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Synthesis and antimalarial activity of new 1,12-bis-(*N*,*N*′-acetamidinyl)dodecane derivatives

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Abstract—Amidoxime and O-substituted derivatives of the bis-alkylamidine 1,12-bis(*N*,*N*′-acetamidinyl)dodecane were synthesized and evaluated as in vitro and in vivo antimalarial prodrugs. The bis-*O*-methylsulfonylamidoxime **8** and the bis-oxadiazolone **9** derivatives show relatively potent antimalarial activity after oral administration.

Malaria has been an ever-present threat to mankind. The past 10 years have been no exception, as this microbial threat has continued to challenge public health. Malaria is endemic in about 100 developing countries and 2.2 billion people are at risk of malaria.[1,2] The most recent estimates indicate that there are more than 500 million clinical cases of malaria annually on the planet, a number that nearly double previous estimates,[3] and the disease measured in disability adjusted life years (DALYs) is estimated at 45 million.[1,4] The lack of a vaccine and the absence of a widely accessible vector control strategy mean that drugs remain the key control measure for malaria. However, parasite resistance of *Plasmodium falciparum*, the most severe form of malaria, has emerged in all classes of antimalarial drugs except the artemisinins, and is responsible for this recent increase in malaria-related mortality.[5–7] Increased efforts in antimalarial drug discovery are urgently needed.[8,9]

We have developed a new strategy of antimalarial treatment targeting the phospholipidic metabolism of the parasite and have successively designed and evaluated three generations of compounds that mimic choline, the polar head of phosphatidylcholine, which accounts for more than 40% of the total lipid content of the parasite.[10–12] The first generation consists of bis-quaternary ammonium salts series.[13–15] The lead compound, 1,16-hexadecamethylenebis(*N*-methylpyrrolidinium)dibromide **G25**, efficiently inhibits pharmacoresistant malaria in vitro[16] and was able to cure rodent malaria[17] as well as *P. falciparum*- and *Plasmodium cynomolgi*-infected monkeys at very low dose after intramuscular administration.[18] However, these ammonium salts are poorly orally absorbed, due to their permanent positive load, preventing their use in the field to a large scale.

To remedy the low per os absorption of quaternary ammonium salts, we then focused on a second generation of compounds consisting of bioisosteric analogues (bis-alkylamidine) that exhibit excellent antimalarial activity.[19] These second-generation compounds were as efficient as the bis-quaternary ammonium salts against the in vitro growth of the human *P. falciparum* parasite (IC_{50} of = 0.3–2 nM). These amidines present an

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equilibrium between protonated and unprotonated forms, and thus they can diffuse more easily through the tissues due to the neutral form. Unfortunately, the in vivo antimalarial activity after oral administration was still low (Vial and Calas, data not shown) indicating that their positive charges are still a drawback for the oral absorption.

In the field of various pharmaceutical applications, amidine derivatives appear very potent compounds. Such therapeutic potential has required neutral surrogate elaboration to temporarily overcome the basic and ionic characters of these drugs. During the last decade, prodrug strategies have been reported for the aromatic amidine that greatly improve both bioavailability and thus, activity after oral administration.[20–22] Modifications of the benzamidine group such as benzamidoxime and derivatives or as carbamate make the prodrugs more lipophilic and less basic with decreased pK_a, overcoming the low oral bioavailability of benzamidines.[22–26] These modifications have little effect on the half-life elimination of these compounds[22] but improve up to 30-fold the bioavailability and up to 20-fold the oral activity.[22,26,27] No particular adverse effects have been observed at comparable benzamidine doses.[22,26] Benzamidoxime groups have been shown to be readily reduced to benzamidine, both in vitro and in vivo, by a microsomal reductase system present in all mammalian species.[28]

In this paper, we modify the amidine group of the lead compound 1,12-bis (*N*,*N*′-acetamidinyl)dodecane **M64** (Fig. 1). This lead compound possesses the linker on the functional nitrogen atom and had potent antimalarial activity both in vitro against the human *P. falciparum* malarial parasites and in vivo against rodent malaria after ip administration, but not after oral administration. We describe the synthesis of amidoxime and O-substituted derivatives of this bis-alkylamidine (Fig. 1). Biological properties in vitro against *P. falciparum* and in vivo against *Plasmodium vinckei* are reported and discussed.

