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Synthesis and *in vitro* bioevaluation of 1,5-Bis(5-substituted benzimidazole) alkanes as antileishmanial agents

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ABSTRACT

*Some 1,5-bis(5-substituted benzimidazole)alkanes **(4a-4f, 5a-5f & 6a-6d)** were synthesized from **3a-3f** substituted benzimidazole and screened towards in vitro antileishmanial activity profile. Some of compounds such as **4c, 5c, 6c & 6d** displayed good antileishmanial activity.*

Key words: 1,5-Bis(5-substituted Benzimidazole), Antileishmanial activity, Promastigotes, Amastigotes, Pentamidine.

INTRODUCTION

The discovery and development of essential drugs for neglected disease such as Chaga's and Leishmaniasis is a major concern in the pharmaceutical world. Recent review on the chemotherapy of Chaga's disease and Leishmaniasis stress the deficiencies of the currently available therapeutic agents and the urgent need for new candidates [1, 2].

Leishmaniasis is a group of tropical disease caused by parasites of about 20 species of the genus Leishmania, which were transmitted by a group of 50 species and sub species of phlebotomine insects [3, 4]. Official data show that there are 12 million infected people around the world, 350 million at risk of acquiring the disease, and 1.5-2 million that will be infected annually [5, 6].

Currently used drugs for treatment of human cutaneous leishmaniasis are quite toxic and cause severe side effects such as pancreatic and cardiac toxicity that have therefore limited their use. Conventional therapy consists in the parenteral administration of pentavalent antimonium and

meglumine antimoniate during 28 days, generally under strict medical supervision, making the treatment unaffordable for most patients.
As happens in Leishmaniasis, the current synthetic drugs such as nifurtimox (a nitrofuran derivative) and benznidazole (a nitroimidazole derivatives) are associated to severe side effects, including cardiac and/or renal toxicity, which accounts for the need to search new effective chemotherapeutic and chemo prophylactic agents [7- 10].

In view of the importance of benzimidazole, many classical methods for the synthesis of 5-substituted benzimidazole have been reported in the literature [11-21]. But we are using selective and eco-friendly method particularly C-C and C-X bond formation in 5- substituted benzimidazole derivatives.

EXPERIMENTAL SECTION

The reported melting point (^oC) are the uncorrected ones. The infrared spectra were recorded using KBr on a Perkin–Elmer model 881. NMR spectra were obtained in $CDCl_3$ (with) Me_4Si as internal standard, Aldrich and are reported in parts per million downfield from Me_4Si, proton. FAB mass were recorded on FAB mass spectrometer model SX-102.

General procedure for formation of 3a – 3f.
Compound **3a-3f** was synthesized by a method reported earlier [20].

Representative procedure for 4a-4f.
A mixture of **3a – 3f** (50 mmol), dry K_2CO_3 (2.5 mol) and dibromopentane (25 mmol) in dry DMF was stirred at room temperature for 24 hrs. After TLC monitoring, till completion of reaction, the reaction mixture was poured in ice water (500ml). It was extracted with DCM (2 × 100 ml). The extracted DCM portion was treated with ice cold water (5 × 100ml) and brine solution (2 × 100ml), dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude product was purified by recrstalization using methanol in chloroform furnished solid compounds of **4a – 4f** in different percentages.

1,5-Bis(5-chloro-1H-benzimidazole)pentane (4a)
Yield: 68%; M.p.168 ^oC; IR (KBr), v, cm^{-1}; 3081, 2956, 1624, 1583, 980, 746; ^1H NMR (200MHz, $CDCl_3$, TMS) δ1.10 (m, 2H), 1.65 (m, 4H), 3.60 (s, 4H), 7.68 – 7.23 (m, 6H, Ar - H), 8.16 (s, 1H, Benz -H); MS m/z: 374 (M^+ +1).

1,5-Bis(5-chloro-2-methyl-1H-benzimidazole)pentane (4b)
Yield: 70%; M.p. 178-180 ^oC; IR (KBr), v, cm^{-1}; 2960, 2913, 1626, 1585, 981, 747; ^1H NMR (200MHz, $CDCl_3$, TMS) δ 1.10 (m, 2H), 1.65 (m, 4H), 2.39 (s, 6H), 3.60 (s, 4H), 7.67 - 7.14 (m, 6H, Ar-H); MS m/z: 402 (M^+ +1).

