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Novel bisbenzimidazoles with antileishmanial effectiveness

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Abstract—A small library of 2,2′-[(α,ω-alkanediylbis(oxyphenylene)]bis-1*H*-benzimidazoles has been prepared and screened in vitro against *Pneumocystis carinii*, *Trypanosoma brucei rhodesiense*, and *Leishmania donovani*. Among the six tested compounds two derivatives emerged as promising hits characterized by IC_{50} values lower than that determined for pentamidine against *L. donovani*.

Whereas the amidine group is seldom encountered in biomolecules, it is present in a number of anticoagulant drug candidates[1] and some pharmaceutical specialties prescribed to cure antifungal and antimicrobial infections.[2] Among those specialties, let us mention (Fig. 1) propamidine (**1**, Brolene®) used in the treatment of eyes infections caused by *Acanthamoeba keratitis*, hexamidine (**2**, Hexomedine®) used as a topical antiseptic found in many skin care compositions, and pentamidine (**3**, Pentacarinat®, Pentam®, NebuPent®), the most representative example of the series, clinically used for the treatment of *Pneumocystis carinii* pneumonia,[3] Human African trypanosomiasis[4] (sleeping sickness), and leishmaniasis.[5] However despite their efficiency those compounds, and pentamidine more particularly, are plagued by important drawbacks including poor bioavailability and some unpleasant side effects.[6] In order to circumvent those drawbacks, many structural variations have already been considered and they essentially deal with modifications of the moiety linking both benzamidine groups. In that sense furamidine[7] (**4**) and 4,4′-(piperazine-1,4-diyl)bisbenzamidine[8] (**5**) have recently emerged as promising drug candidates for the treatment of trypanosomiasis and *P. carinii* pneumonia, respectively

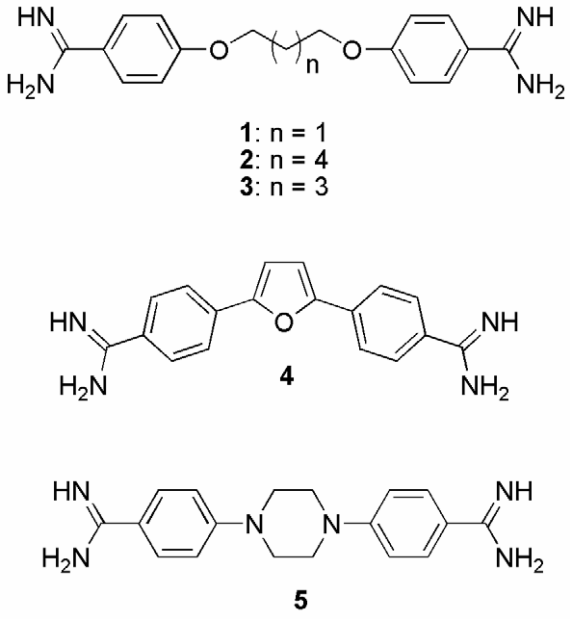

Figure 1. Some pharmacologically important bisbenzamidines.

Keywords: Bisbenzamidine; Bisbenzimidazole; Pentamidine; *Pneumocystis carinii* pneumonia; Human African trypanosomiasis; Leishmaniasis.
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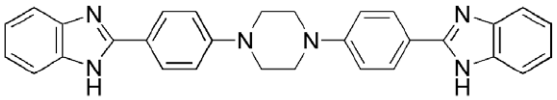


