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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Benzimidazole derivatives: synthesis, leishmanicidal effectiveness, and molecular docking studies

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Abstract Leishmanolysin GP63 is a zinc metalloprotease, expressed at the surface of *Leishmania* promastigotes. Studies on this protein are hindered as only a limited number of effective non-toxic inhibitors of this drug target are known. Present study describes the identification of a variety of 2-aryl- and 5-nitro-2-arylbenzimidazoles as new GP63 inhibitors. All the compounds were tested for in vitro activity against the promastigote form of *Leishmania major* and showed very good activity. 2-(Thiophen-2-yl)-1*H*-benzimidazole (**19**) and 2-(1*H*-indol-3-yl)-5-nitro-1*H*-benzimidazole (**34**) with IC_{50} value of 0.62 μg/mL were identified as lead of this library. Molecular docking studies were performed on binding site of GP63 to study the binding mode of compounds. The results of both in vitro and in silico studies clearly indicated that benzimidazoles may serve as new drug candidates in the combat against leishmaniasis.

Keywords Benzimidazoles · Antileishmanial activity · *Leishmania major* · Leishmanolysin · Molecular docking · Ro5

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Introduction

Leishmaniasis is a chronic infectious disease caused by protozoan parasites belonging to the genus *Leishmania* having sandfly as its vector. *Leishmania* is digenetic and has several diverse clinical manifestations. It disseminates as self-healing cutaneous leishmaniasis to the progressive mucocutaneous and the potentially lethal visceral leishmaniasis (Murray *et al*., 2005). Leishmaniasis is endemic in more than 88 countries throughout Africa, Asia, Southern Europe, and Latin America. It has an estimated prevalence of 12 million cases worldwide, which continues to increase, over 1.5–2 million new cases each year (Ameen, 2010).

Conventional therapies used for the treatment of leishmaniasis are based on pentavalent antimonials such as sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®). However, owing to their toxic nature, requiring long term treatment and most importantly due to development of resistance, the search for new drugs has become imperative. A liposomal glycomacrolide, amphotericin B (Fungilin®), is a drug of choice for the treatment of leishmaniasis, but its cost is prohibitively high and is out of reach of poor people. More recently, miltefosine (Miltex®), an alkylphospholipid which is known to be effective in cancer chemotherapy too, has emerged as an oral leishmanicidal drug that is highly effective in children, but is plagued with the drawback of causing serious gastrointestinal complications and teratogenic effects (Murray *et al*., 2005; Ameen, 2010; Minodier and Parola, 2007; Reithinger *et al*., 2007; Singh and Sivakumar, 2004; Desjeux, 2004). Some other drugs, for example, pentamidine, edelfosine (another alkyl-phospholipid) paromomycin, tamoxifen, and simataquine are reported to give variable cure rates (Miguel *et al*., 2008; Cabrera-Serra *et al*., 2008; Singh and Sivakumar, 2004). Early this year,

Fairlamb *et al.* have identified fexinidazole, which is already in clinical trials for treating sleeping sickness, as possible new treatment for leishmaniasis (Wyllie *et al*., 2012). In spite of a variety of drugs being available, the current treatments seem to be far from ideal and innovation of new active compounds on behalf of control, and prevention of leishmaniasis is urgent necessity of the current scenario.

Besides the generation of an adequate armory of drugs to treat leishmaniasis, new and active drug targets are also required to combat the disease. Enzymes present in the parasite, but absent from their mammalian host, are considered as ideal targets for rational drug designing. One such target is pteridine reductase (PTR1) which is extensively studied, and a variety of PTR1 inhibitors are developed (Pandey *et al*., 2010). Another target for leishmaniasis is the zinc metalloprotease called leishmanolysin, also termed as GP63, MSP, and PSP (EC 3.4.24.36). It belongs to the metzincin class, symbolizing the major surface protease of *Leishmania* sp. It is the major protein constituent of the promastigote surface ($\sim 5 \times 10^5$ molecules/cell). GP63, a glycol protein of 63 kDa, contributes to parasite virulence and pathogenesis, participating in the resistance of promastigotes to complement-mediated lysis and in receptor-mediated uptake of *Leishmania*. Furthermore, promastigotes release active GP63 by autoproteolysis of GPI-linked GP63 and/or secretion of intracellular GP63. In fact, GP63 is a drug target and vaccine candidate (Bianchini *et al*., 2006; Schlagenhauf *et al*., 1998; Yao *et al*., 2003).

GP63 (PDB code: 1LML) consists of 478 amino acids and contains predominantly β-sheet secondary structure. It contains three domains: the *N*-terminal, the central, and *C*-terminal. The *N*-terminal domain consists of His264 and His268 catalytic residues within the HisGluXxxXxxHis signature. The central domain comprises the third catalytic His334 residue, a Met-turn (Asp342–Ala348), an α-helix C (H12), and the 62 amino acid insertion (Phe272–Ser333) which is unique within metzincins. The extended *C*-terminal domain of GP63 resides mainly antiparallel β-strand and random coil structure with only slight helical contributions. In the active-site of GP63, the zinc atom is coordinated to the ε-nitrogen atoms of His264, His268, and His334 at a distance of 2.18, 2.18, and 2.12 Å, respectively (Schlagenhauf *et al*., 1998).

Currently, globally, scientists are trying to explore new armors to fight multi-drug resistance. In this combat, benzimidazole received a lot of attention of researchers because they are found numerously in biologically active compounds (Preston, 1974; Touzeau *et al*., 2003; Rondu *et al*., 1997). Moreover, benzimidazole scaffold is found in various synthetic pharmaceuticals revealing a broad spectrum of biologic activity (Cedillo-Rivera and Munoz, 1992; Chavez *et al*., 1992; Navarrete-Vázquez *et al*., 2001). A recent example of fexinidazole, reported to be an effective antileishmanial drug, illustrates the significance of an imidazole ring (Wyllie *et al*., 2012). Nowadays, with the advent of fast computers and sophisticated softwares, in silico tools like molecular modeling and docking have emerged as acumen for the designing of novel scaffolds, besides providing a rationale for the results achieved in wet lab (Ivanenkov *et al*., 2009). Our research group has been engaged, for a long time, in target-based drug designing and has reported the synthesis of diverse classes of small drug-like compounds and quite a few have been identified as potential candidates for the inhibition of enzymes such as urease, cholinesterase, etc. Moreover, the leads identified in different bioassays were subjected to molecular modeling and docking studies and an attempt was made to rationalize the results obtained in different bioassays. Based on these in silico studies, some predictions for the synthesis of new enzyme inhibitors with activity were also made (Ansari *et al*., 2012, 2009; Nawaz *et al*., 2008; Zaheer-Ul-Haq *et al*., 2010). In continuation to our efforts in this drug design paradigm, a library of 2-aryl/heteroaryl-1*H*-benzimidazoles and 2-aryl/heteroaryl-5-nitro-1*H*-benzimidazoles has been synthesized and evaluated for its potential against *L. major*. Although GP63 is a virulent factor for leishmaniasis (Yao *et al*., 2003), however, molecular docking studies using this protein target have not yet been reported. Hence, molecular docking studies of the synthesized benzimidazoles were performed on leishmanolysin (PDB code: 1LML) by means of Molecular Operating Environment (MOE) software (MOE, 2010.10 2010). In the combat against multi-drug resistance, such in silico studies have played a key role in the identification of new drug targets and the designing of new scaffolds as novel drug candidates.

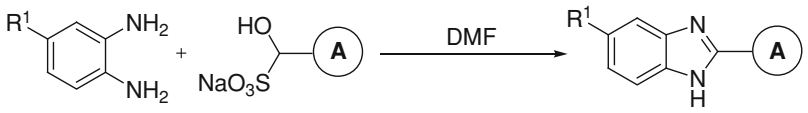
Results and discussion

Chemistry

Benzimidazoles are generally synthesized by the Philips reaction; but, in the case of 2-aryl-benzimidazole, the yield of the reaction is very low and condition of the reaction is also harsh (Hein *et al*., 1957). Another route involves the initial formation of sodium metabisulfite adducts by the reaction of aryl-carboxaldehydes with sodium metabisulfite followed by their reaction with 1,2-phenylenediamine (Ridley *et al*., 1965; Goker *et al*., 2002). This protocol leads to a better yield of benzimidazoles under milder conditions.

Following the strategy (Scheme 1), sodium metabisulfite adducts of 13 different substituted benzaldehydes and six different heteroaryl-carboxaldehydes were synthesized

Scheme 1 Synthesis of 2-aryl- and 5-nitro-2-aryl-1*H*-benzimidazoles



Cpd.	R^1	A
1	H	Phenyl
2	H	4-Chlorophenyl
3	H	2-Chlorophenyl
4	H	2-Nitrophenyl
5	H	3-Nitrophenyl
6	H	4-Nitrophenyl
7	H	3-Methoxyphenyl
8	H	4-Methoxyphenyl
9	H	4-Hydroxyphenyl
10	H	4-Methylphenyl
11	H	4-Fluorophenyl
12	H	4-Butoxyphenyl
13	H	4-*i*-Propylphenyl
14	H	Pyridin-3-yl
15	H	Pyridin-4-yl
16	H	Indol-3-yl
17	H	Pyrrol-2-yl
18	H	5-Methylfuran-2-yl
19	H	Thiophen-2-yl

Cpd.	R^1	A
20	NO_2	Phenyl
21	NO_2	4-Chlorophenyl
22	NO_2	2-Chlorophenyl
23	NO_2	2-Nitrophenyl
24	NO_2	3-Nitrophenyl
25	NO_2	4-Nitrophenyl
26	NO_2	3-Methoxyphenyl
27	NO_2	4-Methoxyphenyl
28	NO_2	4-Methylphenyl
29	NO_2	4-Fluorophenyl
30	NO_2	4-Butoxyphenyl
31	NO_2	4-*i*-propylphenyl
32	NO_2	Pyridin-2-yl
33	NO_2	Pyridin-3-yl
34	NO_2	Indol-3-yl
35	NO_2	Pyrrol-2-yl
36	NO_2	5-Methylfuran-2-yl
37	NO_2	Thiophen-2-yl

in good yield (>90 %). In a subsequent step, benzimidazoles **1**–**19** were prepared by reacting these sodium metabisulfite adducts with 1,2-phenylenediamine. Benzimidazoles **20**–**37** were synthesized by the reaction of the same adducts with 4-nitro-1,2-phenylenediamine. The reaction time recorded was 4–7 h; however, the synthesis of nitro-substituted counterparts required relatively longer time. The yield of all compounds varied from good to excellent. Melting points of literature-reported compounds were verified. The structures of all synthesized compounds were confirmed by IR, ^{1}H-, and ^{13}C-NMR spectroscopic data and LCMS. IR spectroscopic analysis indicated the formation of benzimidazole ring by the absorption peaks corresponding to NH and C=N group at 3,274–3,410 and 1,586–1,625 cm^{-1}, respectively. ^{1}H NMR supported the proposed structures by the appearance of a characteristic NH signal recorded as broad singlet at δ 10.21–11.23 ppm. The presence of the nitro group at C-5 of benzimidazole ring in compounds **20**–**37** was confirmed by a singlet δ 8.39–8.63 ppm corresponding to H-4. LCMS analysis confirmed the identity of synthesized compounds by the appearance of [M+H$^+$] peak in all the cases besides confirming their purity.

