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Identification of a Novel Potent and Selective Anti-*Trichomonas vaginalis* Agent among Libraries of Bisbenzimidazoles

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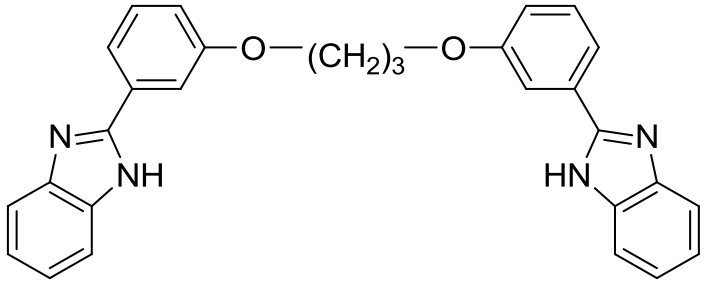
Graphical Abstract



MIC after 48 h under aerobic conditions

T. vaginalis isolate C1: 9 ± 4 µM *vs* 18 ± 5 µM for metronidazole

T. vaginalis isolate 085: 26 ± 7 µM *vs* 145 ± 12 µM for metronidazole

Ability to cure a mouse model infection using

T. vaginalis isolate 286 at 10 mg kg^{-1} day^{-1} for 4 days: 4/5 *vs* 5/5 for metronidazole

T. vaginalis isolate 085 at 10 mg kg^{-1} day^{-1} for 4 days: 4/5 *vs* 0/5 for metronidazole

Abstract:

Small libraries of bisbenzimidazoles structurally related to pentamidine have been synthesized and evaluated against different species of parasites.

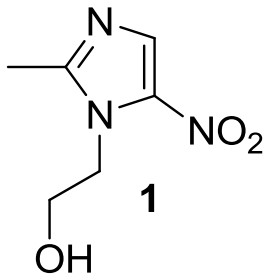
2,2’-[1,3-Propanediylbis(oxy-1,3-phenylene)]bis-1*H*-benzimidazole emerged as a potent and selective anti-*Trichomonas vaginalis* agent from *in vitro* and *in vivo* studies. In particular, *in vitro* under aerobic conditions the compound was more active than metronidazole against both metronidazole-susceptible (C1) and –refractory (085) isolates of *Trichomonas vaginalis. In vivo*, it cured a subcutaneous mouse model infection using both kinds of isolates.

Keywords: bisbenzimidazole; metronidazole; *Trichomonas vaginalis*

Introduction

Metronidazole (**1**) is a nitroimidazole that is clinically used for the treatment of bacterial and parasitic infections [1]. It is effective against the anaerobic bacteria *Bacteroides fragilis* (gram negative), *Helicobacter pylori* (gram negative), and *Clostridium difficile* (gram positive) as well as against *Giardia intestinalis, Entamoeba histolytica*, or *Trichomonas vaginalis*.

The drug is generally well tolerated but emergence of refractory strains have been reported, especially in the case of trichomoniasis [2], a sexually transmitted disease (STD) affecting hundreds of millions of people worldwide.



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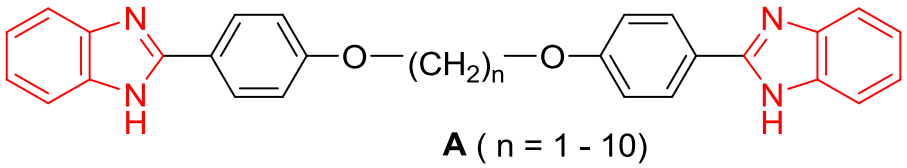
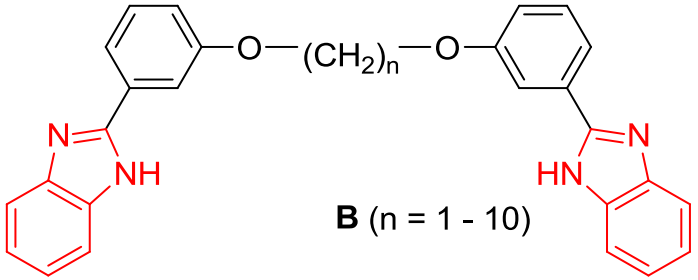
[1] Sobel, R.; Sobel, J.D. *Expert Opin. Pharmacother.* **2015**, *16,* 1109-1115.
[2] Kirkcaldy, R.D.; Augostini, P.; Asbel, L.E.; Bernstein, K.T.; Kerani, R.P.; Mettenbrink, C.J.; Pathela, P.; Schwebke, J.R.; Secor, W.E.; Workowski, K.A.; Davis, D.; Braxton, J.; Weinstock, H.S. *Emerg. Infect. Dis.* **2012**, *18*, 939-943.

