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Identification of a Hit in a Small Library of Potential Antiplasmodial Imidazo[4,5-b]pyridines

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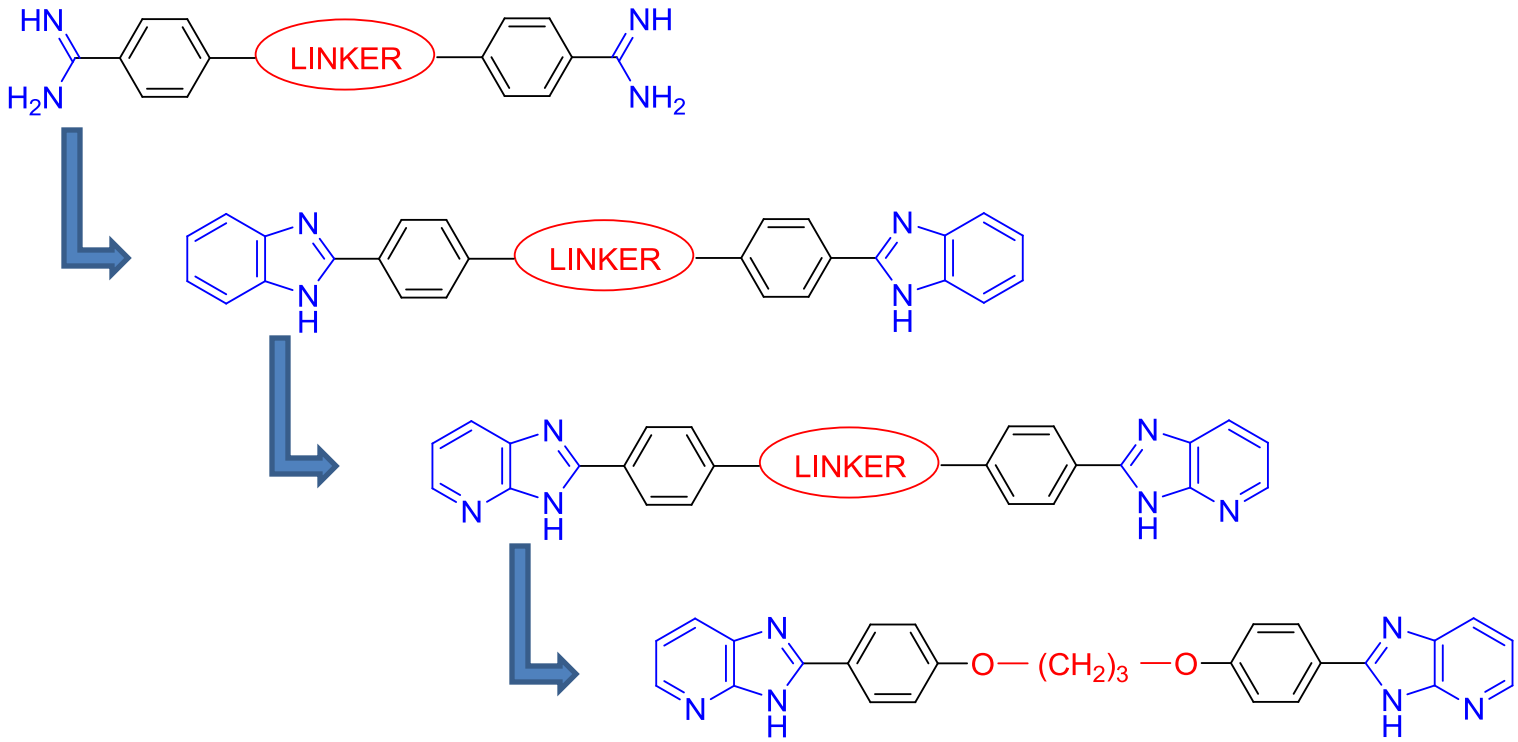
Graphical Abstract



Abstract:

Recently we have demonstrated that some bis(oxyphenylene)benzimidazoles constituted potential antiplasmodial candidates but they are characterized by a high lipophilic character. To circumvent that drawback, two series of structural analogs have been prepared. In the first series, oxygen atoms have been introduced in the linker separating both pharmacophores. In the second series, the heterocyclic skeletons have been replaced by imidazo[4,5-b]pyridine moieties. The antiplasmodial activity of the newly synthesized compounds has been evaluated against the chloroquine-sensitive strain NF-54. Their cytotoxicity in the presence of L6 rat skeletal muscle cells has also been determined.