Antileishmanial activity

In order to establish the pharmacological profile of the synthesized benzimidazoles, these compounds were subjected to in vitro study against the promastigote form of *L. major* by the method reported by Zhai et al. (1999) using amphotericin B as standard control in the assay. The 50 % inhibitory concentrations (IC_{50}) of *Leishmania* growth were calculated by GraphPad Prism software and the data are reported as the mean ± SD in Table 1.

IC_{50} values of the tested compounds **1**–**37** indicated that all the compounds exhibited significant activity (IC_{50} 0.62–0.92 μg/mL) against *L. major* as compared to the standard drug amphotericin B (IC_{50} 0.56 μg/mL). Of the entire series of compounds, 2-heteroarylbenzimidazoles were found to be most active (**17**, **19,** and **34**). Furthermore, 2-arylbenzimidazoles were found to have stronger inhibitory potential than their 5-nitro-substituted counterparts. However, compounds **21**, **34,** and **36** were an exception to this observation. Nitro group is well recognized as potential candidates for chemotherapy of the hypoxic cells. The cytotoxicity of nitro compounds against hypoxic cells may be attributed to the reduction of the nitro group yielding several reactive metabolites such as nitroanion, hydronitroxide, etc. (Romero-Castro *et al*., 2011). It may, therefore, be anticipated that these nitro benzimidazoles can be potential candidates in cytotoxicity studies too. The general trend in the leishmanicidal activity due to a nitro group on the 2-aryl ring was *ortho* > *para* > *meta* (**4**, **5**, **6**, **23**, **24,** and **25**). Of the chloro-substituted benzimidazoles, the *p*-isomers were found to be more effective than the *o*-isomers (**2**, **3**, **21**, and **22**).

Molecular docking

As mentioned earlier, leishmanolysin (GP63) is a virulent factor for leishmaniasis; however, to date, no molecular

Table 1 In vitro antileishmanial activity of synthesized library against promastigote form of *L. major* and in silico studies on GP63

Compd.	IC_{50} (μg/mL)	London dG score (kcal/mol)	Amino acids	Interaction	Distance (Å)	Energy (kcal/mol)
1	0.68 ± 0.02	−7.2453	His268	Arene–arene	3.91	–
			Ala227	H–bonding	3.14	−2.6
			Ser273	Arene–H	3.70	−0.6
2	0.72 ± 0.08	−7.2570	Ser273	H–bonding	3.02	−1.4
3	0.78 ± 0.09	−7.6713	His268	Arene–arene	3.87	–
4	0.69 ± 0.08	−7.9387	Zn	Metal contact	2.64	−0.6
5	0.82 ± 0.10	−8.3562	Zn	Metal contact	2.68	−0.8
			Ala349	H–bonding	3.44	−1.6
			Gly222	H–bonding	3.23	−2.6
6	0.71 ± 0.03	−7.6344	Zn	Metal contact	2.63	−1.2
			Thr229	Arene–H	4.52	−1.0
7	0.77 ± 0.10	−7.7883	Ser273	H–bonding	3.15	−0.8
			Thr228	H–bonding	3.12	−0.9
8	0.81 ± 0.02	−7.5160	His268	Arene–arene	3.82	–
			Ser273	Arene–H	3.82	−1.1
9	0.92 ± 0.10	−8.4845	Glu265	H–bonding	2.74	−4.2
10	0.79 ± 0.05	−7.2669	His264	Arene–arene	3.39	–
			Ala350	Arene–H	4.14	−0.6
11	0.79 ± 0.02	−7.8308	His264	Arene–arene	3.36	–
			Ala350	Arene–H	4.16	−0.6
12	0.65 ± 0.10	−8.7365	His268	Arene–H	4.37	−0.8
			Zn	Metal contact	2.52	−2.5
13	0.64 ± 0.02	−7.5399	His268	Arene–arene	3.95	–
14	0.64 ± 0.01	−7.8907	His268	Arene–arene	3.82	–
			Ala227	H–bonding	3.14	−2.4
			Ser273	Arene–H	3.69	−2.1
15	0.88 ± 0.07	−7.6183	His264	Arene–arene	3.51	–
			Ala349	Arene–H	3.44	−0.6
			Ala350	Arene–H	4.07	−1.2
16	0.87 ± 0.05	−8.4532	His268	Arene–arene	3.93	–
			Pro347	H–bonding	3.82	−1.5
17	0.63 ± 0.04	−7.0279	His264	Arene–arene	3.82	–
			Pro347	H–bonding	2.86	−2.5
18	0.84 ± 0.10	−7.4853	His264	Arene–arene	3.85	–
			His264	Arene–arene	3.92	
19	0.62 ± 0.05	−7.0881	His268	Arene–arene	3.97	–
			Pro347	H–bonding	3.81	−1.4
20	0.80 ± 0.07	−7.9371	Glu265	H–bonding	3.17	−2.3
			Val223	Arene–H	4.35	−0.7
21	0.64 ± 0.09	−8.5248	His268	Arene–arene	3.90	–
			Ala227	H–bonding	3.51	−0.7
			Gly329	H–bonding	2.89	−2.2
22	0.91 ± 0.04	−7.7101	Glu265	H–bonding	3.56	−1.7
23	0.73 ± 0.09	−8.4132	Zn	Metal contact	2.64	−0.6
24	0.79 ± 0.10	−8.7291	Zn	Metal contact	2.68	−2.4
			Ala349	Arene–H	4.6	−0.8
25	0.75 ± 0.01	−8.3911	Thr228	H–bonding	2.84	−2.4

Table 1 continued

Compd.	IC_{50} (μg/mL)	London dG score (kcal/mol)	Amino acids	Interaction	Distance (Å)	Energy (kcal/mol)
26	0.82 ± 0.01	−8.5942	Gly329	H–bonding	2.76	−3.0
27	0.85 ± 0.04	−8.7449	Gly329	H–bonding	2.98	−5.1
28	0.88 ± 0.05	−8.2060	Gly329	H–bonding	2.95	−4.5
29	0.77 ± 0.22	−8.0795	His268	Arene–arene	3.94	–
			Gly329	H–bonding	2.89	−5.0
30	0.76 ± 0.07	−8.8546	His268	Arene–H	4.13	−1.1
			Ser273	Arene–H	4.10	−0.7
			Zn	Metal contact	2.59	−1.6
			Thr229	Arene–H	4.73	−0.7
31	0.80 ± 0.09	−8.3534	Ser273	Arene–H	4.16	−1.2
			Zn	Metal contact	2.64	−0.9
			Thr229	Arene–H	4.98	−0.6
32	0.73 ± 0.03	−8.0065	Zn	Metal contact	2.66	−0.9
33	0.79 ± 0.01	−7.9663	Thr229	H–bonding	3.14	−2.9
34	0.62 ± 0.07	−8.7914	Ala227	H–bonding	3.48	−0.7
			Gly329	H–bonding	2.65	−4.7
35	0.91 ± 0.08	−7.8610	Ala349	H–bonding	3.66	−0.6
			Gly222	H–bonding	3.13	−3.1
			Gly222	H–bonding	3.14	−2.8
36	0.64 ± 0.06	−7.7326	His268	Arene–arene	3.90	–
			Ala227	H–bonding	3.48	−0.8
			Gly329	H–bonding	2.85	−2.9
37	0.82 ± 0.06	−8.0428	Gly329	H–bonding	3.05	−4.6
			Gly329	H–bonding	3.53	−0.6
Standard[a]	0.56 ± 0.04	−11.0916	Zn	Metal contact	2.49	−1.3
			Glu265	H–bonding	3.61	−0.5
			Glu220	H–bonding	2.94	−1.1

[a] Amphotericin B

docking studies on this target have been reported. Hence, molecular docking studies were carried out by means of MOE program of Chemical Computing Group for the standard drug amphotericin B and benzimidazoles **1**–**37**. The crystal structure of GP63 was obtained from protein data bank (PDB code: 1LML). Since this structure does not contain any co-crystallized ligand molecular docking studies were, therefore, conducted by means of the site finder option given in MOE program. In order to visualize the binding pocket, Alpha spheres were created followed by the generation of dummy atoms on the centers of these spheres.

Structurally, GP63 consists of three domains: *N*-terminal, central, and *C*-terminal. The binding pocket of GP63 is built by His264, His268, and Zn metal from *N*-terminal domain, while His334, Asp342–Ala348, and Phe272–Ser333 from the central domain (Fig. 1). The key amino acids of the active His264, His268, and His334, are found coordinated with Zn (Fig. 2) (Schlagenhauf *et al*., 1998).

