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N-substituted bis-*C*-alkyloxadiazolones as dual effectors: Efficient intermediates to amidoximes or amidines and prodrug candidates of potent antimalarials

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ABSTRACT

A convenient route to N-substituted bis-*C*-alkylamidines possessing antiplasmodial activity and their oxadiazolone and amidoxime prodrug candidates, is described. These three families of compounds were available after a key N-alkylation step of the parent oxadiazolone **1a**. Testing of the three compound classes in vitro and in vivo is also presented.

Widespread strains of *Plasmodium falciparum* are becoming resistant to most available antimalarial drugs.[1] Faced with this problem, bis-alkylamidines have been developed as a potential new chemotherapy (Fig. 1).[2,3] Bis-alkylamidines share the same novel mechanism of action as bis-thiazolium salts (**T3**).[4–6] By mimicking choline structure, these compounds target the parasitic de novo phosphatidylcholine biosynthesis.[6–8] Thus, the mechanism of action of bis-alkylamidine drugs differs from benzamidines which also possess anti parasitic activities including antimalarial activities.[9–11] In contrast, benzamidines are supposed to exert their activity as DNA minor groove binder, by means of their planar and rigid structure.[12] Bis-alkylamidines, where **M34** is the lead compound, possess potent in vitro and in vivo antimalarial activities. However, they are not active after oral administration due to their cationic charges and/or their very polar heads.

Strategies to improve oral bioavailability of amidines have focused on the design of prodrugs, attempting to temporarily mask the positive charges. Clement's group originally applied the prodrug principle on pentamidine, an anti-parasitic drug with a benzamidine moiety.[13] This group introduced an oxygen atom to mask the cationic charge of benzamidines.[14] The resulting amidoximes were therefore less basic and not protonated under physiological conditions. These amidoxime neutral prodrugs possess improved bioavailability. Clement's group showed that these benzamidoximes are readily reduced by cytochrome P450 enzymatic system to the active aromatic amidines.[15]

We previously described the bis-*C*-alkyloxadiazolone **1a** and the bis-*C*-alkylamidoxime **2a**.[16] (Fig. 2) They were able to tempo-

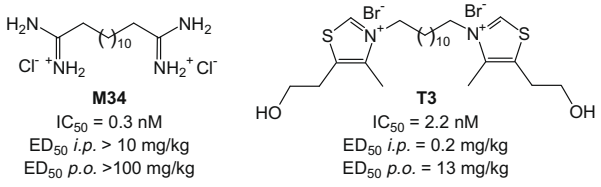

Figure 1. Bis-cationic antimalarial drugs.

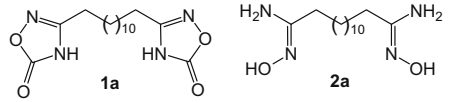

Figure 2. Bis-*C*-alkyloxadiazolone and bis-*C*-alkylamidoxime antimalarial prodrugs.

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rarily mask the basic character allowing oral delivery of the bis-*C*-alkylamidine drug **M34**.

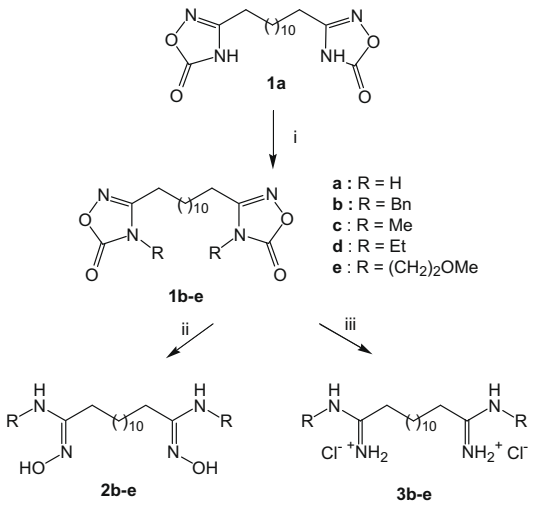
It is noteworthy that the **M34** alkylamidine drug presents excellent growth inhibition of the virulent *P. falciparum* parasite with an IC_{50} in the sub-nanomolar range while having no in vivo activity against *Plasmodium vinckei* after intraperitoneal (*ip*) administration. The introduction of N-substituents on the amidine function might improve in vivo antimalarial activity. Indeed, these modulations influence both the pK_a[17] and, above all, the lipophilicity of amidines drugs. Thus, the application of the amidoxime-based prodrug strategy may improve the oral activity of the resulting drugs. The oxadiazolone derivatization was one of the O-substituents needed to obtain molecules with a relevant oral antimalarial activity in alkylamidine series.[16,18] In the same way, Kitamura et al. described oxadiazolone derivatives of platelet aggregation inhibitors.[19] The derivatizations of the benzamidino group resulted in prodrugs with improved oral bioavailability.