As part of our research on analogs of pentamidine (**2**), another drug in clinical use, we focused our attention on derivatives in which the amidine functions are included into a ring and designed small libraries of bisbenzimidazoles of structures **A** – **D**.

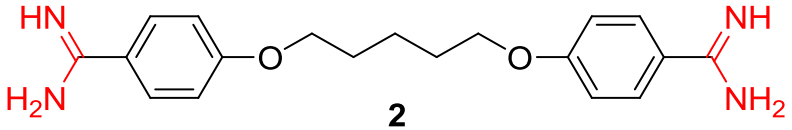
Hereafter we briefly report on the synthesis and biological evaluation of those compounds.



A (n = 1 - 10)



B (n = 1 - 10)



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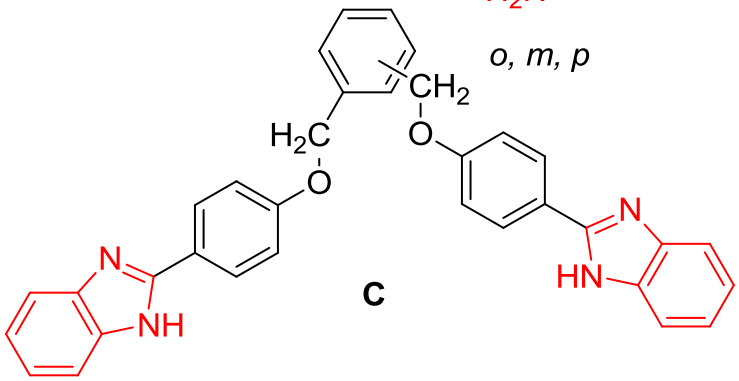
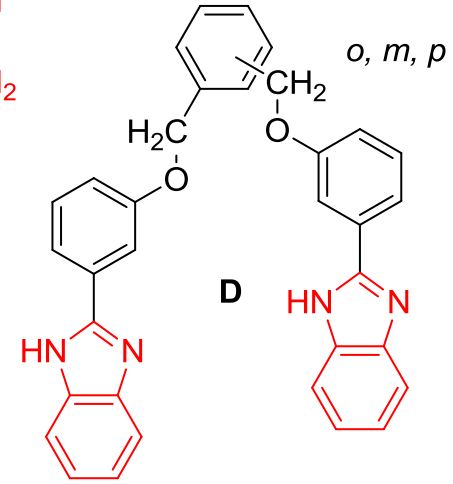