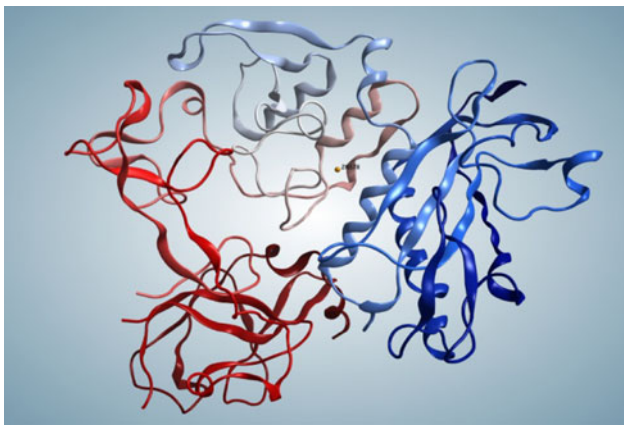


Fig. 1 The ribbon form of GP63 showing three domains (Color figure online)

After having identified the active site, the standard drug amphotericin B was successfully docked in the binding pocket with the rmsd value of 1.02. It was noted that the

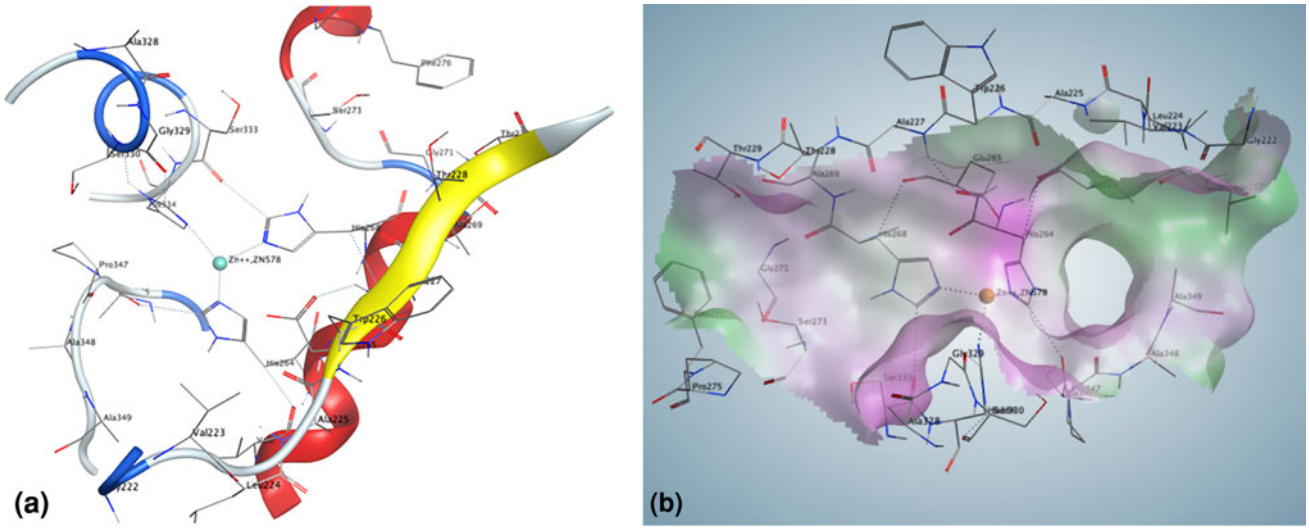


Fig. 2 **a** The ribbon form of the binding pocket of GP63. **b** A close-up view of binding pocket showing lipophilic (*magenta*) and hydrophilic (*green*) surface (Color figure online)

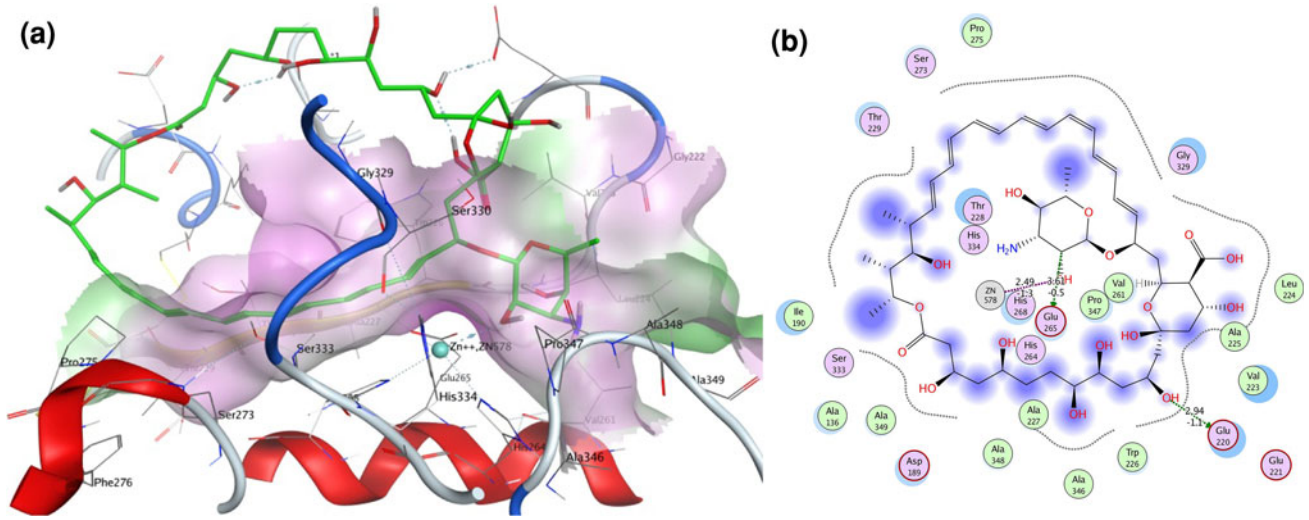


Fig. 3 **a** Docking pose of amphotericin B in the binding pocket. **b** A 2D representation of the same showing interactions with amino acids of the active site (Color figure online)

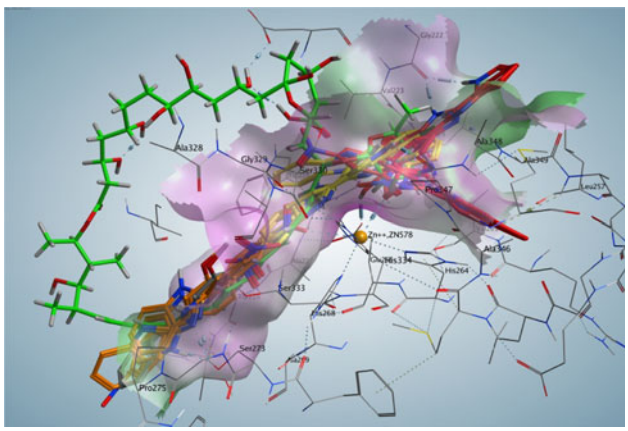


Fig. 4 Binding pocket of GP63 showing superimposition of benzimidazoles and amphotericin B (Color figure online)

amino acids Glu265, Glu220, and Zn were involved in binding with the docked standard drug through a network of hydrogen bonding (Fig. 3). Our target compounds (**1–37**) were also docked successfully in the binding pocket. As all the compounds of the library exhibited promising activity with a narrow range of IC_{50} value (0.62–0.92 μg/mL), no significant correlation was found with the London dG score (−7.0279 to −8.8546 kcal/mol).

All the active compounds were found to have an interesting binding mode in the docked pocket. The superimposition of these benzimidazoles together with amphotericin B is shown in Fig. 4. As shown in figure, the most active group of benzimidazoles (yellow) with IC_{50} values from 0.62 to 0.68 μg/mL was found fitted beautifully in the center of the pocket. However, the docked

compounds (orange) with intermediate IC_{50} values (0.69–0.78 μg/mL) occupied the left side, while the group (red) with the highest values (0.79–0.92 μg/mL) docked to the right of the gorge.

The most important ligand-enzyme binding interactions were arene–arene, arene–H, hydrogen bonding, and metal ligation with Zn578. One of the common binding features of the most potent docked compounds was that all of them showed arene–arene interactions either with His268 (e.g., **1**, **13**, **14**, **19**, **21,,** and **36**) or with His264 (**12**, **17**, and **34**). Another general observation was the hydrogen bonding of these ligands through NH or H-4 of benzimidazole ring with Ala227 or, in a few cases, with Gly329 (e.g., **21** and **34**). Zn metal ligation was observed with the oxygen atom of the nitro group present on the 2-aryl ring of benzimidazoles such as **4**, **5**, and **6** in series1 and the same pattern was visible in **23**, **24**, and **25** of the other series. However, in compounds **30** and **31,** the metal ligation was due to the involvement of 5-nitro functionality of benzimidazole ring. It is anticipated that this metal ligation may be responsible for the leishmanicidal effect of these nitro-benzimidazoles.

The highest activity (IC_{50} = 0.62 μg/mL) of the docked compounds **19** and **34** was supported by the fitting pattern and binding interactions with the amino acids in the gorge. The binding interactions observed in the docked compound **19** (Fig. 5) were hydrophobic interaction with His268 at distance of 3.97 Å and H-bonding interaction between S of thiophene moiety (as hydrogen bond acceptor) with Pro347

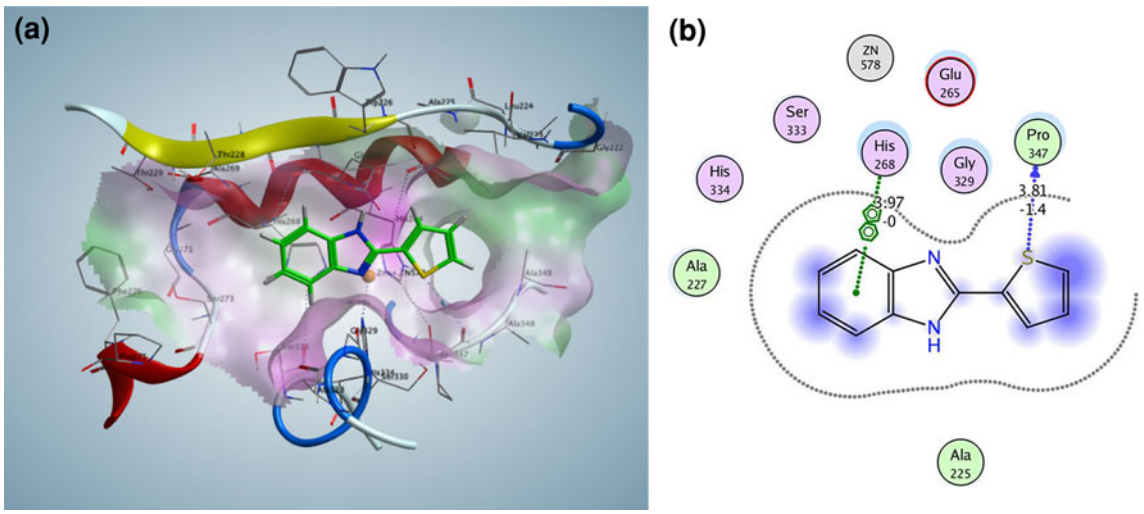


Fig. 5 **a** The close-up view of the binding pocket containing 2-(thiophen-2-yl)-1*H*-benzimidazole (**19**). **b** A 2D representation of the same showing interaction with amino acids of the active site (Color figure online)