1,5-Bis(5-nitro-1H-benzimidazole)pentane (4c)
Yield: 69 %; M.p. 174-176 ^oC; IR (KBr), v, cm^{-1}; 3085, 2982, 1626, 1592, 1368, 980; ^1H NMR (200MHz, $CDCl_3$, TMS) δ 1.12 (m, 2H), 1.70 (m, 4H), 3.65 (s, 4H), 7.96 - 7.4 (s, 6H, Ar-H), 8.20 (s, 2H, Benz - H); MS m/z: 395(M^+ +1).

1,5-Bis(5-nitro-2-methyl-1H-benzimidazole)pentane (4d)
Yield: 72 %; M.p. 166-168 ^oC; IR (KBr), v, cm^{-1}; 2987, 2925, 1623, 1597, 1367, 982, 741; ^1H NMR (200MHz, $CDCl_3$, TMS) δ 1.12 (m, 2H), 1.70 (m, 4H), 2.39 (s, 6H), 3.65 (s, 4H), 7.96 - 7.46 (s, 6H, Ar-H); MS m/z: 423(M^+ +1).

1,5-Bis(5-methyl-1H-benzimidazole)pentane (4e)
Yield: 69 %; M.p. 156-158 ^{o}C; IR (KBr), v, cm^{-1}; 3067, 2980, 2924, 1621, 1596, 1415, 980,754 ; ^{1}H NMR (200MHz, $CDCl_3$, TMS) δ 1.10 (m, 2H), 1.65 (m, 4H), 2.43 (s, 6H), 7.9 – 7.41 (m, 6H, Ar - H), 8.02 (s, 1H, Benz -H); MS m/z: 333(M^+ +1).

1,5-Bis(2,5-dimethyl-1H-benzimidazole)pentane (4f)
Yield: 70 %; M.p. 169-170 ^{o}C; IR (KBr), v, cm^{-1}; 2982, 2918, 1620, 1592, 1410, 980, 756;^{1}H NMR (200MHz, $CDCl_3$, TMS) δ1.10 (m, 2H), 1.65 (m, 4H), 2.40 (s, 12H), 7.40 – 7.10(m, 6H, Ar-H); MS m/z: 371 (M^+ +1).

Representative procedure for 5a – 5f.
A mixture of **3a - 3f** (50 mmol), dry K_2CO_3 (2.5 mol) and dibromohexane (25 mmol) in dry DMF was stirred at room temperature for 24 hrs. After TLC monitoring, till completion of reaction, the reaction mixture was poured in ice water (500ml). It was extracted with DCM (2 x 100 ml). The extracted DCM portion was treated with ice cold water (5 × 100ml) and brine solution (2 × 100ml), dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude product was purified by recrstalization using methanol in chloroform furnished solid compounds of **5a – 5f** in different percentages.

1,6-Bis(5-chloro-1H-benzimidazole)hexane (5a)
Yield: 66 %; M.p.148-150 ^{o}C; ^{1}H NMR (200MHz, $CDCl_3$, TMS) δ1.10 (m, 2H), 1.65 (m, 4H), 3.60 (s, 4H), 7.64 – 7.12 (m, 6H, Ar - H), 8.16 (s, 1H, Benz -H); MS m/z: 388(M^+ +1).

1,6-Bis(5-chloro-2-methyl-1H-benzimidazole)hexane (5b)
Yield: 65 %; M.p.157-158 ^{o}C; ^{1}H NMR (200MHz, $CDCl_3$, TMS) δ1.10 (m, 2H), 1.65 (m, 4H), 2.39 (s, 6H), 3.60 (s, 4H), 7.64 - 7.10 (m, 6H, Ar-H); MS m/z: 416 (M^+ +1).

1,6-Bis(5-nitro-1H-benzimidazole)hexane (5c)
Yield: 69 %; M.p.166-168 ^{o}C; IR (KBr), v, cm^{-1}; 3087, 2982, 1624, 1595, 1366, 982; ^{1}H NMR (200MHz, $CDCl_3$, TMS) δ1.12 (m, 2H), 1.70 (m, 4H), 3.65 (s, 4H), 7.96 - 7.46 (s, 6H, Ar-H), 8.20 (s, 2H, Benz - H); MS m/z: 409 (M^+ +1).

1,6-Bis(5-nitro-2-methyl-1H-benzimidazole)hexane (5d)
Yield: 72 %; M.p.182-184 ^{o}C; IR (KBr), v, cm^{-1}; 2982, 2923, 1620, 1592, 1368, 982; ^{1}H NMR (200MHz, $CDCl_3$, TMS) δ1.12 (m, 2H), 1.70 (m, 4H), 2.39 (s, 6H), 3.65 (s, 4H), 7.96 - 7.50 (s, 6H, Ar-H); MS m/z: 437 (M^+ +1).