Among the active derivatives, 2,2'-[propane-1,3-diylbis(oxy-1,4-phenylène)]bis-1*H*-imidazo[4,5-b]pyridine emerged as the most promising hit.

Keywords:

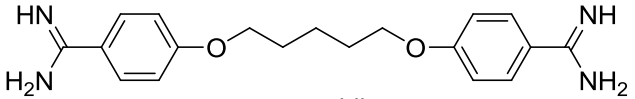
amidine; benzimidazole; imidazo[4,5-b]pyridine; malaria; pentamidine.

Introduction

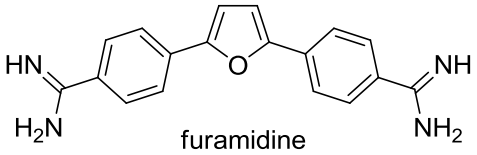
It is estimated that over one billion people (one sixth of the world population) suffers from one or more tropical protozoal infections. Among them malaria, caused by *Plasmodium falciparum*, is the most dangerous disease with the highest rates of complications and mortality. The World Health Organization reports that in 2015 there will be 214 million cases of the sickness causing 438,000 deaths; most of these cases (89 %) and deaths (91 %) occur in sub-Saharan Africa. Fight against female *Anopheles* mosquitoes, the vector of *Plasmodium*, can be affected by indoor spraying with insecticides or distribution of insecticide treated nets to cover beds. Chemoprophylaxis and chemotherapy, including the artemisin-based combination therapies (ACT) constitute other ways to eliminate the parasite. However excessive use of those chemical weapons led to numerous mutations and resistance in *Plasmodium*, thus justifying a continuous search for novel anti-malarial agents [1].

[1] www.who.int/malaria (October 2015)

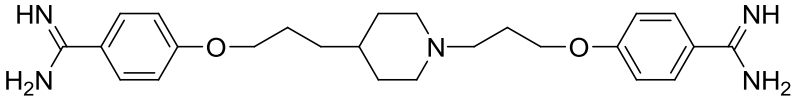
As soon as in 1940, pentamidine, a bisbenzamidine exhibiting a strong activity against several protozoan infections, was successfully tested in the treatment of birds and monkeys malaria [2]. Since that time, many other diamidines have been evaluated for their ability to inhibit growth of *Plasmodium falciparum*, the parasite responsible for human malaria. Among them, mention can be made of furamidine synthesized by Das and Boykin in 1977 [3] and T2307 described by Mitsuyama *et al.* in 2008 [4].



pentamidine



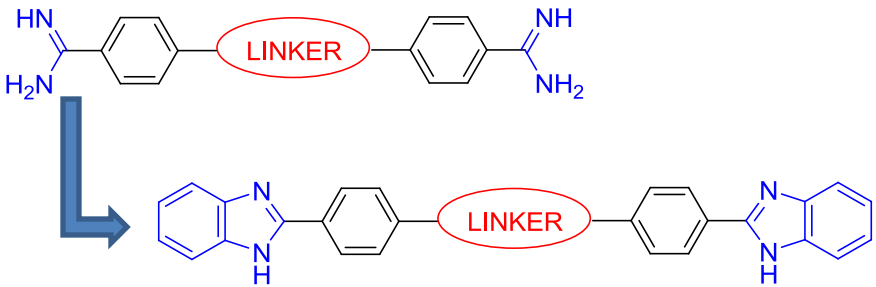
furamidine



T-2307

[2] Fulton, J.D. *Ann. Trop. Med. Parasitol.* **1940**, *34*, 53-66.
[3] Das, B.P.; Boykin, D.W. *J. Med. Chem.* **1977**, *20*, 531-536.
[4] Mitsuyama, J.; *et al. Antimicrob. Agents Chemother.* **2008**, *52*, 1318-1324.

