Bioorganic & Medicinal Chemistry Letters 19 (2009) 624–626

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of amidoxime derivatives for orally potent *C*-alkylamidine-based antimalarial agents

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ARTICLE INFO

Article history:
Received 5 December 2008
Accepted 14 December 2008
Available online 24 December 2008

Keywords:
C-Alkylamidine
Design of prodrug
Specific *O*-substitutions
Alkylamidoxime
Clearance of parasitemia
Oral antimalarial agent

ABSTRACT

Within the frame of the design of prodrug candidates to deliver a *C*-alkylamidine antimalarial agent, we showed that specific *O*-substitutions were needed on the alkylamidoxime structure. Among the newly synthesized molecules, bis-oxadiazolone and bis-*O*-methylsulfonylamidoxime derivatives induced a complete clearance of parasitemia in mice after oral administration.

Malaria is a problem of public health, with 300 to 500 million people worldwide affected by this potentially lethal disease. Moreover, *Plasmodium falciparum* parasites become resistant to most marketed antimalarials, except to artemisinin and its derivatives.[1] Cocktails of antimalarials are now recommended to prevent chemoresistances. Unfortunately, the number of drug classes is very restricted. This emphasizes that alternative drugs with a novel mechanism of action need to be urgently developed.

Vial and coll. first developed bis-quaternary ammonium salts as new antimalarial chemotherapeutic agents. These are bis-cationic choline analogues targeting the phospholipidic metabolism.[2,3] These agents were able to inhibit plasmodial phosphatidylcholine de novo biosynthesis in infected erythrocytes.[4–9] This strategy was then optimized with bis-thiazolium salts active against rodent and non-human primate malaria (**T3**, Fig. 1).[10,11] The test subjects displayed rapid and complete healing without recrudescence. However, these potent molecules contain permanent cationic charges, which may be the cause of their low oral absorption. Thus, bis-alkylamidine derivatives were designed as bioisosteric analogues, which do not possess any permanent cationic charges.[12] Indeed, amidines are strong bases (pK_a ~13–14) and exist under physiological conditions mainly as protonated species. Two chemical series of bis-cationic agents have been developed: *N*-alkylamidines, as **M64**, and *C*-alkylamidines, as **M34** (Fig. 1). These compounds also mimic choline and inhibit the *P. falciparum* parasite's multiplication in the low nanomolar range, similarly to **T3**.[12,13]

The antimalarial activity of these bis-alkylamidines relies on the cationic character of the hydrophilic head,[12] this property may also prevent traversal of the intestinal barrier. This limitation presents a compelling target for a prodrug strategy. The amidoxime prodrug principle was originally developed by Clement and coll. for pentamidine,[14,15] and later applied to other benzamidines such as

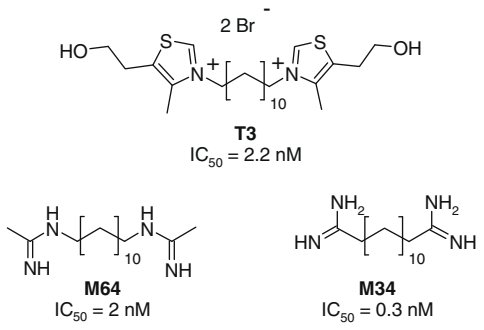

Figure 1. Bis-cationic antiplasmodial compounds.

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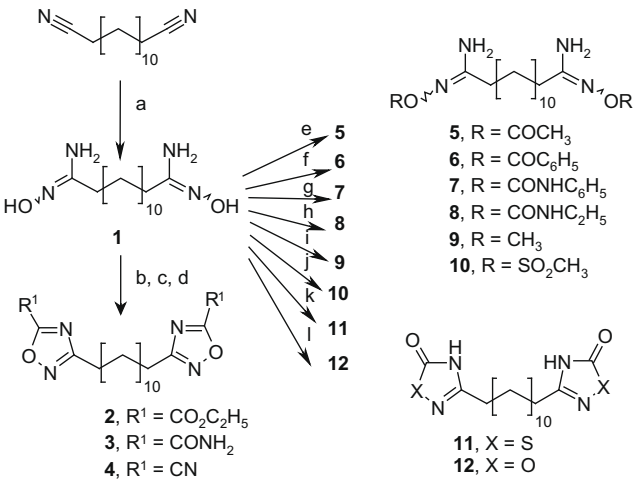
doi:10.1016/j.bmcl.2008.12.058