1,6-Bis(5-methyl-1H-benzimidazole)hexane (5e)
Yield: 74 %; M.p.167-168 ^{o}C; ^{1}H NMR (200MHz, $CDCl_3$, TMS) δ1.12 (m, 2H), 1.70 (m, 4H), 3.65 (s, 4H), 7.56 - 7.96 (s, 6H, Ar-H), 8.20 (s, 2H, Benz - H); MS m/z: 347 (M^+ +1).

1,6-Bis(2,5-dimethyl-1H-benzimidazole)hexane (5f)
Yield: 71 %; M.p.190-192 ^{o}C; ^{1}H NMR (200MHz, $CDCl_3$ TMS) δ1.10 (m, 2H), 1.65 (m, 4H), 2.40 (s, 12H), 7.10 - 7.40 (m, 6H, Ar-H); MS m/z: 385(M^+ +1).

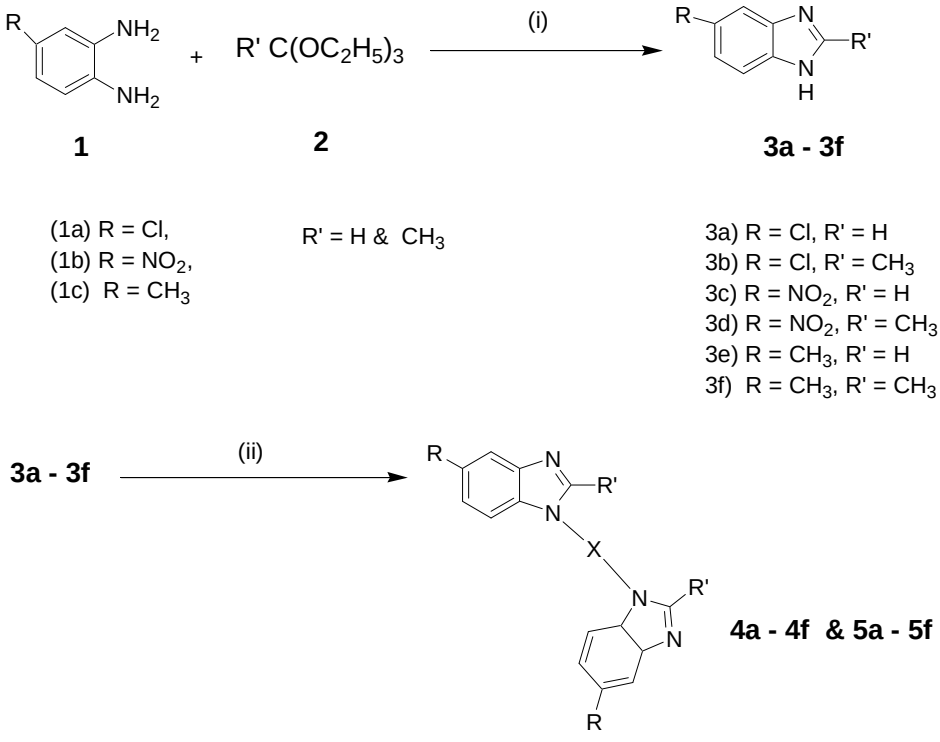
Representative procedure for 6a – 6d.
Synthesized compounds **4c, 4d, 5c & 5f** was hydrogenated in at 45 pci over 10% palladium on carbon in ethanol. Added 300 ml of ethanol and 0.4 -0.5 g of catalyst were used, and the reaction time was 2h. After hydrogenation the catalyst was filtered off, and the filtrate was treated with ethanolic HCl (10–15 ml) to give a precipitate of final products **6a -6d**.

1,5-Bis(5-amino-1H-benzimidazole)pentane (6a)
Yield: 68 %; M.p.172-174 ^{o}C; IR (KBr), v, cm^{-1}; 3437-3359, 3041, 2982, 1620, 1592, 1410; ^{1}H NMR (200MHz, $CDCl_3$, TMS) δ1.12 (m, 2H), 1.70 (m, 4H), 3.65 (s, 4H),6.32(s, 4H), 7.56 - 7.02 (s, 6H, Ar-H), 8.24 (s, 2H, Benz - H); MS m/z: 319(M^+ +1).

