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

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ABSTRACT

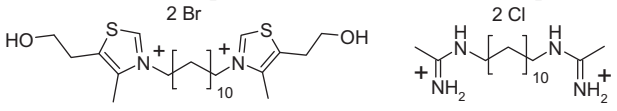
In the frame of the development of bis-cationic choline analogs, the RSA of bis-*N*-alkylamidines were studied and a new series of reverse-benzamidine derivatives was designed. Contrary to the lipophilicity, the basicity of alkylamidine compounds directly influences their antimalarial potencies.

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.[1] Although WHO recommends the use of artemisinin-based combination therapies (ACTs),[2] the malaria control is threatened by the increase of *Plasmodium falciparum* strains resistant to most of available antimalarials, including artemisinin and derivatives.[3,4] To overcome the parasite multichemoresistance, alkylamidine-based choline analogs have been developed as a potential new chemotherapy against *P. falciparum* or *P. vivax*.[5–7] Indeed, Vial and co-workers studied the parasite's phospholipidic metabolism and defined the phosphatidylcholine de novo biosynthesis as a novel antiplasmodial target.[8]

The bis-thiazolium salt **T3** is the first choline analog to undergo phase 3 clinical trials, validating the strategy of using biscationic antimalarial agents.[9–12] Amidines are strong bases (pK_a ~13–14) and exist under physiological conditions mainly as protonated species. Bis-alkylamidines are thus bioisosteres 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 antiplasmodial activity, and optimized the length of the alkyl linker to 12 methylenes.[6] **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**.[13] Nevertheless, **M64** revealed to be quite unstable in vivo.[14]

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)imino]-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-



T3
IC_{50} = 2.2 nM

M64
IC_{50} = 2 nM

Figure 1. Biscationic antiplasmodial compounds.

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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 antiplasmodial activity. Besides, we have developed another series of *C*-alkylamidines and reported previously that N-substitutions could improve their in vivo antimalarial potencies.[15] These results may be related to the resulting pK_a and/or lipophilicity. Thus, we have introduced aromatic substituents and investigated the influence of the lipophilicity and the pK_a 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-dodecanediamine in the presence of triethylamine. The triethylamine was added to neutralize the rest of hydrogen chloride that prevented generating the targeted reversed amidines **3a–p**. The different reversed alkyl-/benz-amidine molecules **3a–p** were purified as free amidines by washing with acetonitrile, 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 amidines were obtained starting, respectively, from commercially available propionitrile, *iso*-butyronitrile, cyclopropylcyanide or hydroxyacetonitrile **1a–d**. In the second series of reverse-benzamidines, the aromatic ring was introduced by starting from the appropriate benzonitriles.[16] 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-sensitive strain of *P. falciparum* (Nigerian strain).[17,18] Results are given in Table 1. Both the *N*-alkylamidines and the reverse-benzamidines possessed potent antimalarial activities, in the nanomolar range, except **3e**, **3g**, and **3h**. The *N*-alkylamidine series is quite homogeneous, with IC_{50} <20 nM, pK_a >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 hydrosolubility (**3d**). **M64** is still the most potent compound among the *N*-alkylamidines. Regarding the IC_{50} and the pK_a values, the reverse-benzamidines are a more heterogeneous series as compared to the *N*-alkylamidines. Indeed, the pK_a values ranged between 10 and 14 and the antiplasmodial activities of **3e**, **3g**, and **3h** were weak (IC_{50} >450 nM), **3i–k** and **3n** possessed significantly higher potencies (60 nM < IC_{50} < 20 nM), while **3f**, **3l**, **3m, 3o**, and **3p** were the most potent compounds (IC_{50} <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 antiplasmodial activity (**3e**: IC_{50} = 715 nM), we have no more used a furan ring. In contrast, the compound **3f** possessing a phenyl ring revealed a good potency (IC_{50} = 13.5 nM). We have then developed ten substituted reverse-benzamidines **3f–3p** 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 IC_{50} of *N*-alkylamidines nor reverse-benzamidines and their lipophilicity.

