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Reverse-benzamidine antimalarial agents: Design, synthesis, and biological evaluation

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ABSTRACT

In the frame of the development of bis-cationic choline analogs, the RSA of bis-N-alkylamidines were studied and a new series of reverse-benzamidine derivatives was designed. Contrary to the lipophilicity, the basicity of alkylamidine compounds directly influences their antimalarial potencies.

Keywords:
Bis-N-alkylamidines
Antimalarial agents
Total clearance of parasitemia
Chemical design
Reverse-benzamidines

Malaria is an infectious disease widespread in tropical and subtropical regions. The World Health Organization (WHO) estimates that half the world population is at risk of malaria. Although WHO recommends the use of artemisinin-based combination therapies (ACTs), the malaria control is threatened by the increase of Plasmodium falciparum strains resistant to most of available antimalarials, including artemisinin and derivatives. To overcome the parasite multichemoresistance, alkylamidine-based choline analogs have been developed as a potential new chemotherapy against P. falciparum or Plasmodium vivax. Indeed, Vial and Calas studied the parasite’s phospholipid metabolism and defined the phosphatidylcholine de novo biosynthesis as a novel antimalarial target.

The bis-thiazolium salt T3 is the first choline analog to undergo phase 3 clinical trials, validating the strategy of using bis-cationic antimalarial agents. Amidines are strong bases ($pK_a$ ~13–14) and exist under physiological conditions mainly as protonated species. Bis-alkylamidines are thus biososeters of the bis-thiazolium salts (T3, Fig. 1), sharing the same mechanism of action.

Calas et al. previously defined that the duplication of the cationic heads is necessary for good antimalarial activity, and optimized the length of the alkyl linker to 12 methylenes. M64 was the lead compound of the reversed N-alkylamidine compounds, in which the alkyl chain is attached to the functional nitrogen atom (Fig. 1). Whereas T3 possesses a permanent cationic charge, bis-alkylamidines exhibit non permanent charges and the amidoxime (hydroxylated amidine) derivatization temporarily reduces the basic character of amidine function. The resulting compounds were able to orally deliver the active bis-N-alkylamidine M64. Nevertheless, M64 revealed to be quite unstable in vivo.

The aim of this work was to investigate structural variations on the cationic center of the N-alkylamidines. Alkyl or aryl substituents were introduced on the carbon atom of the amidine function of N-alkylamidines (1,12-bis-[alkyl]or aryl]-iminoo]-aminododecane derivatives). Depending on the modulation thus introduced, two groups can be distinguished. (i) In the usual reversed N-alkylamidines (3a-c) an alkyl group is attached to the imino group of the amidine function. (ii) As opposed to the N-alkylamidines, the compounds whose imino group is attached to an aromatic ring, are re-

![Figure 1. Biscaticonic antimalarial compounds.](image-url)
ferred to as "reverse-benzamidines" (3d-o). We aimed to improve the stability of the alkylamidine cationic heads, while maintaining their in vivo antimalarial potencies.

Reverse-benzamidines constitute a new series of alkylamidines. They have been designed to evaluate how the aromatic ring introduced within the cationic heads may influence the antiparasitic activity. Besides, we have developed another series of C-alkylamidines and reported previously that N-substitutions could improve their in vivo antimalarial potencies. These results may be related to the resulting pKa and/or lipophilicity. Thus, we have introduced aromatic substituents and investigated the influence of the lipophilicity and the pKa of the resulting reverse-benzamidine compounds on their in vitro and in vivo antimalarial potencies.

Molecules were synthesized as described in Figure 2. The appropriate alkyl-/benzo-nitriles 1a-p were treated under Pinner's conditions (HCl/EtOH) and converted into the ethyl alkyl-/benzimidates 2a-p. The late derivatives 2a-p were very unstable. The crude product reacted immediately with 1,12-dodecane disulphide in the presence of trietylamine. The triethylamine was added to neutralize the last of hydrochloric acid that prevented generating the targeted reverse amides 3a-p. The different reverse alkyl-/benz-amidine molecules 3a-p were purified as free amides by washing with acetone, water, and diethyl ether. They were then isolated and conserved as hydrochloride salts (washed with anhydrous diethyl ether). In the N-alkylamidines, alkyl groups such as ethyl (3a), iso-propyl (3b), and cyclopropyl (3c) were firstly introduced, as well as hydroxylated function (3d). The amides were obtained starting, respectively, from commercially available propionitrile, iso-butynitrile, cyclopropylcarboxamide or hydroxyacetoni tetrrole 1a-d. In the second series of reverse-benzamidines, the aromatic ring was introduced starting from the appropriate benzamidines. Most of the benzamidines were isolated as their respective hydrochloride salt in good yields, except for 3j that is more hindered, and for 3o due to problems of purification.

The in vitro antimalarial activities were evaluated against a chloroquine-resistant strain of P. falciparum (Nigerian strain). Results are given in Table 1. Both the N-alkylamidines and the reverse-benzamidines possess potent antimalarial activities, in the nanomolar range, except 3e, 3g, and 3h. The N-alkylamidine series is quite homogeneous, with IC50 <20 nM, pKa >12.5, and Log p <5.