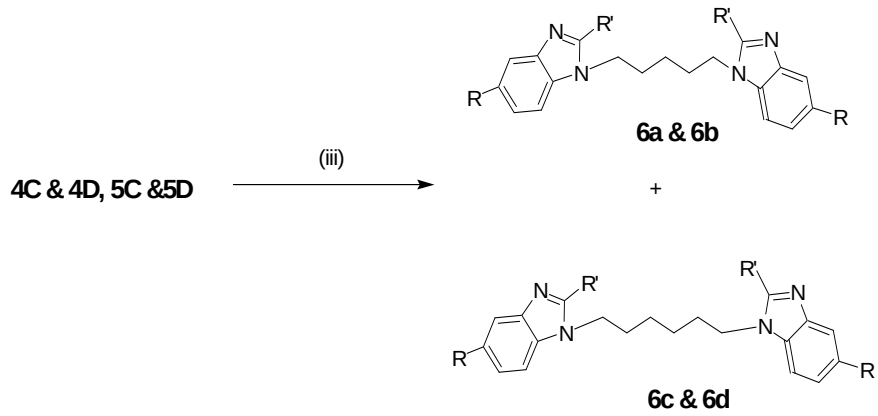
1,5-Bis(5-amino-2-methyl-1H-benzimidazole)pentane (6b)
Yield: 64 %; M.p.190-192 ^{o}C; IR (KBr), v, cm^{-1}; 3440-3362, 3042, 2982, 2924, 1620, 1592, 1410; ^{1}H NMR (200MHz, $CDCl_3$, TMS) δ1.12 (m, 2H), 1.70 (m, 4H), 2.42 (s, 6H), 3.65 (s, 4H), 6.45(s, 4H), 7.88 – 7.14 (s, 6H, Ar-H); MS m/z: 347 (M^+ +1).

1,6-Bis(5-amino-1H-benzimidazole)hexane (6c)
Yield: 66 %; M.p.183-184 ^{o}C; IR (KBr), v, cm^{-1}; 3443-3365, 3039, 2989, 2922, 1625, 1597, 1416, 980; ^{1}H NMR (200MHz, $CDCl_3$, TMS) δ1.12 (m, 2H), 1.70 (m, 4H), 3.65 (s, 4H), 6.08 (s, 4H), 7.82 - 7.12 (s, 6H, Ar-H), 8.90 (s, 2H, Benz – H); MS m/z: 333 (M^+ +1).

1,6 -Bis(5-amino-2-methyl-1H-benzimidazole)hexane (6d)
Yield: 70 %; M.p.194-196 ^{o}C; IR (KBr), v, cm^{-1}; 3444-3369, 3040, 2988, 1623, 1592, 1418, 980, 761; ^{1}H NMR (200MHz, $CDCl_3$, TMS) δ1.12 (m, 2H), 1.72 (m, 4H), 2.39 (s, 6H), 3.65 (s, 4H), 6.23 (s, 4H), 7.56 - 7.20 (s, 6H, Ar-H); MS m/z: 361 (M^+ +1).



(i) MV, $ZrOCl_2.8H_2O$
(ii) K_2CO_3, DMF, RT, Stirring



iii) H_2, 10% Pd/C, EtOH, 2h, then EtOH/HCl

6a) R =NH_2, R' =H
6b) R =NH_2, R' =CH_3
6c) R =NH_2, R' =H
6d) R =NH_2, R' =CH_3

Scheme. 1

Biological activity

Antipromastigote activity

The *Leishmania donovani* promastigote were transfected with firefly luciferase gene, and the transfectants were maintained in medium 199 supplemented with 10% foetal calf serum, 1% penicillin (50 µg/ml), and streptomycin (50 µg/ml) solution (sigma) under pressure of G 418 (Sigma) [22]. The in vitro effect of the compound on the growth of promastigotes was assessed by monitoring the luciferase activity of viable cells after the treatment. The transgenic promastigotes of late log phase were seeded at 5×10^5/100 µl medium 199 per well in 96–well flat bottomed microtitre plates and incubated for 72 h in medium alone or in the presence of serial dilutions of drugs (2.5-50 µg/ml) in DMSO. Parallel dilutions of DMSO were used as controls. After incubation, an aliquot (50 µl) of promastigote suspension was aspirated from each well of a 96–well plate and mixed with an equal volume of steady Glo® reagent (Promega) and luminescence was measured by a luminometer. The inhibition of parasitic growth was determined by comparing the luciferase activity of drug treated parasites with that of untreated controls by the general formula;

Percentage Inhibition = N – n / N ×100

Where N is average relative luminescence unit (RLU) of control wells and n is average RLU of treated wells.