Table 1
Evaluation of reverse-benzamidine compounds as new antimalarials

Compounds	Calculated pK_a[a]	*P. falciparum* IC_{50}[b] (nM)	*P. vinckei* ED_{50}[c] (mg/kg)
M64[d]	12.65	2.2	3.1
3a	12.67	18	>10
3b	12.28	10.15	7
3c	12.67	16	5
3d	14.15	9.15	8.5
3e	11.53	715	ud
3f	12.08	13.5	>10
3g	10.96	455	ud
3h	10.36	520	ud
3i	12.67	26.5	>>20
3j	11.46	24.5	12
3k	10.65	55.5	12
3l	12.41	6.7	>5[e]
3m	11.81	6.6	5
3n	11.55	39	5
3o	13.53	14	2.2
3p	12.45	10	3.1

[a] pK_a were calculated using ACD/pK_a DB, version 6.0, Advanced Chemistry Development Inc.
[b] IC_{50} against the in vitro growth of *P. falciparum* are means of at least two independent experiments conducted in duplicate.
[c] Antimalarial activities (Efficient Dose 50, ED_{50}) were determined after ip administration of the compounds once daily for four consecutive days to infected mice (3 mice/dose).
[d] Compound **M64** was previously described.[13]
[e] Toxicity is observed at higher doses.

As opposed to Log *P* values, we have noticed the influence of the basicity of *N*-alkylamidines and reverse-benzamidines on their in vitro antiplasmodial activity. The Figure 3 illustrates that the observed IC_{50} against the human *P. falciparum* parasite were strongly related to the calculated pK_a values of the compounds. This link is likely more pronounced in the reverse-benzamidines series. Indeed, the reverse-benzamidines sharing electro-withdrawing groups like $-CF_3$ (**3g**) or $-NO_2$ (**3h**) possessed decreased basicity.

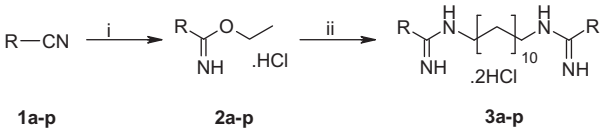

Figure 2. Synthesis of the *N*-alkylamidines and reverse-benzamidine compounds. Reagents and conditions: (i) gazeous HCl, EtOH, 20 h, 0 °C to rt; (ii) 1,12-dodecanediamine, EtOH, Et_3N, 24 h, rt. [a]Log *P* were calculated using ACD/Log *P* DB, Advanced Chemistry Development Inc. [b]**M64** compound was previously described.[6]

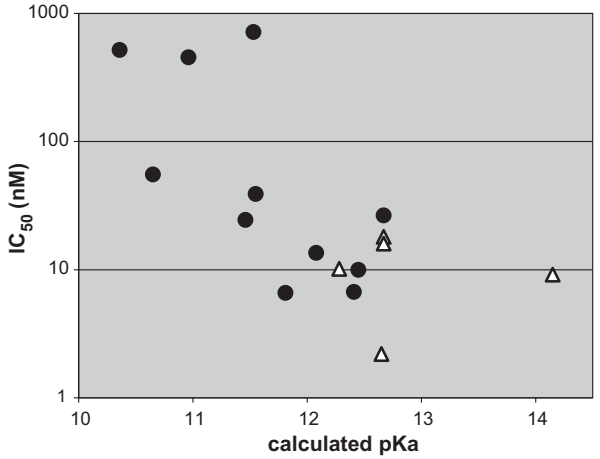

Figure 3. In vitro antimalarial activity (IC_{50}) of *N*-alkylamidine (△) and of reverse-benzamidine (●) as a function of basicity of the cationic heads (calculated pK_a). pK_a were calculated using ACD/pK_a DB, version 6.0, Advanced Chemistry Development Inc.