sibafiban[16] and melagatran[17] and to furamidine, an anti-protozoan agent.[18] In this approach, amidoxime functions mask the positive charge of amidines, which enables these compounds to be efficiently absorbed from the gastrointestinal tract while inhibiting their biological activity. This biological activity is recovered by the in vivo reduction of inactive prodrugs into active bis-cationic drugs.[19] The clinical application of this concept led to the oral use of the amidoxime ximelagatran.[20,21] Although the amidoxime prodrug strategy was widely studied for benzamidine drugs, little is known about its application to alkylamidine antimalarial drugs. We previously reported the successful use of amidoxime derivatives to temporarily reduce the basic character and to produce orally active bis-*N*-alkylamidine **M64.**[22]

The aim of this study was to extend these results to **M34**, the lead compound of bis-*C*-alkylamidines. We were interested in developing **M34** prodrugs because this drug revealed higher in vitro activity than **M64**. Moreover, the **M34** bis-*C*-alkylamidine structure allows a broader range of modulations, in particular it enables the synthesis of 1,2,4-oxadiazole derivatives,[23] which is not possible for **M64**.

The targeted compounds were obtained as outlined in Scheme 1. Bis-alkylamidoxime **1** was prepared by heating 1,12-dicyanododecane with an excess of hydroxylamine hydrochloride in an ethanolic solution of NaOH 1 M and was used as a key-intermediate for compounds **2**–**12**. The 1,2,4-oxadiazole **2** was generated by heating **1** with oxalylethyl chloride and pyridine in chloroform. In the presence of ammonia in ethanol, **2** was converted into the corresponding carbamoyl-oxadiazole **3**. The cyanooxadiazole **4** was prepared from **3** using trifluoroacetic anhydride and pyridine in dioxane. The bis-*O*-acetylamidoxime **5** was obtained from bis-alkylamidoxine **1** using an excess of acetic anhydride. The bis-*O*-benzoylamidoxime **6** was synthesized in the presence of benzoyl chloride and triethylamine in chloroform. Bis-*O*-carbamoyl derivatives **7** and **8** were prepared with the corresponding isocyanate in heterogeneous conditions using chloroform–K_2CO_3. The bis-*O*-methylamidoxime derivative **9** was obtained from **1** with dimethylsulfate and NaOH 1 M in ethanol. Bis-*O*-methylsulfonyl-amidoxime **10** was synthesized with methylsulfonyl chloride in chloroform in the presence of pyridine. The thiadiazolone **11** was obtained from **1** using thiocarbonyldiimidazole (TCDI) in tetrahydrofuran (THF), followed by diethylether trifluoroborate in THF. The bis-oxadiazolone **12** was generated in two steps: **1** reacted with ethylchloroformiate in the presence of triethylamine, and then, the intermediate compound was cyclized by heating in xylene. These reactions occurred chemoselectively at the OH group of amidoxime **1**.[24] Indeed, in amidoximes, the $-NH_2$ group is electron deficient as observed in amides.[25] All structures were fully characterized by ^1H and ^{13}C NMR, MS (FAB or ESI) and in particular by FTIR.[26] The two characteristic bands at 3490–3400 and 3374–3315 cm^{-1} were observed and corresponded to symmetrical and asymmetrical NH_2 stretching modes.[27]