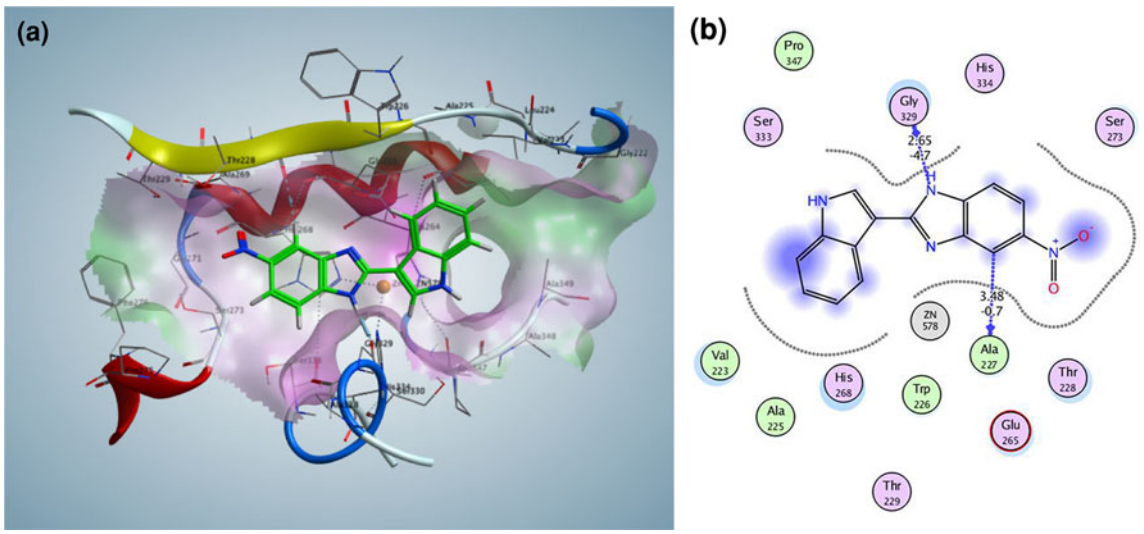


Fig. 6 **a** Docking pose of the 2-(1*H*-indol-3-yl)-5-nitro-1*H*-benzimidazole (**34**). **b** A 2D ligand interaction diagram of the same compound showing interaction with amino acids of the active site (Color figure online)

at a distance of 3.81 Å with the energy released −1.4 kcal/mol. In the case of docked compound **34** (Fig. 6), the ligand-enzyme complex was stabilized by a strong H-bonding interaction of NH of benzimidazole ring with the oxygen of Gly329 at distance of 2.65 Å with the energy released −4.7 kcal/mol. Further, H-4 of benzimidazole ring was also found interacting with carbonyl oxygen of Ala227 at distance of 3.48 Å and energy released −0.7 kcal/mol. Since the precise role of GP63 in parasite physiology is not well understood, these molecular docking studies (expected to be the first report) may enable the designing of new scaffolds for GP63 inhibition.

Lipinski's rule of five

According to Lipinski's Ro5, most drug-like molecules have molecular weight ≤500, logarithm of the octanol/water partition coefficient (log *P*) ≤5, total polar surface area (TPSA) < 140 $Å^2$, number of hydrogen bond donors (HBD) ≤ 5, and hydrogen bond acceptor (HBA) ≤ 10 (Lipinski *et al*., 1997). Further modifications in the Ro5 were made by Veber et al. who suggested the number of rotatable bond (NOR) of drug-like molecule must be fewer or equal to 10 (Veber *et al*., 2002). Molecules violating more than one of these rules may have problem with bio-availability. These molecular descriptors were calculated for all the synthesized compounds by the ligand property calculation function of MOE software and all of them were found to obey Lipinski's Ro5 cut-off limits (Table 2), thereby confirming that these drug-like compounds may be potential drug candidates against leishmaniasis. Log *P* of all synthesized compounds ranges from 2.47 to 4.4, reflected no absorption risk as oral drug administration. Furthermore, the compounds do not accumulate in deep tissue compartments and clearance is majorly via renal (as most compounds have MW < 300 amu). All drugs have HBD < 4, and hence are not prone to phase-II metabolism.

Table 2 Physico-chemical properties for the synthesized library

Compd.	Mol. wt.	Log *P*	TPSA	HBD	HBA	NOR
1	194.24	3.23	28.68	1	1	1
2	228.68	3.88	28.68	1	1	1
3	228.68	3.88	28.68	1	1	1
4	239.23	3.14	74.50	1	1	2
5	239.23	3.14	74.50	1	1	2
6	239.23	3.14	74.50	1	1	2
7	224.26	3.24	37.91	1	2	2
8	224.26	3.24	37.91	1	2	2
9	210.24	2.94	48.91	2	2	1
10	208.26	3.54	28.68	1	1	1
11	212.23	3.37	28.68	1	1	1
12	266.34	4.40	37.91	1	2	5
13	236.32	4.35	28.68	1	1	2
14	195.23	2.63	41.57	1	2	1
15	195.23	2.63	41.57	1	2	1
16	233.27	3.71	44.47	2	1	1
17	183.21	2.56	44.47	2	1	1
18	198.23	3.13	41.82	1	1	1
19	200.27	3.29	28.68	1	1	1
20	239.23	3.14	74.50	1	1	2
21	273.68	3.79	74.50	1	1	2
22	273.68	3.79	74.50	1	1	2
23	284.23	3.05	120.32	1	1	3
24	284.23	3.05	120.32	1	1	3
25	284.23	3.05	120.32	1	1	3
26	269.26	3.15	83.73	1	2	3
27	269.26	3.15	83.73	1	2	3
28	253.26	3.45	74.50	1	1	2
29	257.22	3.28	74.50	1	1	2
30	311.34	4.32	83.73	1	2	6
31	281.32	4.26	74.50	1	1	3
32	240.22	2.53	87.39	1	2	2
33	240.22	2.53	87.39	1	2	2
34	278.27	3.62	90.29	2	1	2
35	228.21	2.47	90.29	2	1	2
36	243.26	3.04	87.64	1	1	2
37	245.26	3.20	74.50	1	1	2
Standard[a]	924.09	2.67	445.14	13	17	3

[a] Amphotericin B

Conclusion

Leishmaniasis has become a major health problem worldwide because the current chemotherapy is far from being satisfactory and urges the search of new, safe, affordable, and effective drugs. However, besides generating an armory of drugs for treating any disease, there is also need of new and effective drug targets to combat the disease. This work targets GP63 protein which is a virulent factor for leishmaniasis. The leishmanicidal effect of 37 membered library of benzimidazole library against promastigote form of clinical isolate of *L. major* was studied. All compounds of library exhibited significant antileishmanial activity with IC_{50} value of 0.62–0.92 μg/mL. These results were corroborated by an in silico study on leishmanolysin also known as GP63 (PDB code: 1LML). Since the precise role of GP63 in parasite physiology is not well understood, these molecular docking studies (expected to be the first report), may enable the designing of new scaffolds for GP63 inhibition. The current study is an encouraging advance in the search of new drug candidates for GP63 inhibition. The easy and economic synthesis of

benzimidazoles, together with their drug-like behavior urges further in vivo studies for their assessment as effective and affordable drug candidates.

Experimental

Chemistry

Melting points are uncorrected and were recorded by Open Capillary tube method using Gallenkamp melting point apparatus. The IR spectra were obtained on Thermo Scientific Nicolet Spectrophotometer model 6700 (samples taken in original form). ^{1}H- and ^{13}C NMR spectra were recorded on Bruker spectrometer at 300 and 75 MHz, respectively, and chemical shifts (δ) were reported in parts per million (ppm) relative to tetramethyl silane (TMS) as an internal standard. Mass spectra were recorded on Agilent Technologies 1200 series HPLC comprising G1313 DAD and Ion Trap LCMS G2445D SL. All the chemicals and solvents were purchased from Sigma-Aldrich or Fluka. The library of 2-substituted-benzimidazole derivatives was synthesized by the reported procedure (Ridley *et al*., 1965; Goker *et al*., 2002).

*General procedure for the synthesis of **1–37***

Appropriate aryl- and heteroaryl-carboxaldehydes (15 mmol) were dissolved in 50 mL ethanol followed by the addition of 10 mL (16 %) aqueous solution of sodium metabisulfite in portions along with vigorous stirring. During the course of reaction, more ethanol was added. After that, the mixture was kept in refrigerator for several hours to yield precipitates which were filtered and dried in oven at 50 °C (yield > 90 %). The mixture of the obtained sodium metabisulfite adduct (7 mmol) and appropriate 1,2-phenylenediamine (5 mmol) in DMF (15 mL) was heated at 110 °C under reflux condition for 4–7 h. The reaction mixture was then cooled to room temperature and poured into the ice cold water (150 mL); the resulting solid was filtered and recrystallized by a mixture of ethanol and water.

*2-Phenyl-1H-benzimidazole (**1**)* White solid; Yield: 89 %; m.p. 291–292 °C (Gogoi and Konwar, 2006). IR (KBr, cm^{-1}): 3,365 (NH); 3,057 (CH aromatic); 1,612 (C=N); 1,542 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.22 (m, 1H, H-4′); 7.26 (m, 2H, H-5, H-6); 7.31 (m, 2H, H-3′, H-5′); 7.51 (m, 2H, H-2′, H-6′); 7.71(m, 2H, H-4, H-7); 11.01 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 115.25; 123.22; 127.57; 128.78; 129.92; 130.95; 138.75; 152.70. LCMS: 195.1 [M+H^{+}].