The target compounds were synthesized as outlined in Scheme 1. Bis-alkylamidoxime **1** was prepared by reaction of 1,12-diaminododecane with ethyl *N*-hydroxyacetimidate hydrochloride. It is used as a key intermediate to obtain compounds **2**–**9**. Experimental reactions were optimised by varying solvent, base and temperature. The compounds were characterized by ^{1}H and ^{13}C NMR, MS (FAB or ESI), FTIR and the data were consistent with the structure. The bis-*O*-methylamidoxime derivative **2** was obtained from **1** with methyl iodide and NaOH 1 N in ethanol. The bis-*O*-acetylamidoxime **3** was prepared using an acetic anhydride excess as reagent and solvent of the reaction. The bis-*O*-benzoylamidoxime **4** was synthesized by reacting **1** with benzoyl chloride and triethylamine in chloroform. A similar reaction occurs for synthesis of compound **7** with diethylchlorophosphate in DMF. With methylsulfonyl chloride in chloroform, pyridine is used to obtain bis-*O*-methylsulfonylamidoxime **8**. Bis-*O*-carbamoyl derivatives **5** and **6** were synthesized with the corresponding isocyanate in heterogeneous medium chloroform–K_2CO_3. Under the same conditions, bis-alkylamidoxime **1** with methylchloroformate gives the bis-oxadiazolone **9**. At room temperature, the reaction is followed by TLC and heterocyclisation is completed by gentle heating at 25–50 °C.

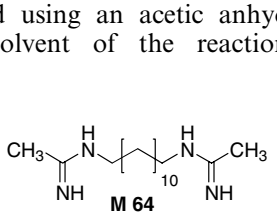
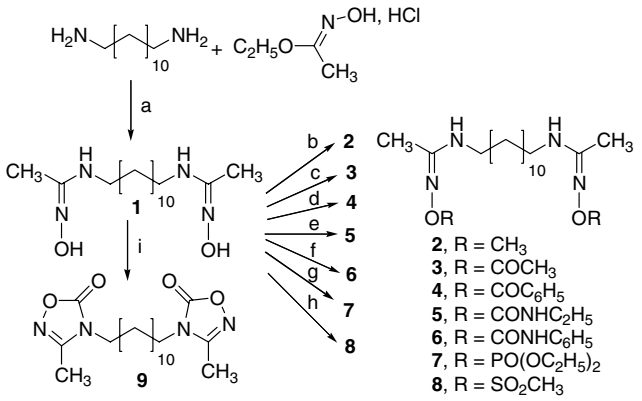


Figure 1. 1,12-bis(*N*,*N*′-acetamidinyl)dodecane **M64**.



Scheme 1. Synthesis of the bioprecursor compounds **1**–**9**. Reagents and conditions: (a) NaOH·EtOH, 80 °C (yield: 46%); (b) CH_3I, EtOH/NaOH 1 N, 5–25 °C (yield: 96%); (c) $(CH_3CO_2)_2O$ (yield: 96%); (d) $ClCOC_6H_5/CHCl_3$, TEA, 5–25 °C (yield: 76%); (e) $C_2H_5NCO/CHCl_3$, K_2CO_3, 25 °C (yield: 90%); (f) $C_6H_5NCO/CHCl_3$, K_2CO_3, 25 °C (yield: 90%); (g) $ClPO(OC_2H_5)_2$/DMF, TEA, 0–25 °C (yield: 60%); (h) $CH_3SO_2Cl/CHCl_3$, Pyridine, 0–15 °C (yield: 90%); (i) $ClCO_2CH_3/CHCl_3$, K_2CO_3, 25–50°C (yield: 75%).

In vitro antimalarial activities are evaluated against a chloroquine-sensitive strain of *P. falciparum* (Nigerian strain) and in vivo against the *P. vinckei petteri* strain (279BY) in female Swiss mice.[29,30] Results are given in Table 1.

N-hydroxylation of the bis-alkylamidine **M64** (IC_{50} = 2 nM)[19] into bis-alkylamidoxime **1** (IC_{50} = 310 nM) reduces in vitro antimalarial activity. The heterocyclisation of **1** in bis-oxadiazolone **9** (IC_{50} = 7100 nM) or the O-substitution of **1** by the methyl group (**2**: IC_{50} = 4350 nM) and the acetyl group (**3**: IC_{50} = 1800 nM) also strongly decreases in vitro antimalarial activity. Changing the O-substituted group on the compound **1** by the phenylcarbamoyl (**6**: IC_{50} = 135 nM), ethylcarbamoyl (**5**: IC_{50} = 93 nM) or benzoyl (**4**: IC_{50} = 68.7 nM) antimalarial activity was gradually improved by a factor of 2–5 compared to **1**. Diethylphosphate (**7**: IC_{50} = 9.6 nM) and methylsulfonyl (**8**: IC_{50} = 12 nM) substituents strongly increase antimalarial activity by a factor of 32 and 26 compared to **1**. These derivatives are less active than the original drug **M64**. The weaker activity is in agreement with the fact that the chemical modifications of the drug lead to less basic prodrugs and that activity (IC_{50}) of the series of bis-alkylamidine is pK_a