Scheme 1. Synthesis of the bioprecursor compounds **1**–**12**. Reagents and conditions: (a) $NH_2OH.HCl$, EtOH/NaOH 1 M, 5–25 °C (96%); (b) $ClCOCO_2C_2H_5$/Pyridine, $CHCl_3$, 85 °C (62%); (c) EtOH/NH_3 10%, 25 °C (91%); (d) $(CF_3CO)_2O$/Pyridine, dioxane, 25 °C (61%); (e) $(CH_3CO)_2O$, NaOH 2 M (90%); (f) $ClCOC_6H_5/CHCl_3$, TEA, 5–25 °C (87%); (g) $C_6H_5NCO/CHCl_3$, K_2CO_3, 25 °C (81%); (h) $C_2H_5NCO/CHCl_3$, K_2CO_3, 25 °C (74%); (i) $(CH_3)_2SO_4$, EtOH/NaOH 1 M, 5–25 °C (48%); (j) CH_3SO_2Cl/Pyridine, $CHCl_3$, 0–15 °C (92%); (k) TCDI/THF, then $BF_3.OEt_2$/THF, 25 °C (63%); (l) 1—ClCOOEt, TEA, $CHCl_3$, 25 °C (75%); 2—xylene, reflux (95%).

The in vitro antimalarial activities (Table 1) were evaluated against a chloroquine-sensitive strain of *P. falciparum* (Nigerian strain).[28] Since the introduction of either an oxygen or a sulfur atom leads to the loss of the compounds' basicity, the compounds **1**–**12** should not be able to act as choline analogues. The neutral compounds **1**, **3**–**9**, and **12** exhibited very weak intrinsic antiplasmodial activity compared to **M34**. Contrary to the previous compounds, the methylsulfonate derivative **10** and, to a lesser extent, the thiadiazolone **11** were the only compounds to have in vitro antimalarial activities occurring in the same nanomolar range as the parent alkylamidine **M34**. Indeed, these molecules revealed IC_{50} lower than 20 nM (**10**: IC_{50} = 2.5 nM and **11**: IC_{50} = 17.5 nM). Since they do not possess any cationic character, their in vitro antimalarial activity raises the question if they are metabolized into active drugs or if they possess intrinsic antimalarial activity.

The in vivo antimalarial activities of our compounds were investigated against the *Plasmodium vinckei petteri* strain (279BY) in female Swiss mice.[29] The mice were treated with compounds either intraperitoneally (ip) or orally (po) once daily for four consecutive days (days 1–4 post-infection). After ip administration (20 mg/kg), no antimalarial effect could be detected with most of the *O*-protected derivatives **1**, **3**–**9**, and **12.** However, with the thiadiazolone **11**, parasitemia was reduced to 50% compared to control and a total clearance of parasitemia was observed with the methylsulfonate derivative **10** (ED_{50} ip = 7 mg/kg). Since the compounds **10** and **11** exert their antimalarial activity at the same nanomolar range (in vitro) or with the same ED_{50} ip (in vivo) as bis-cationic bis-alkylamidines, these prodrug candidates are likely efficiently converted into **M34** drug either by chemical way or eventually by enzymatic systems differing from cytochrome P450 reductases.

No antimalarial activities could be detected after either ip or oral administration of compounds **3**–**7** at the tested doses, which

Table 1
In vitro and in vivo antiplasmodial activities of compounds **1**–**12**

Compound	*Plasmodium falciparum* IC_{50} (nM)[a]	*Plasmodium vinckei* ED_{50} (mg/kg)[b]	
		ip	po
M34	0.3	>10	>100
1	3500	nd	120
3	7000	>20	nd
4	100,000	>20	nd
5	1400	>10	>90
6	1100	>10	>90
7	nd	>20	>180
8	180	>20	90
9	6800	>20	180
10	2.5	7	40
11	17.5	20	>120
12	9200	>20	100

[a] IC_{50} values are means of at least two independent experiments, each of them were conducted in duplicate. (nd, not determined.)

[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.