Antiamastigote activity

For assessing the activity of compounds against the amastigote stage of the parasite, mouse macrophage cell line infected with promastigotes expressing luciferase firefly reporter gene was used [22]. Cells were seeded in a 96 well plate (5×10^4 cell/100 µl/well) in RPMI – 1640 containing 10% foetal calf serum and the plates were incubated at 37 ^oC in a CO_2 incubator. After 24 h, the medium was replaced with fresh medium containing stationary-phase promastigotes (2.5×10^5 /100µl/well). Promastigotes invade the macrophage and are transformed into amastigotes. The tested material in appropriate concentrations (2.5–50 µg/ml) in complete medium was added after replacing the previous medium and the plates were incubated at 37 ^oC in a CO_2 incubator for 72 h. After incubation, the drug-containing medium was decanted and 50 µl PBS was added in each well and mixed with an equal volume of steady Glo® reagent. After

gentle shaking for 1-2 min, the parasitic growth was taken in a luminometer. The inhibition of parasitic growth was determined as described above.

***Tab.1*. In vitro antileishmanial activity profile of 1,5-Bis(5-substituted benzimidazole) alkanes against promastigote and amastigotes**

S. No.	Compound	Antipromastigote activity (µg/ml)	Antiamastigote activity (µg/ml)
		IC_{50} (C.I.)	IC_{50} (C.I.)
1	4a	43.65 (28.05-67.66)	ND
2	4c	7.37 (6.48-8.38)	8.52 (7.02-10.32)
3	4d	9.98 (8.77-11.37)	12.89 (9.32-17.83)
4	4e	13.55 (11.78-15.57)	ND
5	5a	22.68 (20.40-25.22)	ND
6	5c	6.91 (6.33-7.55)	8.57 (7.07-10.39)
7	5d	12.59 (15.56-19.90)	ND
8	5e	15.71 (13.84-17.83)	ND
9	6a	4.57 (3.86-5.35)	13.16 (9.77-17.73)
10	6b	4.59 (4.13-5.10)	22.80 (20.11-25.87)
11	6c	1.58 (1.55-1.60)	5.32 (4.31-6.56)
12	6d	2.04 (1.49-2.69)	6.15 (5.24-7.22)
13	Pentamidine	0.58 (0.55-0.60)	-
14	Amphotericine-B	-	$6.46x10^{-3}$
15	Miltefocine	-	33.90 (29.90-38.44)

ND = note done.

IC $_{50}$ was calculated by Probit analysis [23]. Compounds with more than 15 µg/ml IC_{50} were considered as inactive while compound with IC_{50} between 15 and 5 µg/ml were considered as moderately active and less than 5µg/ml IC_{50} as highly active.

RESULTS AND DISCUSSION

The synthesis of 5-substituted benzimidazoles **3a-3f** was carried out by the condensation between the appropriate *o*- phenylenediamine and orthoesters **2** in presence of catalytic amount of $ZrOCl_2.8H_2O$ (10 mole %) gives quantitative yield (75-85%). In conventional method formation of benzimidazoles require ethyl alcohol at RT over a long period of time, resulted low yield of final compound.

The microwave irradiated method for formation of 5-substituted benzimidazole **3a-3f** without catalyst isolated yield only 20-30%.

The dimeric form of intermediates 3a -3f in presence of K_2CO_3 and DMF at RT furnished to yield **4a-4f** (68-72 %) and **5a-5f** (65–74%). The nitro group of compounds **4c, 4d, 5c & 5d**, were converted in to amino group. All the compounds were characterized by IR, ^{1}H NMR and MASS.

The 1,5-Bis(5-substituted benzimidazole)alkanes and diamine derivatives of it, were subjected to in vitro antileishmanial screening against promastigote and amastigote model. Amongst the 12 compounds tested for *in vitro* antipromastigote activity, several compounds **(4c, 4d, 5c, 6a, 6b, 6c & 6d**) have shown significant activity ($IC_{50,}$ Table-1).

The compounds which showing significant activity also evaluated against amastigote stage in macrophages. Of these, four compounds (**4c, 5c, 6c & 6d**) have shown encouraging results (Table-1). Both of drug miltefosine and amphotericin-B, treated as reference drug, because these are new lead in antileishmanial chemotherapy and may be very useful for future optimization work in the area of drug development against leishmaniasis.

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