*2-(4-Chlorophenyl)-1H-benzimidazole (**2**)* Off-white solid; Yield: 87 %; m.p. 287–289 °C (Patil *et al*., 2011). IR (KBr, cm^{-1}): 3,363 (NH); 3,052 (CH aromatic); 1,617 (C=N); 1,539 (C=C); 747 (Ar–Cl). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.27 (m, 2H, H-5, H-6); 7.34 (m, 2H, H-3′, H-5′); 7.43 (m, 2H, H-2′, H-6′); 7.70(m, 2H, H-4, H-7); 10.97 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 115.31; 123.19; 128.59; 128.93; 129.78; 134.92; 138.87; 152.94. LCMS: 229.2 [M+H^{+}].

*2-(2-Chlorophenyl)-1H-benzimidazole (**3**)* Off-white solid; Yield: 86 %; m.p. 233–234 °C (Hein *et al*., 1957). IR (KBr, cm^{-1}): 3,369 (NH); 3,054 (CH aromatic); 1,613 (C=N); 1,542 (C=C); 743 (Ar–Cl). ^{1}H NMR (300 MHz, DMSO-d_6): δ; 7.16–7.34 (m, 6H, H-5, H-6, H-3′, H-4′, H-5′, H-6′); 7.73 (m, 2H, H-4, H-7); 10.93 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 115.29; 123.04; 127.21; 128.59; 129.48; 130.31; 138.26; 138.93; 152.74. LCMS: 229.1 [M+H^{+}].

*2-(2-Nitrophenyl)-1H-benzimidazole (**4**)* Brown solid; Yield: 81 %; m.p. 240–241 °C (Sharma *et al*., 2009). IR (KBr, cm^{-1}): 3,361 (NH); 3,079 (CH aromatic); 1,615 (C=N); 1,553 (C=C); 1,367 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.27–7.46 (m, 3H, H-5, H-6, H4′); 7.70–7.74 (m, 4H, H-4, H-7, H-5′, H-6′); 8.25 (m, 1H, H-3′); 10.89 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 115.92; 121.94; 123.56; 128.30; 132.31; 138.88; 146.74; 152.83. LCMS: 240.1 [M+H^{+}].

*2-(3-Nitrophenyl)-1H-benzimidazole (**5**)* Brown solid; Yield: 80 %; m.p. >300 °C (Patil *et al*., 2011). IR (KBr, cm^{-1}): 3,361 (NH); 3,079 (CH aromatic); 1,619 (C=N); 1,548 (C=C); 1,358 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.28 (m, 2H, H-5, H-6); 7.58 (m, 1H, H-5′); 7.73 (m, 2H, H-4, H-7);7.89 (m, 1H, H-6′); 8.15 (m, 1H, H-4′); 8.42 (s, 1H, H-2′); 11.12 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 115.39; 121.11; 122.53; 123.39; 130.36; 131.94; 134.10; 139.18; 148.93; 152.72. LCMS: 240.2 [M+H^{+}].

*2-(4-Nitrophenyl)-1H-benzimidazole (**6**)* Brown solid; Yield: 81 %; m.p. >300 °C (Sharma *et al*., 2009). IR (KBr, cm^{-1}): 3,359 (NH); 3,082 (CH aromatic); 1,609 (C=N); 1,547 (C=C); 1,357 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.25 (m, 2H, H-5, H-6); 7.72–7.74 (m, 4H, H-4, H-7, H-2′, H-6′); 8.21 (m, 2H, H-3′, H-5′); 11.10 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 116.13; 121.72; 123.70; 128.29; 136.63; 138.64; 148.19; 152.59. LCMS: 240.2 [M+H^{+}].

*2-(3-Methoxyphenyl)-1H-benzimidazole (**7**)* White solid; Yield: 89 %; m.p. 212–213 °C. IR (KBr, cm^{-1}): 3,354

(NH); 3,091 (CH aromatic); 1,621 (C=N); 1,559 (C=C); 1,209 (C–O–C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.78 (O–CH_3); 6.73 (m, 1H, H-4′), 7.01–7.04 (m, 2H, H-2′, H-6′); 7.23 (m, 1H, H-5′); 7.27 (m, 2H, H-5, H-6); 7.71 (m, 2H, H-4, H-7) 10.86 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 55.73; 112.31, 114.67; 115.69; 120.13; 123.10; 130.54; 131.76; 138.89; 152.71, 161.26. LCMS: 225.2 [M+H$^+$].

2-(4-Methoxyphenyl)-1H-benzimidazole ***(8)*** Off-white solid; Yield: 92 %; m.p. 224–225 °C (Gogoi and Konwar, 2006). IR (KBr, cm^{-1}): 3,345 (NH); 3,103 (CH aromatic); 2,918 (CH aliphatic); 1,623 (C=N); 1,563 (C=C); 1,214 (C–O–C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.75 (O–CH_3); 6.81 (m, 2H, H-3′, H-5′), 7.26–7.37 (m, 4H, H-5, H-6, H-2′, H-6′); 7.72 (m, 2H, H-4, H-7); 10.96 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 55.92; 114.80; 116.17; 123.61; 124.34; 129.93; 138.75; 152.90; 161.31. LCMS: 225.2 [M+H$^+$].

2-(4-Hydroxyphenyl)-1H-benzimidazole ***(9)*** White solid; Yield: 78 %; m.p. 225–226 °C (Sharma *et al*., 2009). IR (KBr, cm^{-1}): 3,343 (NH); 3,479 (OH); 3,087 (CH aromatic); 1,613 (C=N); 1,557 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.78 (m, 2H, H-3′, H-5′), 7.27–7.30 (m, 4H, H-5, H-6, H-2′, H-6′); 7.71 (m, 2H, H-4, H-7); 7.91 (bs, 1H, OH) 10.96 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 115.94; 116.76; 123.23; 124.76; 128.94; 138.78; 152.80; 159.12. LCMS: 211.1 [M+H$^+$].

2-(4-Methylphenyl)-1H-benzimidazole ***(10)*** White solid; Yield: 91 %; m.p. 275–276 °C (Gogoi and Konwar, 2006). IR (KBr, cm^{-1}): 3,359 (NH); 3,067 (CH aromatic); 2,917 (CH aliphatic); 1,619 (C=N); 1,561 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.36 (s, 3H, CH_3); 7.17–7.33 (m, 6H, H-5, H-6, H-2′, H-3′, H-5′, H-6′); 7.73 (m, 2H, H-4, H-7); 10.45 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 26.33; 116.12; 123.38; 127.25; 127.91; 138.18; 138.94; 152.45. LCMS: 209.2 [M+H$^+$].

2-(4-Fluorophenyl)-1H-benzimidazole ***(11)*** White solid; Yield: 91 %; m.p. 258–259 °C (Rashid *et al*., 2007). IR (KBr, cm^{-1}): 3,347 (NH); 3,061 (CH aromatic); 1,612 (C=N); 1,560 (C=C); 1,037 (Ar–F). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.04 (m, 2H, H-3′, H-5′); 7.26 (m, 2H, H-5, H-6); 7.48 (m, 2H, H-2′,H-6′); 7.71 (m, 2H, H-4, H-7); 10.62 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 115.88; 117.13; 123.10; 126.73; 129.49; 138.73; 152.91. LCMS: 213.2 [M+H$^+$].

2-(4-Butoxyphenyl)-1H-benzimidazole ***(12)*** Off-white solid; Yield: 87 %; m.p. 223–224 °C. IR (KBr, cm^{-1}): 3,367 (NH); 3,065 (CH aromatic); 2,909 (CH aliphatic); 1,612 (C=N); 1,560 (C=C); 1,237 (C–O–C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.97 (t, 3H, CH_3″); 1.34 (q, 2H, CH_2-3″); 1.71 (m, 2H, CH_2-2″); 3.95 (t, 2H, CH_2–O); 6.82 (m, 2H, H-3′, H-5′); 7.28–7.35 (m, 4H, H-5, H-6, H-2′, H-6′); 7.73 (m, 2H, H-6, H-7); 10.57 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 15.18; 19.35; 32.12; 69.78; 113.52; 115.80; 123.19; 123.63; 128.36; 138.92; 152.54; 158.47. LCMS: 267.3 [M+H$^+$].

2-(4-i-Propylphenyl)-1H-benzimidazole ***(13)*** Off-white solid; Yield: 81 %; m.p. 259–260 °C. IR (KBr, cm^{-1}): 3,364 (NH); 3,062 (CH aromatic); 2,913 (CH aliphatic); 1,614 (C=N); 1,563 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.29 (d, 6H, CH_3); 3.14 (q, 1H, CH); 7.19–7.37 (m, 6H, H-5, H-6, H-2′, H-3′, H-5′, H-6′); 7.71 (m, 2H, H-6, H-7); 10.64 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 24.36; 36.49; 115.44; 123.07; 126.72; 127.36; 128.31;138.84; 148.77; 152.49. LCMS: 237.2 [M+H$^+$].

2-(Pyridin-3-yl)-1H-benzimidazole ***(14)*** Light yellow solid; Yield 89 %; m.p. 248–249 °C (Schiffmann *et al*., 2006). IR (KBr, cm^{-1}): 3,354 (NH); 3,060 (CH aromatic); 1,601 (C=N); 1,564 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.21–7.44 (m, 3H, H-5, H-6, H-3′); 7.74–7.92 (m, 3H, H-4, H-7, H-6′); 8.57 (m, 1H, H-4′); 8.75 (s, 1H, H-2′); 11.04 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 116.34; 123.27; 124.20; 133.35; 134.13; 138.66;148.73; 149.48; 152.72. LCMS: 196.1 [M+H$^+$].

2-(Pyridin-4-yl)-1H-benzimidazole ***(15)*** Yellow solid; Yield: 88 %: m.p. 218–219 °C (Huang *et al*., 2004). IR (KBr, cm^{-1}): 3,331 (NH); 3,057 (CH aromatic); 1,597 (C=N); 1,556 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.25 (m, 2H, H-5, H-6); 7.63–7.74 (m, 4H, H-4, H-7, H-2′, H-6′); 8.67 (m, 2H, H-3′, H-5′); 10.94 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 115.93; 121.59; 123.36; 134.43; 138.97; 150.14; 152.91. LCMS: 196.1 [M+H$^+$].