C

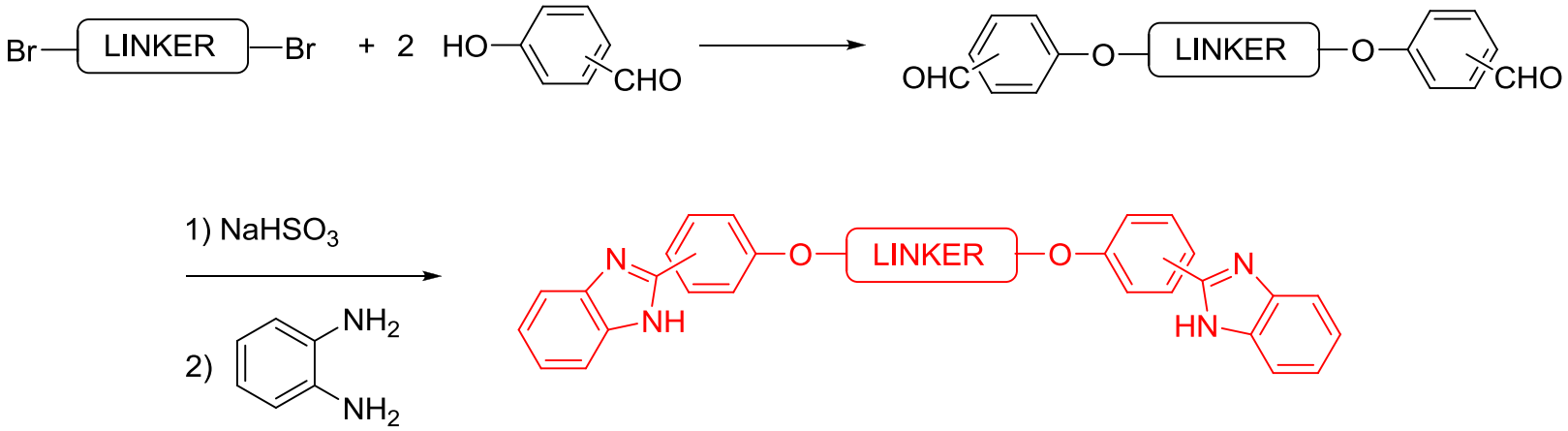


D

Results and discussion

1. Synthesis

The targeted compounds were prepared by a two-step sequence involving (i) an α,ω –dibromo compound generating the internal linker and an hydroxybenzaldehyde; (ii) activation of the so-obtained dialdehydes with sodium bisulfite and further reaction with a 1,2-phenylenediamine. Advantageously, both steps were optimized [3,4] and could be performed within minutes under microwave irradiation (Initiator® Biotage).



[3] Mayence, A.; Pietka, A.; Collins, M.S.; Cushion, M.T.; Tekwani, B.L..; Huang, T.L.; Vanden Eynde, J.J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2658-2661.

[4] Cappoen, D.; Forge, D.; Vercammen, F.; Mathys, V.; Kiass, M.; Roupie, V.; Anthonissen, R.; Verschaeve, L.; Vanden Eynde, J.J.; Huygen, K. *Eur. J. Med. Chem*. **2013**, *63*, 731-738.

2. Biological evaluation – *In vitro* study

The bisbenzimidazoles of structures A – D were evaluated [5,6] for their inhibitory activity toward:

- *Pneumocystis cariniii*
- *Trypanosoma brucei rhodesiense*
- *Trypanosoma cruzi*
- *Leishmania donovani*
- *Plasmodium falciparum K1*
- *Giardia intestinalis*
- *Entemoeba hystolytica Hk-9*
- *Trichomonas vaginalis* isolate 286
- *Trichomonas vaginalis* isolate 085.

Interestingly, 2,2'-[1,3-propanediylbis(oxy-1,3-phenylene)]bis-1H-benzimidazole (**3**) is more active than the control drugs only in the presence of the metronidazole-susceptible and metronidazole-refractory *T. vaginalis* isolates under aerobic conditions.

[5] Mayence, A.; Vanden Eynde, J.J.; Kaiser, M.; Brun, R.; Yarlett, N.; Huang, T.L. B*ioorg. Med. Chem*. **2011**, *19*, 7493-7500.
[6] Korosh, T.; Bujans, E.; Morada, M.; Karaaglioglu, C.; Vanden Eynde, J.J.; Mayence, A.; Huang, T.L.; Yarlett, N. *Chem. Biol. Drug Design* **2017**, *90*, 489-495.

