WHO, 2016).

The main drugs available for the treatment of leishmaniasis are the pentavalent antimonials (SbV), Sodium Stiboglucanate (Pentostam), Meglumine Antimoniate (Glucantime), and Amphotericin B (Fungizone). Amphotericin B is the first drug of choice for visceral leishmaniasis in regions with high resistance to treatment with SbV. Miltefosine is the most recent antileishmanial drug in the market and the first effective oral treatment against VL, being recommended as first line drug for childhood VL (Freitas-Junior et al., 2012). Chemotherapy currently available for leishmaniasis treatment is far from satisfactory and has several problems including many adverse side effects, high costs and toxicity (Savoia, 2015; Varela-M et al., 2012). Furthermore, drug resistance to all known antileishmanial drugs has been reported (Bhattacharya et al., 2016; Fernandes et al., 2016: Mondelaers et al., 2016; Shaw et al., 2016; Coelho et al., 2014; Kumar et al., 2014). Therefore, the identification of specific therapeutic targets and the development of new drugs are urgently needed.

In this regard, benzimidazole derivatives are of wide interest because of their biological activities and clinical applications (Alhtar et al., 2016). Their use as antibacterial, antifungal, antimalarial, antileishmanial as well as anti-inflammatory and anticancer agents was reviewed by Keri et al. (2015). Our research group has demonstrated the antiprotozoal activity of benzimidazole derivatives against *Giardia intestinalis*, *Entamoeba histolytica*, *Trichomonas vaginalis*. *L. mexicana* and *Trypanosoma cruzi* (Melchor-Doncel de la Torre et al., 2017; Velázquez-López et al., 2016; Díaz-Chiguer et al., 2012; Hernández-Luis et al., 2010; Navarrete-Vázquez et al., 2001; Valdez et al., 2002). Among them, 2-(trifluoromethyl)-1*H*-benzimidazole derivatives showed promising *in vitro* activity against *L. mexicana* with IC_{50} values in the range of 4–24 μM (Hernández-Luis et al., 2010). The antileishmanial activity of benzimidazole derivatives has also been demonstrated in other studies (Méndez-Cuesta et al., 2016; Mota et al., 2014; Oh et al., 2014). Recently, 2-arylbenzimidazole derivatives have proven to be good candidates as antileishmanial agents (Keurulainen et al., 2015); however, this kind of compounds have high carboaromatic rings and rigid systems, these properties are associated with low solubility and pharmacokinetics/pharmacodynamics problems. Besides, the target of these derivatives on *Leishmania* was not characterized.

Among druggable targets of the parasite, important for parasite survival and proliferation, is the enzyme arginase (ARG) that participates in the polyamine pathway (Balaña-Fouce et al., 2012). L-ornithine, the amino acid from which polyamines are generated, is produced from the hydrolysis of L-arginine by ARG. Inhibition of ARG by *N*-hydroxyarginine (NOHA) reduces polyamine levels in *Leishmania* amastigotes and parasite load (Iniesta et al., 2001). *Leishmania* ARG shares 39–43% identity with human ARG (Ilari et al., 2015); therefore, it is considered a therapeutic target for the treatment of leishmaniasis.

Previously, we performed a virtual screening study of ZINC database on the LmARG in order to find inhibitors. From this study, compounds with *N*-benzyl-1*H*-benzimidazole-2-amine scaffold were identified as potential LmARG inhibitors (Méndez-Cuesta et al., 2012). Inspired in these results and continuing our search for benzimidazole derivatives with antileishmanial activity, a new series of *N*-benzyl-1*H*-benzimidazol-2-amine derivatives was synthesized. The main features in these compounds are the substituent at position 1 of the benzimidazole nucleus, from C_4- alkyl groups to hydrogen; the substitution in the benzenoid ring, with or without chlorine; and a substituted benzyl group on the 2-amino to increase the flexibility of the compounds. Nine compounds are not substituted at positions 5 and/or 6 of the benzimidazole nucleus, but the others have one or two chlorine atoms at positions 5 and 6. These compounds will give information about how the activity is affected with the different substitutions, especially, when positions 5 and 6 of the benzimidazole nucleus are substituted, since it is known that these positions undergo the first step metabolism, which could increase the half-life of a possible drug.

The biological activity of the new compounds was initially evaluated against promastigotes of *L. mexicana* and those with the highest antileishmanial activity and lower cytotoxicity than miltefosine and amphotericin B were further tested against the promastigote and amastigote of *L. braziliensis*, and *L. donovani* as well as *L. mexicana* amastigotes. In addition, the effect of compounds **7** and **8** on LmARG activity was also evaluated, and in order to know how these benzimidazole derivatives inhibit the LmARG activity, additional *in silico* docking study was performed.

2. Materials and methods

2.1. Chemistry

All *N*-benzyl-1*H*-benzimidazol-2-amine derivatives **1**–**28** were synthesized by our research group thought a reductive amination method between 1*H*-benzimidazol-2-amines **29**–**39** and aldehydes **40**–**47**. The structure of target compounds is shown in

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