In previous work [5-7], we reported on the antiplasmodial activity of a library of bisbenzamidines connected by various conformationally restricted linkers. We then determined the importance of the amidine functions on that activity by focusing our attention on bisbenzimidazoles [8, 9]. Indeed, in such heterocyclic structures the nitrogen atoms of the amidine group are involved in a conjugated system so that their basicity is dramatically reduced. Interestingly our results indicated that some bisbenzimidazoles were as efficient against *P. falciparum* as their non-heterocyclic analogs.



[5] Mayence, A.; *et al. J. Med. Chem.* **2004**, *47*, 2700-2705.
[6] Vanden Eynde, J.J.; *et al. Bioorg. Med. Chem. Lett.* **2004**, *14*, 1625-1628.
[7] Huang, T.L.; *et al. J. Pharmacy Pharmacol.* **2006**, *58*, 1033-1042.
[8] Mayence, A.; *et al. Bioorg. Med. Chem.* **2011**, *19*, 7493-7500.
[9] Vanden Eynde, J.J.; *et al. Eur. Patent* EP 2194981 (Nov. 23, 2011).

Among the compounds tested against chloroquine resisitant K1 *P. falciparum*, eight derivatives exhibited an activity characterized by IC_{50} values in the range of 180–410 nM (0.11–0.21 µg/mL) and selectivity indexes (IC_{50} rat skeletal myoblasts L6 cells *vs* IC_{50} K1 strain) varying between 92 and more than 450. However those substances are plagued by an excessive hydrophobic character, what is largely recognized as an obstacle to further development.

To circumvent that drawback and to increase the hydrophilic properties, we decided to introduce an ether bound or an hydroxy group in the linker, to add a carboxylic acid function on the benzimidazole moieties, and additionally to replace the benzimidazole systems by imidazo[4,5-b]pyridine fused rings.

Thus, targeted candidates were:

1

2

3

LINKER

a $O-CH_2\cdot CH_2\cdot CH_2\cdot O$

b $O-CH_2\cdot CH(OH)-CH_2\cdot O$

c $O-CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot O$

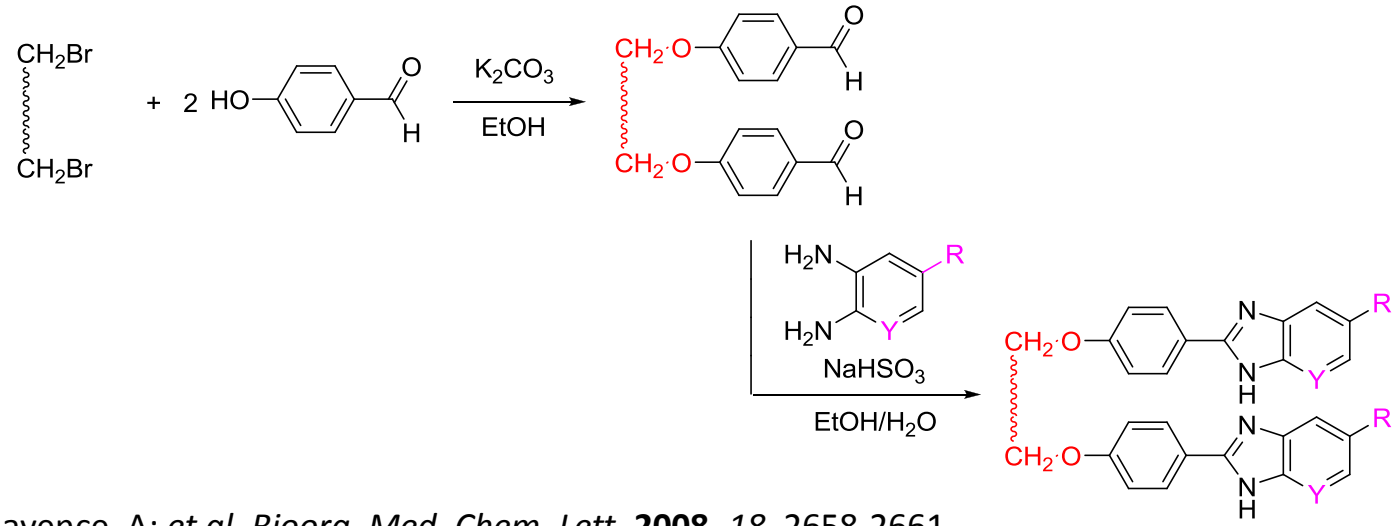
d $O-CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot O$