2-(1H-indol-3-yl)-1H-benzimidazole ***(16)*** Off-white solid; Yield: 75 %; m.p. 228–229 °C. IR (KBr, cm^{-1}): 3,335 (NH); 3,061 (CH aromatic); 1,594 (C=N); 1,543 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.01–7.52 (m, 7H, H-5, H-6, H-2′, H-4′, H-5′, H-6′, H-7′); 7.74 (m, 2H, H-4, H-7); 10.94 (bs, 1H, NH); 11.23 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO): 107.38; 112.15; 116.10; 119.13; 120.16; 122.42; 123.18; 128.67; 131.69; 135.53; 138.92; 153.27. LCMS: 234.2 [M+H$^+$].

2-(1H-pyrrol-2-yl)-1H-benzimidazole ***(17)*** Orange solid; Yield: 79 %; m.p. 205–206 °C. IR (KBr, cm^{-1}): 3,356 (NH); 3,091 (CH aromatic); 1,607 (C=N); 1,534 (C=C).

^{1}H NMR (300 MHz, DMSO-d_6): δ 6.12–6.53 (m, 3H, H-3′, H-4′, H-5′); 7.29 (m, 2H, H-4, H-7); 7.69 (m, 2H, H-4, H-7); 10.61 (bs, 1H, NH); 10.92 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO): 106.33; 108.90; 115.16; 118.38; 123.15; 129.92; 138.68; 154.65. LCMS: 184.1 [M+H$^+$].

2-(5-Methylfuran-2-yl)-1H-benzimidazole ***(18)*** Grayish pink solid; Yield: 92 %; m.p. 175–177 °C. IR (KBr, cm^{-1}): 3,354 (NH); 3,101 (CH aromatic); 2,925 (CH aliphatic); 1,612 (C=N); 1,527 (C=C); 1,218 (C–O–C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.19 (s, 3H, CH_3); 5.91–6.13 (m, 2H, H3′, H-4′); 7.24 (m, 2H, H-4, H-7); 7.73 (m, 2H, H-4, H-7); 10.90 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.38; 101.99; 107.67; 116.31; 123.40; 138.92; 141.74; 151.96; 153.17. LCMS: 199.2 [M+H$^+$].

2-(Thiophen-2-yl)-1H-benzimidazole ***(19)*** Off-white solid; Yield: 90 %; m.p. >300 °C (Schiffmann *et al.*, 2006). IR (KBr, cm^{-1}): 3,348 (NH); 3,122 (CH aromatic); 1,613 (C=N); 1,530 (C=C); 945 (C–S). ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.98–7.14 (m, 3H, H3′, H-4′, H-5′); 7.27 (m, 2H, H-4, H-7); 7.70 (m, 2H, H-4, H-7); 10.87 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 116.10; 124.17; 125.43; 127.51; 127.87; 138.82; 141.58; 143.80. LCMS: 201.1 [M+H$^+$].

2-Phenyl-5-nitro-1H-benzimidazole ***(20)*** Light yellow solid; Yield: 79 %; m.p. 148–149 °C (Navarrete-Vázquez *et al.*, 2010). IR (KBr, cm^{-1}): 3,380 (NH); 3,126 (CH aromatic), 1,614 (C=N); 1,545 (C=C); 1,367 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.47–7.58 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′); 7.94–8.70 (m, 3H, H-4, H-6, H-7); 10.21 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 112.37; 115.53; 119.82; 127.58; 128.64; 129.83; 130.91; 140.65; 142.93; 143.19 152.81. LCMS: 240.1 [M+H$^+$].

2-(4-Chlorophenyl)-5-nitro-1H-benzimidazole ***(21)*** Yellow solid; Yield: 77 %; m.p. 275–276 °C (Jain *et al.*, 2010). IR (KBr, cm^{-1}): 3,274 (NH); 3,056 (CH aromatic); 1,614 (C=N); 1,545 (C=C); 1,377 (NO_2); 742 (Ar–Cl). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.24–7.57 (m, 4H, H-2′, H-3′, H-5′, H-6′); 7.91–8.63 (m, 3H, H-4, H-6, H-7); 10.86 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 113.15; 116.21; 119.54; 127.71; 129.57; 130.32; 134.56; 140.61; 142.97; 143.19; 152.83. LCMS: 274.1 [M+H$^+$].

2-(2-Chlorophenyl)-5-nitro-1H-benzimidazole ***(22)*** Light yellow solid; Yield: 79 %; m.p. 223–224 °C. IR (KBr, cm^{-1}): 3,376 (NH); 3,090 (CH aromatic); 1,613 (C=N); 1,542 (C=C); 1,356 (NO_2); 744 (Ar–Cl). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.33–7.46 (m, 4H, H-3′, H-4′, H-5′, H-6′); 7.99–8.61 (m, 3H, H-4, H-6, H-7); 10.63 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 112.38; 116.31; 118.55; 127.32; 128.66; 129.45; 130.33; 133.59; 136.30; 139.83; 143.18; 144.37; 152.71. LCMS: 274.1 [M+H$^+$].

2-(2-Nitrophenyl)-5-nitro-1H-benzimidazole ***(23)*** Orange solid; Yield: 75 %; m.p. 130–131 °C (Romero-Castro *et al.*, 2011). IR (KBr, cm^{-1}): 3,365 (NH); 3,133 (CH aromatic); 1,613 (C=N); 1,541 (C=C); 1,368 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.81–7.92 (m, 3H, H-7, H-4′, H-5′); 8.01–8.17 (m, 3H, H-6, H-3′, H-6′); 8.53 (bs, 1H, H-4); 10.81 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 110.93; 116.28; 118.14; 122.3; 128.612; 129.84; 132.19; 135.30; 139.72; 142.86; 144.59; 147.15; 152.81. LCMS: 285.2 [M+H$^+$].

2-(3-Nitrophenyl)-5-nitro-1H-benzimidazole ***(24)*** Orange solid; Yield: 72 %; m.p. 281–282 °C (Romero-Castro *et al.*, 2011). IR (KBr, cm^{-1}): 3,359 (NH); 3,069 (CH aromatic); 1,613 (C=N); 1,553 (C=C); 1,359 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.86–7.97 (m, 3H, H-7, H-5′, H-6′); 8.17–8.36 (m, 3H, H-6, H-2′, H-4′); 8.63 (bs, 1H, H-4); 10.97 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 111.34; 116.15; 118.30; 121.97; 123.62; 130.35; 131.75; 134.66; 139.92; 142.60; 144.31;148.21; 152.50. LCMS: 285.2 [M+H$^+$].

2-(4-Nitrophenyl)-5-nitro-1H-benzimidazole ***(25)*** Brown solid; Yield: 76 %; m.p. 265–266 °C (Romero-Castro *et al.*, 2011). IR (KBr, cm^{-1}): 3,371 (NH); 3,121 (CH aromatic); 1,612 (C=N); 1,537 (C=C); 1,361 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.75–7.96 (m, 3H, H-7, H-2′, H-6′); 8.21–8.25 (m, 3H, H-6, H-3′, H-5′); 8.63 (bs, 1H, H-4); 11.03 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 112.63; 116.30; 118.58; 122.34; 128.90; 135.18; 139.61; 142.70; 144.32; 148.85; 152.81. LCMS: 285.2 [M+H$^+$].

2-(3-Methoxyphenyl)-5-nitro-1H-benzimidazole ***(26)*** Light yellow solid; Yield: 81 %; m.p. 187–189 °C. IR (KBr, cm^{-1}): 3,367 (NH); 3,086 (CH aromatic); 1,608 (C=N); 1,534 (C=C); 1,337 (NO_2); 1,215 (C–O–C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.81 (s, 3H, O–CH_3); 5.11 (bs, 1H, NH); 6.81–7.01 (m, 4H, H-2′, H-4′, H-5′, H-6′); 7.95–8.15 (m, 2H, H-6, H-7); 8.59 (bs, 1H, H-4); 10.71 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 55.68; 112.18; 113.95; 114.92; 116.46; 118.18; 121.19; 129.30; 132.71; 139.77; 142.67; 145.14; 152.73; 161.34. LCMS: 270.2 [M+H$^+$].

2-(4-Methoxyphenyl)-5-nitro-1H-benzimidazole ***(27)*** Yellow solid; Yield: 83 %; m.p. 237–239 °C (Estrada-Soto *et al.*,

2006). IR (KBr, cm^{-1}): 3,342 (NH); 3,139 (CH aromatic); 2,921 (CH aliphatic); 1,620 (C=N); 1,564 (C=C); 1,369 (NO_2); 1,225 (C–O–C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 3H, O–CH_3); 6.91–7.21 (m, 4H, H-2′, H-3′, H-5′, H-6′); 7.76–8.11 (m, 2H, H-6, H-7); 8.49 (bs, 1H, H-4); 10.90 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 55.49; 110.61; 114.67; 115.94; 118.72; 122.78; 128.52; 139.93; 142.81; 144.74; 152.68; 160.73. LCMS: 270.2 [M+H$^+$].

2-(4-Methylphenyl)-5-nitro-1H-benzimidazole ***(28)*** Light yellow solid; Yield: 84 %; m.p. 205–207 °C (Jain *et al.*, 2010). IR (KBr, cm^{-1}): 3,258 (NH); 3,050 (CH aromatic); 2,916 (CH aliphatic); 1,608 (C=N); 1,563 (C=C); 1,366 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.33 (s, 3H, CH_3); 7.12–7.35 (m, 4H, H-2′, H-3′, H-5′, H-6′); 7.86-8.13 (m, 2H, H-6, H-7); 8.39 (bs, 1H, H-4); 10.44 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 25.97; 112.22; 115.95; 118.73; 127.18; 127.63; 128.90; 137.72; 140.10; 142.61; 144.93; 152.65. LCMS: 254.2 [M+H$^+$].