Trypanosoma cruzi

IC_{50} = 8.9 uM

IC_{50} benznidazole = 1.13 uM

Leishmania donovani

IC_{50} = 3.91 uM

IC_{50} miltefosine = 0.45 uM

Trypanosoma brucei rhodesiense

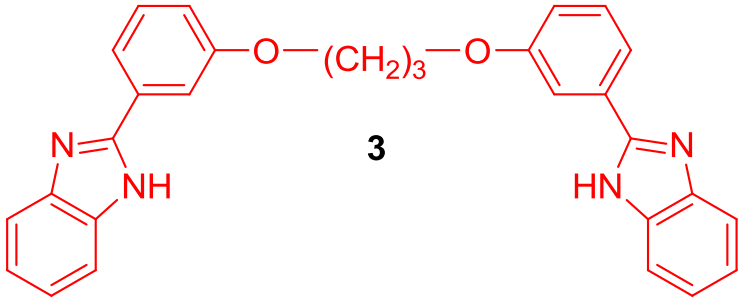
IC_{50} = 8.01 uM

IC_{50} melarsoprol = 0.01 uM

Plasmodium falciparum K1

IC_{50} = 2.41 uM

IC_{50} chloroquine = 0.18 uM



3

Entemoeba hystolytica Hk-9

IC_{50} = 78.26 uM

IC_{50} metronidazole = 5.82 uM

Giardia intestinalis

IC_{50} = 56.07 uM

IC_{50} metronidazole = 1.56 uM

***Trichomonas vaginalis* isolate C1**

IC_{50} = 9 uM

IC_{50} metronidazole = 18 uM

***Trichomonas vaginalis* isolate 085**

IC_{50} = 26 uM

IC_{50} metronidazole = 145 uM

2. Biological evaluation – *In vivo* study

The ability of benzimidazole **3** to cure an *in vivo* subcutaneous mouse model infection using *T. vaginalis* isolates 286 (metronidazole-susceptible) and 085 (metronidazole-refractory) was evaluated [6] at two doses: 10 mg/day/kg and 25 mg/kg/day. Five animals per group were dosed once per day for 4 days. On the sixth day the abscesses were removed and a portion checked microscopically for viable parasites at 24 and 48 hr. Compound **3** appeared to be as effective against both isolates and no sign of behavioral toxicity (loss of appetite, huddling in the corner, moving in circles, lethargy) was observed.

	***T. Vaginalis* 286**		***T. Vaginalis* 085**	
	10 mg/kg/day	25 mg/kg/day	10 mg/kg/day	25 mg/kg/day
	cured animals			
3	4/5	5/5	**4/5**	**5/5**
metronidazole	5/5	5/5	**0/5**	**2/5**

[6] Korosh, T.; Bujans, E.; Morada, M.; Karaaglioglu, C.; Vanden Eynde, J.J.; Mayence, A.; Huang, T.L.; Yarlett, N. *Chem. Biol. Drug Design* **2017**, *90*, 489-495.

Conclusion

We found that, among small libraries of bisbenzimidazoles, 2,2'-[1,3-propanediylbis(oxy-1,3-phenylene)]bis-1*H*-benzimidazole (**3**) emerged as an effective *in vitro* and *in vivo* anti-*Trichomonas vaginalis* agent. It is noteworthy that no sign of behavioral toxicity was observed during the *in vivo* study. In addition, contrary to some lower and higher homologues or structurally related analogues, **3** was characterized by an interesting selectivity because it was poorly active against other fungi/parasites including *Pneumocystis cariniii, Trypanosoma brucei rhodesiense, Trypanosoma cruzi, Leishmania donovani, Plasmodium falciparum* K1*, Giardia intestinalis,* and *Entemoeba hystolytica Hk-9*.

It is well known that benzimidazoles can target the β-tubulin of microtubules, resulting in disruption of mitotic spindle formation, cytoskeleton structures, and disruption of cilia and flagella biosynthesis. As with many genes in *T. vaginalis,* there are multiple β-tubulin gene copies and three of these have conserved Tyr167 and Phe200 residues required for benzimidazole binding. Let us also mention that in our hands, it was found that compound **3** was poorly susceptible to reduction by pyruvate:ferredoxin oxidoreductase.