e $O-CH_2\cdot CH_2\cdot O-CH_2\cdot CH_2\cdot O$

Results and discussion

Strategy of synthesis

The targeted derivatives were prepared by a two-steps sequence following described procedures [10, 11]. In the first step a dibromo precursor was treated with 4-hydroxybenzaldehyde in the presence of a base. In the second step, the aldehyde was activated by conversion into a bisulfitic adduct, which was reacted, without prior isolation, with the appropriate (hetero)aromatic diamine.



[10] Mayence, A; *et al. Bioorg. Med. Chem. Lett.* **2008**, *18*, 2658-2661.
[11] Ridley, H.F.; *et al. J. Heterocycl. Chem.* **1965**, *2*, 453-456.

1: Y = CH; R = H
2: Y = CH; R = CO_2H
3: Y = N; R = H

All compounds were evaluated at the Swiss Tropical Institute for their activity against a line of strain NF54 of chloroquine sensitive *P. falciparum* and for their citotoxicity in the presence of L6 rat skeletal muscle cells.
From the results, it appeared that

Antiplasmodial activity

The five compounds bearing a carboxylic function (**2**) were poorly active against the parasite (IC_{50} > 12 µg/mL *vs* 0.003 µg/mL for chloroquine);

The five (unsubstituted) bisbenzimidazoles (**1**) were fairly active against the parasite (IC_{50} ranging from 0.033 to 0.274 µg/mL);

The five bisimidazopyridines (**3**) were less than ten-fold less active than the corresponding bisbenzimidazoles against the parasite (IC_{50} ranging from 0.235 to 2.1 µg/mL).

From the results, it appeared that

Cytotoxicity

The five compounds bearing a carboxylic function (**2**) were not cytotoxic (IC_{50} > 100 µg/mL *vs* 0.007 µg/mL for podophyllotoxin);

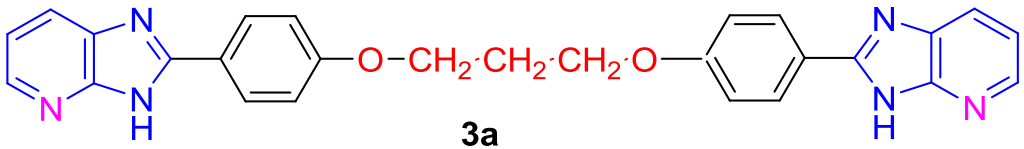
The five (unsubstituted) bisbenzimidazoles (**1**) were poorly cytotoxic (IC_{50} ranging from 1.85 to 14.60 µg/mL);

The five bisimidazopyridines (**3**) were less cytotoxic than the corresponding bisbenz-imidazoles (IC_{50} ranging from 29.5 to > 100 µg/mL).