2-(4-Fluorophenyl)-5-nitro-1H-benzimidazole ***(29)*** Light yellow solid; Yield: 86 %; m.p. 264–265 °C (Goker *et al.*, 2002). IR (KBr, cm^{-1}): 3,410 (NH); 3,146 (CH aromatic); 1,623 (C=N); 1,545 (C=C); 1,366 (NO_2); 1,040 (Ar–F). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.10–7.42 (m, 4H, H-2′, H-3′, H-5′, H-6′); 7.83–8.16 (m, 2H, H-6, H-7); 8.45 (bs, 1H, H-4); 10.95 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 113.59; 115.98; 116.77; 118.13; 126.82; 129.27; 139.93; 142.66; 145.34; 152.90; 171.15. LCMS: 258.2 [M+H$^+$].

2-(4-Butoxyphenyl)-5-nitro-1H-benzimidazole ***(30)*** Light yellow solid; Yield: 81 %; m.p. 191–193 °C. IR (KBr, cm^{-1}): 3,361 (NH); 3,143 (CH aromatic); 2,883 (CH aliphatic); 1,618 (C=N); 1,556 (C=C); 1,378 (NO_2); 1,232 (C–O–C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.96 (t, 3H, CH_3″); 1.34 (q, 2H, CH_2-3″); 1.70 (m, 2H, CH_2-2″); 3.93 (t, 2H, CH_2–O); 6.87–7.37 (m, 4H, H-2′, H-3′, H-5′, H-6′); 7.86–8.17 (m, 2H, H-6, H-7); 8.53 (bs, 1H, H-4); 11.01 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 15.13; 21.28; 31.70; 69.27; 110.54; 114.45; 116.34; 118.36; 124.73; 128.30; 139.59; 142.81; 144.97; 151.90; 157.69. LCMS: 312.2 [M+H$^+$].

2-(4-i-Propylphenyl)-5-nitro-1H-benzimidazole ***(31)*** Yellow solid; Yield: 80 %; m.p. 213–214 °C. IR (KBr, cm^{-1}): 3,258 (NH); 3,050 (CH aliphatic); 2,926 (CH aliphatic); 1,614 (C=N); 1,563 (C=C); 1,366 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.30 (d, 6H, CH_3); 3.12 (q, 1H, CH); 7.21–7.41 (m, 4H, H-2′, H-3′, H-5′, H-6′); 7.83–8.11 (m, 2H, H-6, H-7); 8.47 (bs, 1H, H-4); 10.83 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 23.63; 37.19; 110.64; 116.23; 118.16; 126.87; 127.50; 128.33; 139.73; 142.81; 144.79; 150.10; 152.83. LCMS: 282.2 [M+H$^+$].

2-(Pyridin-2-yl)-5-nitro-1H-benzimidazole ***(32)*** Pink solid; Yield: 81 %; m.p. 212–213 °C (Schiffmann *et al.*, 2006). IR (KBr, cm^{-1}): 3,328 (NH); 3,056 (CH aromatic); 1,625 (C=N); 1,564 (C=C); 1,351 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.61–7.82 (m, 3H, H-3′, H-4′, H-5′); 7.94–8.13 (m, 2H, H-6, H-7); 8.45–8.56 (m, 2H, H-4, H-6′); 10.61 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 112.13; 116.15; 117.98; 123.83; 120.93; 124.11; 136.82; 139.90; 142.73; 144.61; 149.69; 155.14. LCMS: 241.2 [M+H$^+$].

2-(Pyridin-3-yl)-5-nitro-1H-benzimidazole ***(33)*** Off-white solid; Yield: 79 %; m.p. 260–261 °C (Jain *et al.*, 2010). IR (KBr, cm^{-1}): 3,329 (NH); 3,059 (CH aromatic); 1,590 (C=N); 1,562 (C=C); 1,350 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.42 (m, 1H, H-5′); 7.96–8.13 (m, 3H, H-6, H-7, H-4′); 8.39–8.49 (m, 2H, H-4, H-6′); 8.78 (s, 1H, H-2′); 10.81 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 112.26; 116.34; 118.19; 124.05; 132.96; 134.23; 138.94; 142.95; 144.34; 147.92; 149.10; 152.68. LCMS: 241.2 [M+H$^+$].

2-(1H-indol-3-yl)-5-nitro-1H-benzimidazole ***(34)*** Light brown solid; Yield: 68 %; m.p. 213–215 °C. IR (KBr, cm^{-1}): 3,334 (NH); 3,067 (CH aromatic); 1,586 (C=N); 1,552 (C=C); 1,343 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.05–7.55 (m, 5H, H-2′, H-4′, H-5′, H-6′, H-7′); 7.93–8.13 (m, 2H, H-6, H-7); 8.39 (s, 1H, H-4); 10.23 (bs, 1H, NH); 10.91 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 106.72; 110.43; 111.19; 116.37; 118.12; 119.27; 121.23; 122.70; 128.55; 131.76; 135.29; 138.74; 142.61; 144.82; 152.98. LCMS: 279.2 [M+H$^+$].

2-(1H-pyrrol-2-yl)-5-nitro-1H-benzimidazole ***(35)*** Orange solid; Yield: 71 %; m.p. 186–188 °C. IR (KBr, cm^{-1}): 3,363 (NH); 3,108 (CH aromatic); 1,615 (C=N); 1,548 (C=C); 1,358 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.12–6.59 (m, 3H, H-3′, H-4′, H-5′); 7.96–8.19 (m, 2H, H-6, H-7); 8.49 (s, 1H, H-4); 10.13 (bs, 1H, NH); 10.35 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 106.75; 108.53; 110.98; 116.14; 118.21; 120.33; 122.94; 139.79; 142.90; 145.06; 154.86. LCMS: 229.1 [M+H$^+$].

2-(5-Methylfuran-2-yl)-5-nitro-1H-benzimidazole ***(36)*** Brown solid; Yield: 78 %; m.p. 149–150 °C. IR (KBr, cm^{-1}): 3,342 (NH); 3,139 (CH aromatic); 2,916 (CH aliphatic); 1,612 (C=N); 1,527 (C=C); 1,369 (NO_2); 1,225 (C–O–C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.16 (s, 3H, CH_3);

6.12–6.24 (m, 2H, H-3′, H-4′); 7.87–8.08 (m, 2H, H-6, H-7); 8.45 (s, 1H, H-4); 10.43 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.28; 102.13; 107.87; 112.34; 116.79; 118.10; 139.92; 141.31; 142.74; 144.89; 150.90; 152.14. LCMS: 244.1 [M+H$^+$].

2-(Thiophen-2-yl)-5-nitro-1H-benzimidazole (***37***) Light yellow solid; Yield: 79 %; m.p. 146–147 °C. IR (KBr, cm^{-1}): 3,380 (NH); 3,126 (CH aromatic); 1,613 (C=N); 1,530 (C=C); 1,367 (NO_2); 940 (C–S). ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.97–7.25 (m, 3H, H-3′, H-4′, H-5′); 7.92–8.15 (m, 2H, H-6, H-7); 8.43 (s, 1H, H-4); 10.81 (bs, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 110.78; 116.25; 118.01; 125.31; 127.68; 128.14; 139.73; 141.59; 142.64; 143.51; 144.89. LCMS: 246.1 [M+H$^+$].

Antileishmanial activity

Antileishmanial activity of the library was assayed by following the method reported by Zhai et al. (1999) using a pre-established culture of clinical isolate of *L. major* obtained from National Institute of Health (NIH), Islamabad, Pakistan. Promastigotes were cultured in medium 199 (Cassion Laboratories, Inc. USA) containing 10 % fetal bovine serum (ICN Flow, UK), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), sodium bicarbonate (Sigma), penicillin (ICN Biochemicals, Inc. Germany), and streptomycin (Sigma). Incubation and growth of the parasite were carried out at 24 °C. Briefly after 6–7 days of the culture, the promastigotes were centrifuged at 3,000 rpm for 3 min, the supernatant was discarded and the pellet was washed three times with phosphate buffer saline (MP-Biomedicals, Inc. France) and resuspended in medium 199 at 2 × 10^6 cell/mL.

Promastigotes were seeded in 96-well round bottom microtiter plates (TPP, Switzerland) containing serial dilution of the compound and media 199. Amphotericin B (BioChemica, Germany) and DMSO were used as positive and negative controls, respectively. The plates were incubated for 72 h at 24 °C in shaker incubator. All the compounds were assayed in triplicate and the number of alive parasites was determined by counting in Neubauer chamber. The inhibitory concentrations for the 50 % of the inhibition IC_{50} were calculated by GraphPad Prism (GraphPad Prism Software, Inc. USA) software and data reported as the mean ± SD.

Molecular docking

All the molecular docking studies and ligand property calculations were performed by means of "MOE version 2010.10," Chemical Computing Group Inc., 1010 Sherbrook Street West, Suite 910, Montreal, H3A 2R7, Canada. The program operated under "Windows Server 2003 R2" operating system installed on an IBM System ×3400 with four Intel(R) Xeon(TM) CPU 3.00 GHz processors and 2048 RAM.

The target compounds were built using the builder interface of the MOE program and subjected to energy minimization tool by means of the included MOPAC 7.0. The produced model was subjected Systematic Conformational Search where all the atoms were set as default with RMS gradient of 0.01 kcal/mol and RMS distance of 0.1 Å.

The data of high resolution crystal structure of GP63 were obtained from Protein Data Bank (PDB code: 1LML) (Schlagenhauf *et al.*, 1998). The crystal structure was imported into MOE and all hydrogen atoms were added to structure with their standard geometry followed by their energy minimization using MOPAC 7.0. The water molecules were removed using sequence editor interface of the MOE program. The resulting model was subjected to systematic conformational search at default parameters with RMS gradient of 0.01 kcal/mol using Alpha Site Finder for the active site search in the enzyme structure and dummy atoms were created from the obtained Alpha spheres. The docking studies were performed on the prepared enzyme by Triangle Matcher method in conjunction by means of post-placement refinement method Forcefield. Rescoring 1 and 2 were selected London dG and Affinity dG, respectively. The obtained ligand–enzyme complex model was then used in calculating the energy parameters by means of MMFF94x force field energy calculation and predicting the ligand–enzyme interactions at the active site. The lowest energy minimized pose was used for further analysis. Rule of five parameters (Ro5) were calculated by ligand property calculation function of MOE.