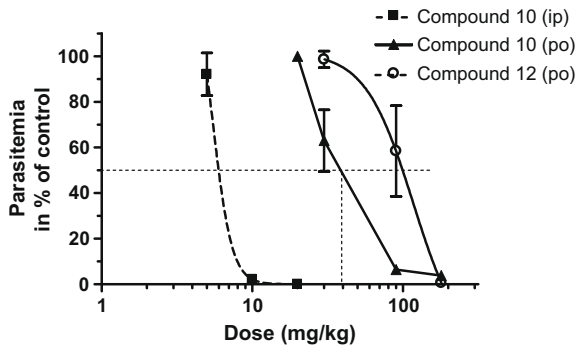


Figure 2. In vivo antimalarial activity of compounds **10** (■, ▲) and **12** (○) against *P. vinckei*-infected mice after ip (dashed line) or oral (straight line) treatment. Mice were iv-infected with 10^7 parasites, leading to parasitemia of 0.5% on day 1. Treatment consisted in one daily administration for four consecutives days (days 1–4 post-infection). Parasitemia were checked on day 5 and expressed in % of control. Results are the mean of three mice per dosage ± SEM. After ip administration, compound **12** do not significantly affect parasitemia until 20 mg/kg.

were limited by the weak solubility of the compounds. The application of the validated prodrug strategies including acetylated amidoxime[30] and oxadiazole,[23] did not lead to any in vivo antimalarial activity. On the other hand, molecules **1** and **8**–**12** revealed significant activities against *P. vinckei* after oral administration. Given orally, 120 mg of the bis-alkylamidoxime **1**, and 180 mg of the bis-*O*-methylamidoxime **9** reduced parasitemia to 50% compared to control. These compounds appeared more efficient by oral administration at 90 mg/kg than the parent drug **M34,** which possessed no significant effects at that concentration. According to these results, the prodrug strategies using the simple amidoxime (**1**) or the *O*-methylamidoxime (**9**), that were validated for benzamidine drugs (melagatran[17] and furamidine[18]), appear less effective at improving the oral bioavailability of *C*-alkylamidine **M34**. The specific enzymes may not recognize alkylamidoxime structures whereas benzamidoxime prodrugs of melagatran and of furamidine are recognized. No data are yet available on biotransformations of alkylamidoximes. Oral administration of 120 mg/kg of the thiadiazolone derivative **11** and of bis-*O*-ethyl-carbamoylamidoxime **8** led to, respectively, 25% and 80% decreases of parasitemia as compared to control. Contrary to the previous compounds, the two derivatives, methylsulfonate **10** and oxadiazolone **12,** were successful, since a total clearance of parasitemia was obtained with 120 mg/kg of both compounds (Fig. 2). Since the oxadiazolone **12** possesses weak in vitro activity, it is likely that the significant oral antimalarial activity of oxadiazolone **12** is linked to its in vivo conversion into **M34**. The results obtained with antithrombotic agents and **M64** confirm this hypothesis.[22,23] Remarkably, the methylsulfonyle substituent greatly increased in vivo antimalarial activity. The bis-*O*-methylsulfonyle compound **10** displays the most potent oral antiplasmodial activity (ED_{50} po = 40 mg/kg) of all tested compounds, totally inhibiting *P. vinckei* growth in infected mice.

In conclusion, we were challenged to develop prodrugs of **M34** that displayed oral antimalarial activity, since this drug exhibited no in vivo antimalarial activity. Strategies developed for benzamidines do not apply to alkylamidine drugs and specific *O*-substituents are necessary to obtain molecules with relevant oral antimalarial activity. In *C*-alkylamidine series, the oxadiazolone **12** and the *O*-methylsulfonate **10** exhibited potent antimalarial activities when given orally. These prodrug candidates may be efficiently converted into active drug. They induce the fall of parasitemia within two log of concentration and the complete clearance of the blood parasite after one daily dose for 4 days.

Acknowledgments

These studies were supported by the European Commission (Antimal integrated project LSHP-CT-2005-018834). We are grateful to Marjorie Maynadier and Frederic Boudou for their assistance in testing the compounds. We thank Aaron Gabriel (Harvard Medical School, Cambridge, USA) for a critical reading of the manuscript.

Supplementary data

^{1}H 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.2008.12.058.

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