Their antiplasmodial potencies are thus dramatically decreased, when compared to **3f**. On the other hand, the derivatives with electro-releasing groups like $-NH_2$ (**3o**) or $-CH_3$ (**3p**) presented increased pK_a values and revealed good antiplasmodial potencies. Since their antimalarial activities are strongly linked to their basicity, the reverse-benzamidine compounds seem to act in a similar way as the alkylamidine choline analogs described by Calas et al.[6] Indeed, they observed the same correlation between the antiplasmodial activities and the pK_a values of the molecules, suggesting that the pK_a values of alkylamidines reflect their ability to form strong bonds with the target. This correlation indicates that the mechanism of action of reverse-benzamidine compounds would be rather linked to the capacity for these bis-cations to mimic the choline structure and to inhibit the phospholipidic metabolism, than associated to the ability of antiparasitic dibenzamidines to bind DNA as pentamidine or furamidine.[19,20]

The in vivo antimalarial activities of our compounds were investigated against the *Plasmodium vinckei petteri* strain (279BY) in female Swiss mice.[21] The in vitro antiplasmodial activity of **3e**, **3g**, and **3h** being too low, they were not evaluated in vivo. The mice were infected on day 0 and treated with compounds either intraperitonally (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*-alkylamidine 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 (ED_{50} 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 ED_{50} 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 prodrug strategies might be applied to the most potent compounds in this new reverse-benzamidine 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-benzamidine compounds exhibited potent antimalarial activities (ED_{50} 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 ^{13}C 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.bmcl.2010.07.124.

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16. For example, in an oven-dried three-neck flask, *p*-tolunitrile **1o** (2.0 g, 17.07 mmol) was solubilized in 30 ml of dry ethanol and the mixture was cooled to 0 °C in an ice-water bath. To saturate the reaction medium with hydrogen chloride, a gaseous hydrogen chloride was bubbled for 20 min and then, the flask was sealed. The mixture was stirred at room temperature for 20 h. After removal of the solvent under reduced pressure, the imidate **2o** was obtained as a white solid. Ethyl (*p*-tolu)imidate hydrochloride **2o**: ^1H (DMSO-d_6, 300 MHz) d: 1.55 (t, 3H, 7.0 Hz); 2.50 (s, 3H); 4.63 (quad, 2H, 7.0 Hz); 7.53 (d, 2H, 8.3 Hz); 8.16 (d, 2H, 8.3 Hz); 12.01 (large s, 2H). In an oven-dried flask, the imidate **2o** (crude, 15.02 mmol) and 1,12-dodecanediamine (1.2 g, 6.01 mmol) was suspended in dry ethanol (40 ml). Then, dry triethylamine (8.4 ml, 60.1 mmol) was added dropwise. The reaction mixture was stirred at room temperature and under nitrogen for 24 h. After removal of the solvent under reduced pressure, the resulting crude was solubilized in an aqueous solution of sodium hydroxide (1 M) to afford the free base. After stirring for 1 h, the generated precipitate was washed with acetonitrile, water, and diethyl ether. In the presence of hydrochloric acid, the amidine hydrochloride **3o** was formed, recovered after removal of the solvent under reduced pressure. Washing the crude with dry diethyl ether led to a white solid (2.76 g, 80%). 1,12-(*p*-tolu)amidinedodecane **3o**: ^1H (DMSO-d_6, 300 MHz) δ: 1.24 (m, 16H); 1.60 (m, 4H); 2.36 (s, 6H) ; 3.43 (m, 4H); 7.35 (d, 4H, 8.5 Hz); 7.74 (d, 4H, 8.5 Hz); 9.26 (large s, 2H); 9.54 (large s, 2H); 10.01 (large s, 2H). ^{13}C (DMSO-d_6, 75 MHz) δ: 21.0; 26.0; 27.3; 28.5; 28.8; 42.4; 125.7; 128.0; 129.1; 143.3; 162.0. FT-IR cm^{-1}: 727; 824; 1377; 1510; 1576; 1618; 1668; 2853; 2923; 3041. ES^+ SM: 435 [M+H$^+$]; 218 [(M+2H$^+$)/2]. HRMS calcd for $C_{28}H_{39}N_4O_4^+$ 435.3488; found 435.3485. Mp: 80–81 °C (Et_2O).
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