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References

Ameen M (2010) Cutaneous leishmaniasis: advances in disease pathogenesis, diagnostics and therapeutics. Clin Exp Dermatol 35(7):699–705

Ansari FL, Wadood A, Ullah A, Iftikhar F, Ul-Haq Z (2009) In silico studies of urease inhibitors to explore ligand-enzyme interactions. J Enzyme Inhib Med Chem 24(1):151–156. doi:10.1080/14756360801945598

Ansari FL, Kalsoom S, Ul-Haq Z, Ali Z, Jabeen F (2012) In silico studies on 2,3-dihydro-1,5-benzothiazepines as cholinesterase inhibitors. Med Chem Res 21(9):2329–2339. doi:10.1007/s00044-011-9754-6

Bianchini G, Bocedi A, Ascenzi P, Gavuzzo E, Mazza F, Aschi M (2006) Molecular dynamics simulation of *Leishmania major* surface metalloprotease GP63 (leishmanolysin). Proteins 64(2):385–390

Cabrera-Serra MG, Valladares B, Pinero JE (2008) In vivo activity of perifosine against *Leishmania amazonensis*. Acta Trop 108(1): 20–25

Cedillo-Rivera R, Munoz O (1992) In vitro susceptibility of *Giardia lamblia* to albendazole, mebendazole and other chemotherapeutic agents. J Med Microbiol 37(3):221–224

Chavez B, Cedillo-Rivera R, Martinez-Palomo A (1992) *Giardia lamblia*: ultrastructural study of the in vitro effect of benzimidazoles. J Protozool 39(4):510–515

Desjeux P (2004) Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis 27(5):305–318

Estrada-Soto S, Villalobos-Molina R, Aguirre-Crespo F, Vergara-Galicia J, Moreno-Díaz H, Torres-Piedra M, Navarrete-Vázquez G (2006) Relaxant activity of 2-(substituted phenyl)-1*H*-benzimidazoles on isolated rat aortic rings: design and synthesis of 5-nitro derivatives. Life Sci 79(5):430–435

Gogoi P, Konwar D (2006) An efficient and one-pot synthesis of imidazolines and benzimidazoles via anaerobic oxidation of carbon–nitrogen bonds in water. Tetrahedron Lett 47(1):79–82

Goker H, Kus C, Boykin DW, Yildiz S, Altanlar N (2002) Synthesis of some new 2-substituted-phenyl-1*H*-benzimidazole-5-carbonitriles and their potent activity against *Candida* species. Bioorg Med Chem 10(8):2589–2596

Hein DW, Alheim RJ, Leavitt JJ (1957) The use of polyphosphoric acid in the synthesis of 2-aryl- and 2-alkyl-substituted benzimidazoles, benzoxazoles and benzothiazoles. J Am Chem Soc 79(2):427–429. doi:10.1021/ja01559a053

Huang XC, Zeng MH, Ng SW (2004) 2-(4-Pyridyl)-1*H*-benzimidazole trihydrate. Acta Crystallogr Sect E Struct Rep Online 60(6):o939–o940. doi:10.1107/s1600536804010244

Ivanenkov YA, Savchuk NP, Ekins S, Balakin KV (2009) Computational mapping tools for drug discovery. Drug Discov Today 14:767–775

Jain P, Sharma P, Rajak H, Pawar R, Patil U, Singour P (2010) Design, synthesis and biological evaluation of some novel benzimidazole derivatives for their potential anticonvulsant activity. Arch Pharm Res 33(7):971–980. doi:10.1007/s12272-010-0701-8

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Delivery Rev 23(1–3):3–25

Miguel DC, Yokoyama-Yasunaka JKU, Uliana SRB (2008) Tamoxifen is effective in the treatment of *Leishmania amazonensis* infections in mice. PLoS Negl Trop Dis 2(6):e249

Minodier P, Parola P (2007) Cutaneous leishmaniasis treatment. Travel Med Infect Dis 5(3):150–158

Molecular Operating Environment (MOE), 2010.10 (2010). (vol 2010.10, 2010.10 edn). Chemical Computing Group Inc., Montreal

Murray HW, Berman JD, Davies CR, Saravia NG (2005) Advances in leishmaniasis. Lancet 366(9496):1561–1577

Navarrete-Vázquez G, Cedillo R, Hernández-Campos A, Yépez L, Hernández-Luis F, Valdez J, Morales R, Cortés R, Hernández M, Castillo R (2001) Synthesis and antiparasitic activity of 2-(trifluoromethyl)benzimidazole derivatives. Bioorg Med Chem Lett 11(2):187–190

Navarrete-Vázquez G, Hidalgo-Figueroa S, Torres-Piedra M, Vergara-Galicia J, Rivera-Leyva JC, Estrada-Soto S, León-Rivera I, Aguilar-Guardarrama B, Rios-Gómez Y, Villalobos-Molina R, Ibarra-Barajas M (2010) Synthesis, vasorelaxant activity and antihypertensive effect of benzo[*d*]imidazole derivatives. Bioorg Med Chem 18(11):3985–3991

Nawaz SA, Umbreen S, Kahlid A, Ansari FL, Choudhary MI (2008) Structural insight into the inhibition of acetylcholinesterase by 2,3,4, 5-tetrahydro-1, 5-benzothiazepines. J Enzyme Inhib Med Chem 23(2):206–212. doi:10.1080/14756360701533080

Pandey VP, Bisht SS, Mishra M, Kumar A, Siddiqi MI, Verma A, Mittal M, Sane SA, Gupta S, Tripathi RP (2010) Synthesis and molecular docking studies of 1-phenyl-4-glycosyl-dihydropyridines as potent antileishmanial agents. Eur J Med Chem 45(6):2381–2388

Patil SV, Patil SS, Bobade VD (2011) A simple and efficient approach to the synthesis of 2-substituted benzimidazole via sp^3 C–H functionalization. Arab J Chem (0). doi: 10.1016/j.arabjc.2011.1006.1017

Preston PN (1974) Synthesis, reactions, and spectroscopic properties of benzimidazoles. Chem Rev 74(3):279–314

Rashid N, Tahir MK, Kanwal S, Yusof NM, Yamin BM (2007) 2-(4-Fluorophenyl)-1*H*-benzimidazole. Acta Crystallogr Sect E Struct Rep Online 63(3):o1402–o1403. doi:10.1107/s160053680700774x

Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S (2007) Cutaneous leishmaniasis. Lancet Infect Dis 7(9):581–596

Ridley HF, Spickett RGW, Timmis GM (1965) A new synthesis of benzimidazoles and aza-analogs. J Heterocycl Chem 2(4):453–456

Romero-Castro A, León-Rivera I, Ávila-Rojas L, Navarrete-Vázquez G, Nieto-Rodríguez A (2011) Synthesis and preliminary evaluation of selected 2-aryl-5(6)-nitro-1*H*-benzimidazole derivatives as potential anticancer agents. Arch Pharm Res 34(2):181–189. doi:10.1007/s12272-011-0201-5

Rondu F, Bihan GL, Wang X, Lamouri A, Touboul E, Dive G, Bellahsene T, Pfeiffer B, Renard P, Guardiola-Lemaitre B, Manechez D, Penicaud L, Ktorza A, Godfroid JJ (1997) Design and synthesis of imidazoline derivatives active on glucose homeostasis in a rat model of type II diabetes. 1. Synthesis and biological activities of *N*-benzyl-*N*′-(arylalkyl)-2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)piperazines. J Med Chem 40(23):3793–3803. doi:10.1021/jm9608624

Schiffmann R, Neugebauer A, Klein CD (2006) Metal-mediated inhibition of *Escherichia coli* methionine aminopeptidase: structure–activity relationships and development of a novel scoring function for metal–ligand interactions. J Med Chem 49(2):511–522. doi:10.1021/jm050476z

Schlagenhauf E, Etges R, Metcalf P (1998) The crystal structure of the *Leishmania major* surface proteinase leishmanolysin (gp63). Structure 6(8):1035–1046

Sharma D, Narasimhan B, Kumar P, Jalbout A (2009) Synthesis and QSAR evaluation of 2-(substituted phenyl)-1*H*-benzimidazoles and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones. Eur J Med Chem 44(3):1119–1127

Singh S, Sivakumar R (2004) Challenges and new discoveries in the treatment of leishmaniasis. J Infect Chemother 10(6):307–315

Touzeau F, Arrault A, Guillaumet G, Scalbert E, Pfeiffer B, Rettori MC, Renard P, Mérour JY (2003) Synthesis and biological evaluation of new 2-(4,5-dihydro-1*H*-imidazol-2-yl)-3,4-dihydro-2*H*-1,4-benzoxazine derivatives. J Med Chem 46(10):1962–1979. doi:10.1021/jm021050c

Ul-Haq Z, Khan W, Kalsoom S, Ansari FL (2010) In silico modeling of the specific inhibitory potential of thiophene-2,3-dihydro-1,5-benzothiazepine against BChE in the formation of beta-amyloid plaques associated with Alzheimer's disease. Theor Biol Med Model 7(1):22. doi:10.1186/1742-4682-7-22

Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD (2002) Molecular properties that influence the oral bioavailability

of drug candidates. J Med Chem 45(12):2615–2623. doi:10.1021/jm020017n

Wyllie S, Patterson S, Stojanovski L, Simeons FRC, Norval S, Kime R, Read KD, Fairlamb AH (2012) The anti-trypanosome drug fexinidazole shows potential for treating visceral leishmaniasis. Sci Transl Med 4(119). doi:10.1126/scitranslmed.3003326

Yao C, Donelson JE, Wilson ME (2003) The major surface protease (MSP or GP63) of *Leishmania* sp. Biosynthesis, regulation of expression, and function. Mol Biochem Parasitol 132(1):1–16

Zhai L, Chen M, Blom J, Theander TG, Christensen SB, Kharazmi A (1999) The antileishmanial activity of novel oxygenated chalcones and their mechanism of action. J Antimicrob Chemother 43(6):793–803. doi:10.1093/jac/43.6.793