From the results, it appeared that

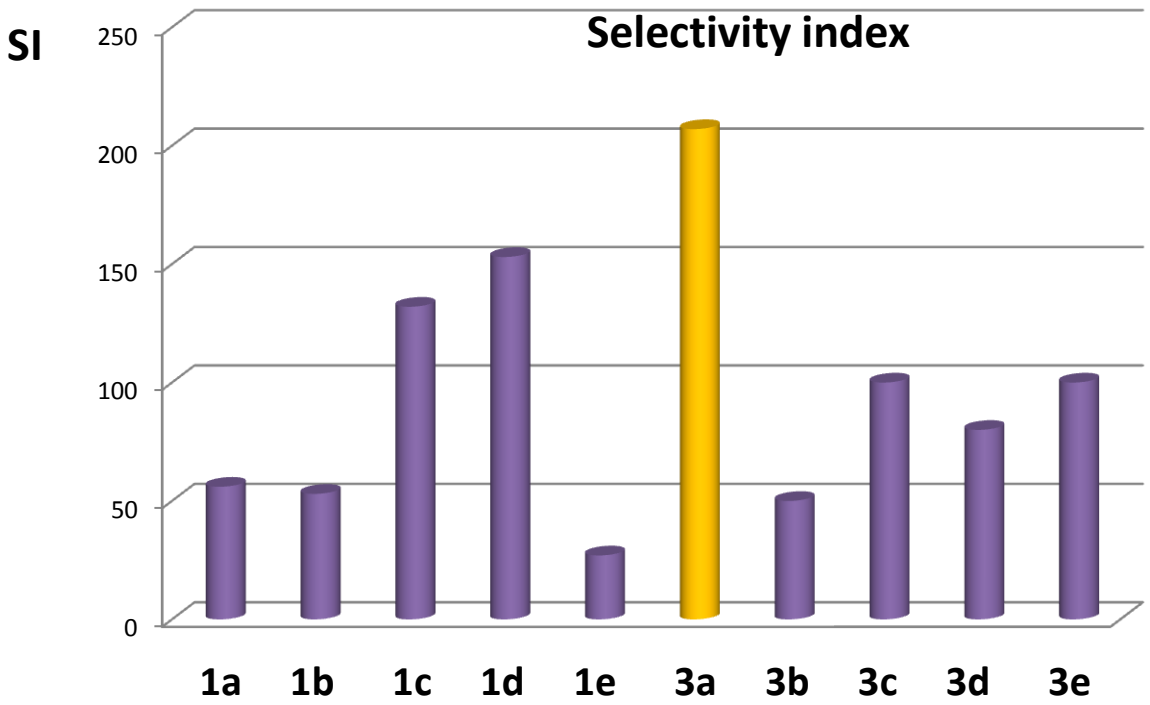
Selectivity index

The selectivity index (SI) has been defined as:
IC_{50} L6 rat skeletal muscle cells / IC_{50} NF54 of chloroquine sensitive *P. falciparum.* Among the ten compounds exhibiting an antiplasmodial activity, 2,2'-[propane-1,3-diylbis(oxy-1,4-phenylène)]bis-1*H*-imidazo[4,5-b]pyridine (**3a**) emerged as the most promising hit. Indeed, it is characterized by the highest selectivity index but also by an acceptable hydrophilic character, as expressed by cLogP.



3a

From the results, it appeared that



1

3

a $O-CH_2\cdot CH_2\cdot CH_2\cdot O$

b $O-CH_2\cdot CH(OH)-CH_2\cdot O$

c $O-CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot O$

d $O-CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot O$

e $O-CH_2\cdot CH_2\cdot O-CH_2\cdot CH_2\cdot O$

Mode of action

Plasmodium falciparum has a life cycle divided into three overall stages (mosquito, liver, and blood stages) that can be selectively targeted by chemotherapeutic agents. Chloroquine is generally thought to act during the blood stage, when the parasite digests hemoglobin to obtain the amino acids it requires. This reaction produces free heme (ferriprotoporphyrin IX, FPIX), which is toxic to *Plasmodium falciparum*. Detoxication of heme can occur in the lysosomal vacuole of the parasite via formation of an insoluble polymer called hemozoin, or malaria pigment. Chloroquine is believed to exert its antimalarial action primarily by inhibiting hemozoin formation either by binding to the free heme or by capping the end of the growing polymer [12].

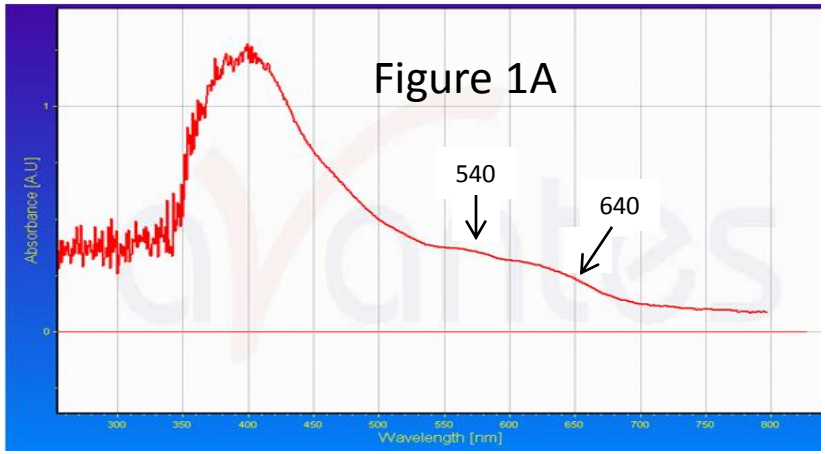
Stead *et al.* [13] suggested that the antiplasmodial action of pentamidine is very similar to that of chloroquine. In order to assess whether bisbenzimidazoles and bisimidazopyridines might act in a similar manner, we analyzed their behavior in the presence of FPIX in a cell-free system [6].