6

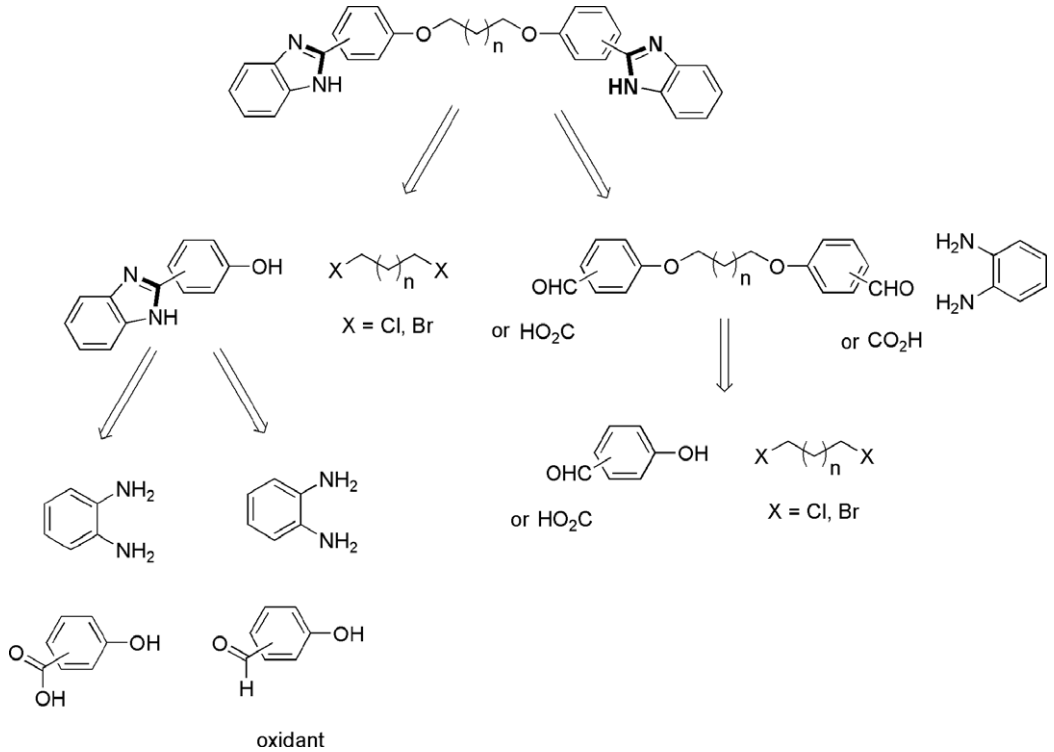
Figure 2. Structure of the bisbenzimidazole **6**.

Our approach is quite different as it consists in masking the amidine functions by incorporation into conjugated cyclic systems and more particularly into benzimidazole systems. In that way, we have already observed that bisbenzimidazole **6** (Fig. 2), structurally related to **5**, was inactive[9] against *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei brucei* but active, and far more active than **5** or even pentamidine **3**, against *Leishmania donovani*.[10] In this report, we extend that preliminary result and we disclose our recent findings on the preparation and the evaluation of other novel bisbenzimidazoles.

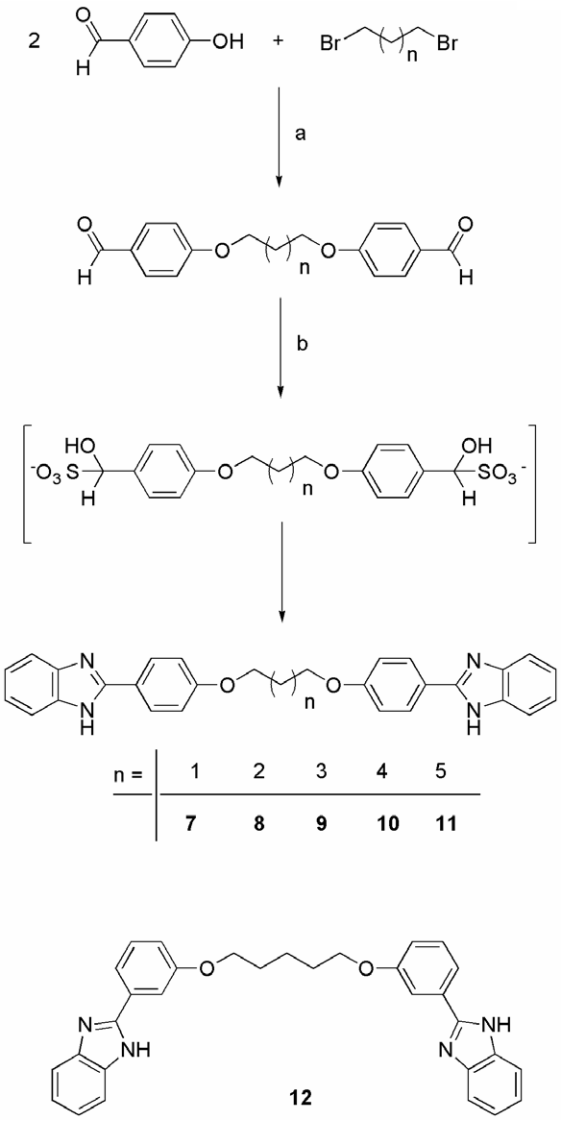
Benzimidazoles are generally prepared[11] (*cfr.* Scheme 1) by the reaction of 1,2-phenylenediamine with carboxylic acid under drastic conditions. Another popular route[11] involves 1,2-phenylenediamines and aldehydes to form transient imines that must be oxidized in a second step. A few papers indicate that starting from the bisulfite adduct of the aldehydes enables to avoid the oxidation step[12] and we decided to test the possibility of exploiting that protocol to prepare bisbenzimidazoles **7**–**12** (*cfr.* Scheme 2). In those substances the heterocyclic systems are linked by oxyphenylene groups separated by a number of methylene units ranging from 3 to 7. Therefore, they are structurally related to the bisbenzamidines **1**–**3**.