Table 1. In vitro and in vivo antimalarial activity of the compounds **1–9**

Compound	IC_{50}[a] (nM) *P. falciparum*			ED_{50}[b] (mg/kg) *P. vinckei*	
	Nigerian strain (CQ^S)	FCM29 strain (CQ^R)	W2 strain (CQ^R)	ip	po
Chloroquine (CQ)	20	158	143		
M64	2	4	8.1	3.1	>200
1	310			9.2	90
2	4350			>10	110
3	1800			7.1	>90[c]
4	68.7			>9	>90
5	93			>10[c]	>90
6	135			10	>90
7	9.6			>9	>90
8	12			4.7	42
9	7100			>9[c]	62

[a] IC_{50} are means of at least two independent experiments conducted in duplicate.
[b] Antimalarial activities (Efficient dose 50, ED_{50}) were determined after intraperitoneal (ip) or oral (po) administration of the compounds once daily for 4 days to infected mice. Range of compound concentrations used to generate data: ip doses 1–9 mg/kg; po doses 10–90 mg/kg.
[c] Moderate effect at this dose.

dependent.[19] However, the in vitro antiplasmodial activity of **4** (68.7 nM), **5** (93 nM), **6** (135 nM) and in particular **7** (9.6 nM) or **8** (12 nM) would be consecutive to their conversion into drugs either chemically or enzymatically, by systems that differ from P450 reductases.[31] If they do not undergo bioconversion, then they are probably bioisosteres of **M64**.

Compounds were tested for activity in mice infected with *P. vinckei*. The mice were treated with compounds intraperitoneally or orally once daily for four consecutive days (days 1–4 post-infection). The O-protected derivatives **2**, **4** and **7** do not induce any antimalarial effect after intraperitoneal administration of 9 mg/kg but compounds **5** and **9** induce a slight antimalarial effect for dosages higher or equal to 9 mg/kg (data not shown). A good antimalarial activity by ip is found for compounds **6**, **1**, **3** and **8**, in order of increasing effectiveness. The last derivative **8** (ED_{50} ip = 4.7 mg/kg) is as efficient as its drug **M64** (ED_{50} ip = 3.1 mg/kg).[19] Derivatives **2** and **4–7** are not very effective against *P. vinckei* with ED_{50} higher or equal to 10 mg/kg after intraperitoneal administration and higher or equal to 90 mg/kg after oral administration. This can probably be attributed to the low conversion of the drug into the prodrug. On the other hand, bis-alkylamidoxime **1** and its bis-O-acetylated derivative **3** are effective with ED_{50} po = 90 mg/kg. The bis-oxadiazolone **9** (ED_{50} po = 62 mg/kg) is even more potent by oral route with an activity at least 3 times more powerful than the corresponding drug **M64**. After intraperitoneal administration, the compound bis-*O*-methylsulfonyl **8** exerts antimalarial activity (ED_{50} ip = 4.7 mg/kg) as potent as **M64** (ED_{50} ip = 3.1 mg/kg). Remarkably, after oral administration, compound **8** showed the most potent antimalarial activity of all tested prodrugs on the parasitized mice, and appeared 5 times more effective than its drug **M64** (ED_{50} po = 42 mg/kg).

Drug M64 and all its bioprecursors are well tolerated by mice in the in vivo test conditions (intraperitoneal or oral administration, once daily for four consecutive days). No clinical toxic effect was detected during the tests independent of the used dose.

Based on strategies previously described, we have designed and evaluated, for antimalarial activity, new modified amidines such as bis-alkylamidoximes, O-substituted bis-alkylamidoximes and bis-oxadiazolones, able to temporarily reduce the basic character and to produce orally active bis-alkylamidine drugs. It is the first time that prodrug strategy is validated for bis-alkylamidines. Among our compounds, the derivative bis-*O*-methylsulfonylamidoxime **8** and the bis-oxadiazolone **9** constitute the best prodrug active on *Plasmodium* in vivo after oral administration. This strategy applied to bis-alkylamidines linked on the functional carbon atom is currently being studied.

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Supplementary data

^1H and ^{13}C NMR, MS (FAB or ESI), FTIR data of new compounds and biological protocol are given. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.11.013.

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