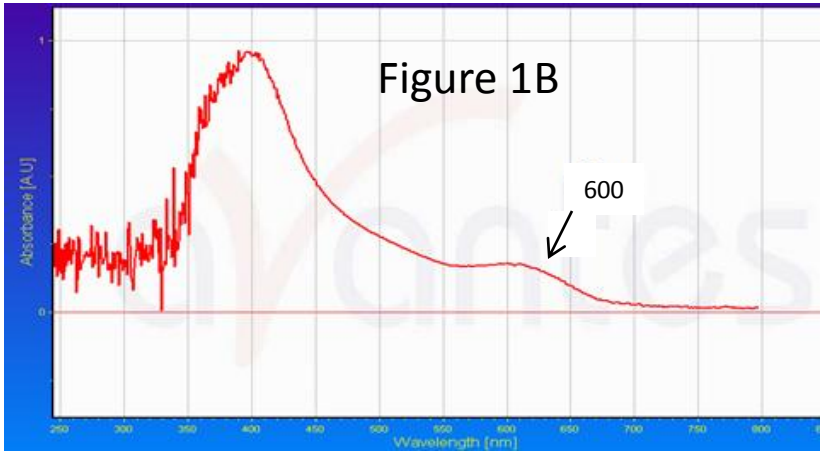
[12] *i.a.* Egan, T. J. *Mini Rev. Med. Chem.* **2001**, 113-123.
[13] Stead, A.M.W.; *et al. Mol. Pharmacol.* **2001**, *59*, 1298-1306.

Indeed, transformation of heme into β-hematin (the synthetic form of hemozoin) can be mimicked in the absence of the parasite under defined experimental conditions (acetate buffer at pH 5.5). In the visible spectrum of the reaction medium, β-hematin is characterized by two shoulders at 540 and 640 nm (Figure 1A).

In the presence of chloroquine (and other antimalarial drugs having the same mode of action), the reaction is inhibited and one maximum only is detected at 600 nm Figure 1B).

Experimentally, we observed that all tested compounds, including **1** and **3**, were found to inhibit the formation of β-hematin, thus suggesting that they could act like chloroquine against the parasite.

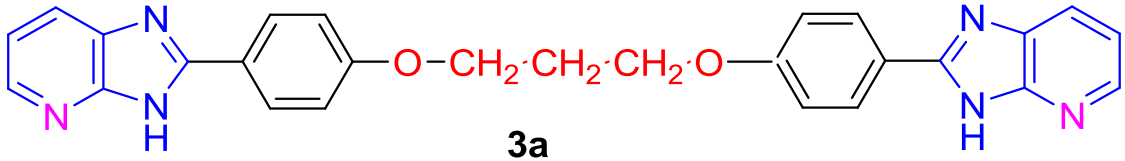




Conclusions

In order to extend our results on the evaluation of the antiparasitic activity of bisbenzamidines and analogs, we synthesized a small library of bisbenzimidazoles and bisimidazopyridines and determined their ability to inhibit growth of *Plasmodium falciparum*, the parasite responsible for human malaria. From the study, 2,2'-[propane-1,3-diylbis(oxy-1,4-phenylène)]bis-1*H*-imidazo[4,5-b]pyridine (**3a**) emerged as the most promising hit. Indeed, it is characterized by a moderate antiplasmodial activity (NF54 chloroquine sensitive strain), a poor cytotoxicity (L6 rat skeletal muscle cells), and an acceptable hydrophilic character, as expressed by cLogP. The compound should behave like chloroquine and be able to inhibit formation of hemozoin in the lysosomal vacuole of the parasite.

Further derivatizations of **3a** are foreseen in order to improve the properties of the hit.



3a