Based on a disconnection strategy, the targeted derivatives can be obtained either (i) by forming 2-(hydroxyphenyl)-1*H*-benzimidazoles and subsequently the ether bonds by reaction with a dihaloalkane or (ii) by reacting a dihaloalkane with an hydroxybenzaldehyde and then only forming the heterocyclic entities. In our hands, the first route led to mixtures of compounds (including *N*-alkylated benzimidazoles) whereas the second synthetic scheme afforded the expected derivatives in good yields (75–98%) and purity. Intermediate dialdehydes have been described in the literature[13] and reaction conditions have been optimized for the cyclization step only. Indeed activation of the dialdehydes by reaction with sodium bisulfite and coupling with the diamines were performed by a one-pot procedure and were advantageously conducted under microwave irradiation within a few minutes[14].

In order to establish the pharmacological profile of compounds **7**–**12** they have been tested in vitro against *P. carinii*, *Trypanosoma brucei rhodesiense*, and *L. donovani*, the three species that can be efficiently killed by the action of pentamidine **3**. Inspection of the data gathered in Table 1 clearly indicates that inclusion of the amidine functions in benzimidazole systems reduced the in vitro activity against *P. carinii* and *Trypanosoma brucei rhodesiense* by a tenfold factor and even more when compared to **3**. For both strains the most active derivative was compound **12** bearing the benzimidazole groups in the *meta* position relative to the ether bonds. Except **7** the other derivatives were inactive against both parasites. The situation was quite different in the screening against *L. donovani*. Indeed the IC_{50} determined for pentamidine **3** was 2.2 μM and the bisbenzimidazoles



Scheme 1. Disconnection approach to the targeted bisbenzimidazoles.



Scheme 2. Preparation of compounds **7**–**12**. Reaction conditions: (a) EtOH, K_2CO_3 (1 equiv), reflux 8 h; (b) EtOH/H_2O (3/1), $Na_2S_2O_5$ (1 equiv), 1,2-phenylenediamine (2 equiv), MW 140 °C, 15 min.

Table 1. Pharmacological activities[19] for compounds **7**–**12** and pentamidine **3**

Compound	*Pneumocystis carinii*[20]	*Trypanosoma brucei rhodesiense*[21]	*Leishmania donovani*[22]		Vero cells[23]
	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{90} (μM)	IC_{50} (μM)
7	10.2	>60	1.5	3.3	27.2
8	169.4	>60	4.9	12.9	>100
9	54.1	>60	1.4	3.1	28.7
10	>100	>60	11.8	53.8	27.9
11	>100	>60	13.6	56.2	46.1
12	5.2	0.85	10.3	45.1	12.3
3	0.5	0.05	2.2	9.8	>100

that we prepared were characterized by IC_{50} values ranging from 1.4 μM, more active than pentamidine, to 13.6 μM, sixfold less active than pentamidine. Among the tested compounds, **7** and **9** emerged as promising hits exhibiting a more efficient antiparasitic behavior than **3**, when either IC_{50} or IC_{90} values are considered. Interestingly, **7** and **9** possess structural features closely related to propamidine **1** and pentamidine **3** itself.

All compounds were subsequently tested for cytotoxicity. Only compound **8** displayed no cytotoxicity in the assay considered in this study. Hits **7** and **9**, effectively active against *L. donovani*, gave rise to modest selectivity indexes expressed as ratio IC_{50} Vero cells/IC_{50} *L. donovani* in the range of 20.

Although it is out of the scope of this preliminary communication to suggest any mechanism of action of the bisbenzimidazoles under study, it is noteworthy that, contrary[15] to bisbenzamidines, they did not exhibit significant binding, if any, to DNA (data not shown). Therefore, their antiparasitic properties cannot be understood in terms of inhibition of DNA dependent enzymes (e.g., topoisomerases[16] and nucleases[17]). The lack of efficacy of the bisbenzimidazoles against the *Trypanosoma* parasite can tentatively be linked to poor interactions with the P2 aminopurine transporter because the substrate structural recognition motif (i.e., the amidine group) is masked in those compounds.[18]

In conclusion we have successfully prepared a series of novel bisbenzimidazoles structurally related to clinically used bisbenzamidines. Although those compounds must be carefully considered due to their potential cytotoxicity, our approach led to the identification of some promising hits that were selectively active against *L. donovani* at doses deserving further investigations. Other screenings involving a larger library of bisbenzimidazoles are currently in progress.