The in vitro antimalarial activity was not improved by bulkier alkyl chains nor by a hydroxyl group introduced to increase the hydrophilicity (3d). M64 is still the most potent compound among the N-alkylamidines. Regarding the IC50 and the pKa values, the reverse-benzamidines are a more heterogeneous series as compared to the N-alkylamidines. Indeed, the pKa values ranged between 10 and 14 and the antimalarial activities of 3e, 3g, and 3h were weak (IC50 >450 nM), 3i-k and 3n possessed significantly higher potencies (60 nM < IC50 < 20 nM), while 3f, 3l, 3m, 3o, and 3p were the most potent compounds (IC50 <15 nM). The Log p values of the reverse-benzamidines were higher than in N-alkylamidines group (Log p >5.5, except for 3k and 3o with Log p ~5). As expected, the introduction of the phenyl aromatic ring led to increased lipophilicity. Since the introduction of the furan dramatically decreased antimalarial activity (3e: IC50 = 715 nM), we have no more used a furan ring. In contrast, the compound 3f possessing a phenyl ring revealed a good potency (IC50 = 13.5 nM). We have then developed ten substituted reverse-benzamidines 3f-p varying their basicity and their lipophilicity. These late constituted a sufficient panel to establish some RSA studies. We did not point out any significant relation between the IC50 of N-alkylamidines nor reverse-benzamidines and their lipophilicity.

As opposed to Log p values, we have noticed the influence of the basicity of N-alkylamidines and reverse-benzamidines on their in vitro antiparasitic activity. The Figure 3 illustrates that the observed IC50 against the human P. falciparum parasite were strongly related to the calculated pKa values of the compounds. This link is likely more pronounced in the reverse-benzamidines series. Indeed, the reverse-benzamidines sharing electro-withdrawing groups like -CF3 (3g) or -NO2 (3h) possessed decreased basicity.

![Figure 2. Synthesis of the N-alkylamidines and reverse-benzamidine compounds. Reagents and conditions: (i) gaseous HCl, EtOH, 20 h, 0 °C to rt; (ii) 1,12-dodecanediamine, EtOH, Et3N, 24 h, rt. *Log p were calculated using ACD/Log P DB, Advanced Chemistry Development Inc. †M64 compound was previously described.](image)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Calculated pKa*</th>
<th>P. falciparum IC50 (nM)</th>
<th>P. vincenti ED50 (mg/kg)</th>
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<tr>
<td>M64†</td>
<td>12.65</td>
<td>2.2</td>
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<tr>
<td>3a</td>
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* pKa were calculated using ACD/pKa DB, version 6.0, Advanced Chemistry Development Inc.
† IC50 against the in vitro growth of P. falciparum are means of at least two independent experiments conducted in duplicate.
‡ Antimalarial activities (Efficient Dose 50, ED50) were determined after ip administration of the compounds once daily for four consecutive days to infected mice (3 mice/dose).
§ Compound M64 was previously described.†
‖ Toxicity is observed at higher doses.

![Figure 3. In vitro antimalarial activity (IC50) of N-alkylamidine (△) and of reverse-benzamidine (●) as a function of basicity of the cationic heads (calculated pKa). pKa were calculated using ACD/pKa DB, version 6.0, Advanced Chemistry Development Inc.](image)
Their antiplasmodial potencies are thus dramatically decreased, when compared to 3f, 3g, and 3h being too low, they were not evaluated in vivo. The mice were infected on day 0 and treated with compounds either intraperitoneally (ip) or orally (po) once daily for four consecutive days (days 1–4 post infection, n = 3 per dose). The parasitemia levels were monitored in mice at day 5. All the reversed N-alkylamidines exhibited potent antiplasmodial activities, except 3a (Table 1). But the modulations performed in N-alkylamidines series did not improve M64 activity. The reverse-benzamidine 3i did not reveal any antimalarial activity, while a slight antimalarial activity could be detected with 3f, 3j, 3k, and 3l (ip administration of 5 mg/kg of 3l decreased parasitemia of 40% as compared to control). After ip administration, the other reverse-benzamidines (3m, 3n, 3o, and 3p) exhibited as potent in vivo antimalarial activities (ED50 ip <10 mg/kg) as the best N-alkylamidines, while having lower in vitro antimalarial activities.

Oral administration of 180 mg/kg of N-alkylamidines or of the reverse-benzamidines 3f, 3j, 3k, 3l, and 3p did not reveal any antimalarial effect. On the other side, significant activities were observed with the other compounds 3m, 3n, and 3o, but the parasitemia clearance was not achieved and no ED50 po could be calculated. Thereby, after administration of 180 mg/kg of 3m, 3n, and 3o, respectively, 42%, 20%, and 58% of decrease of parasitemia could be observed as compared to control. These compounds appeared more efficient by oral administration at 180 mg/kg than the N-alkylamidines, which revealed no significant effects at that concentration. However, further studies to improve the bioavailability, as well as pharmacokinetics experiments of the best compounds are needed to obtain compounds suitable for drug development. For example, specific produg strategies might be applied to the most potent compounds in this new reverse-benzamide series.

In conclusion, the reverse-benzamidines have been designed as a new series of antimalarials. The introduction of a phenyl aromatic ring within the polar head can lead to molecules with improved in vivo antimalarial activity. Indeed, four reverse-benzamide compounds exhibited potent antimalarial activities (ED50 ip <10 mg/kg) and three of them (3m, 3n, and 3o) led to a decrease of parasitemia was detected after oral administration (180 mg/kg). Furthermore we have shown that the antimalarial potency can be strongly modulated by introducing aromatic substituents that modify the basicity of the reverse-benzamidines.

Acknowledgments

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Supplementary data

Supplementary data (1H and 13C NMR, MS (FAB or ESI), FTIR data of new compounds and biological protocol) associated with this article can be found, in the online version, at doi:10.1016/j.bmj.2010.07.124.

References and notes