Acknowledgments

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14. *General procedure.* A mixture of an hydroxybenzaldehyde (5.12 g; 42 mmol), a dibromoalkane (20 mmol), and potassium carbonate (2.76 g; 20 mmol) in ethanol (10 ml) was heated under reflux for 8 h. After cooling, the precipitate was filtered and successively washed with water, ethanol, and ether. The bisbenzaldehyde was pure enough to be engaged in the second step. A mixture of the bisbenzaldehyde (3 mmol), sodium pyrosulfite (0.57 g; 3 mmol), *ortho*-phenylenediamine (6 mmol), and water (3 ml) in ethanol (9 ml) was irradiated in a microwave oven (Biotage) for 15 min at 140 °C. After cooling the precipitate was filtered and thoroughly washed with water, ethanol, and ether. 4,4′-[1,5-Pentanediyl(oxy)]bisbenzaldéhyde: IR (KBr, cm^{-1}): 2945, 1692, 1604, 1576, 1510. ^1H NMR (DMSO-d_6, ppm): 9.9 (s, 2H, CHO); 7.9 (d, 4H, $J = 8$ Hz, C_6H_4:H^2,H^6); 7.1 (d, 4H, $J = 8$ Hz, C_6H_4:H^3,H^5); 4.2 (t, 4H, $J = 6$ Hz, O–CH_2–CH_2–CH_2); 1.8 (m, 4H, $J = 6$ Hz, O–CH_2–CH_2–CH_2); 1.5 (m, 2H, $J = 6$ Hz, O–CH_2–CH_2–CH_2). Mp (°C): 90.7–94.5 °C. Yield: 90%. 2,2′-[1,5-Pentanediylbis(oxy-1,4-phenylène)]bis-1*H*-benzimidazole (**8**): IR (KBr, cm^{-1}): 3623–2400 (N–H bz), 1762, 1611,1500, 1254. ^1H NMR (DMSO-d_6, ppm): 8.1 (d, 4H, $J = 9$ Hz, C_6H_4:$H^{2'}$,$H^{6'}$); 7.6 (AA′BB′, 4H, benz: H^4,H^7); 7.2 (AA′BB′, 4H, benz: H^5,H^6); 7.1 (d, 4H, $J = 9$ Hz, C_6H_4:$H^{3'}$,$H^{5'}$); 4.1 (t, 4H, $J = 6$ Hz, O–CH_2–CH_2–CH_2); 1.8 (m.large, 4H, O–CH_2–CH_2–CH_2); 1.6 (m.large, 2H, O–CH_2–CH_2–CH_2). HRMS (ESI-ToF) $MH^+C_{31}H_{29}N_4O_2$: MW experimental: *m/z* 489.2275; MW calculated: *m/z* 489.2291. Mp (°C): >300 °C. Yield: 94%.
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19. Reported values are means of 2–4 determinations.
20. *Pneumocystis carinii* were obtained from chronically immunocompressed Long Evans and Brown Norway rats. Typically, infected rat lungs yielded up to 2×10^{10} organism nuclei with the vast majority (about 95%) of the life cycle forms present as trophic forms with the remainder (about 5%) being composed of cysts. *P. carinii* preparations were evaluated for microbial contamination, ATP content, karyotype, and host cell content prior to use in the ATP assay. See, Cushion, M. T.; Walzer, P. D.; Collins, M. S.; Rebholz, S.; Vanden Eynde, J. J.; Mayence, A.; Huang, T. L. *Antimicrob. Agents Chemother.* **2004**, *48*, 4209.
21. Assays were performed in 96-well microtiter plates, each well containing 100 μl of culture medium with 8×10^3 bloodstream-form trypomastigotes of the strain *Trypanosoma brucei rhodesiense* STIB 900, which is known to be susceptible to all currently drugs. After 3 days of incubation, parasite growth is assessed fluorimetrically after the addition of resazurin. Fluorescence is measured after 24 h at 37 °C. See, http://www.who.int/tdr/grants/workplans/pdf/.
22. The compounds were dissolved in DMSO at the concentration of 2 mg ml^{-1} and diluted with the culture medium to obtain the desired final concentrations. The solutions were added, in a 96-well microplate assay, to the *L. donovani* promastigotes culture (2×10^6 cells/ml). Controls with corresponding dilutions of DMSO indicated that growth of the parasite was not altered under those experimental conditions. The plates were incubated at 26 °C for 72 h and growth of *Leishmania* promastigotes was determined by Alamar blue assay. See, Mikus, J.; Steverding, D. *Parasitol. Int.* **2000**, *48*, 265.
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