The aim of this study was to develop a convenient route to N-substituted bis-*C*-alkylamidine drugs, the corresponding *C*-alkylamidoximes and *C*-alkyloxadiazolones. The antimalarial activity of these compounds was evaluated to see the ability of the N-substituents to improve the potency of *C*-alkylamidine drugs and the oral activity of the amidoxime and oxadiazolone prodrug candidates.

The target compounds were synthesized as outlined in Scheme 1. The bis-alkyloxadiazolone **1a** was prepared in three steps.[16] **1a** was used as a key intermediate to obtain the N-substituted bis-alkyloxadiazolones **1b–e**. The main assays to optimise the conditions of N-alkylation upon NMR conversion rate are reported in Table 1.

The mild conditions used for oxadiazolone alkylation[20–25] did not succeed due to the insolubility of the starting oxadiazolone **1a** in solvents as acetone or methanol.[26] Thus, dimethylformamide (DMF) was preferred, leading to the N-alkylated bis-oxadiazolones. The best results were obtained with the use of sodium methoxide as a base. N-substitutions were performed using activated halides such as benzyl bromide or non hindered halides like methyl, ethyl and methylethyl ether halide with satisfying yields (61–70% for **1b–e**, Scheme 1).[27]

Our first attempts to reach the corresponding N-substituted bis-alkylamidoximes **2b–e** using classical basic conditions (NaOH 5%) failed.[20,24] However, the N-substituted bis-alkylamidoxime derivatives **2b–e** were obtained from **1b–e** using sodium methoxide in anhydrous methanol with acceptable yields (58–83%). To prepare



Scheme 1. Synthesis of the drugs and prodrug candidates. Reagents and conditions: (i) RX/DMF, MeONa, 25 °C, overnight (yields **1b–e**: 61–70%; (ii) MeONa/MeOH, reflux, 40 h (yields **2b–e**: 58–83%); (iii) H_2/Pd/C 10%, MeOH, HCl 3 N, rt, 4 h (yields **3c–e**: 70–82%) or Zn, MeOH/AcOH, 60 °C, 3 h (yield **3b**: 85%).

the N-substituted bis-alkylamidine drugs **3b–e**, two reduction conditions were developed. The compounds **3c–e** were synthesized by hydrogenating **1c–e** in the presence of catalytic 10% Pd/C, in a methanolic solution of 3 N hydrochloric acid. To prevent debenzylation, the derivative **3b** was generated as an hydrochlorate salt using a Zinc powder suspension in a methanolic solution of acetic acid,[20] followed by treatment with 37% aqueous hydrochloric acid solution in methanol. It is noteworthy that methylated **3c** and ethylated **3d** drugs could be obtained by this method, where we did not succeed using Pinner's conditions.[28] Indeed, because of their basic nature and their facile hydrolysis to the corresponding amides, amidines often necessitate suitable protections to facilitate their synthesis and their purification.[20,21] The reduction of oxygenated derivatives was used several times to generate amidines,[29] and oxadiazolones have been reported as suitable amidines protections.[21,22,30,31] Thus, Moormann et al. described a versatile oxadiazolone synthon for the synthesis of acetamidine derivatives.[20] The compounds were characterized by ^1H and ^{13}C NMR, MS (ESI), FTIR and the data were consistent with the structure.

The in vitro antimalarial activities were evaluated against a chloroquine-sensitive strain of *P. falciparum* (Nigerian strain).[32,33] Results are given in Table 2. All *C*-alkylamidine drugs, including **M34** and N-substituted compounds **3b–e**, presented potent in vitro antimalarial activities, with IC_{50} in the very low nanomolar range. The introduction of N-substituents did not alter the antimalarial potency of alkylamidine drugs. On the other hand, their amidoxime **3b–e** or oxadiazolone **1b–e** prodrug derivatives revealed very weak antimalarial activities. Since these molecules are not protonated in physiological conditions, they were not able to act as choline analogs.

The in vivo antimalarial activities of the compounds were investigated against the *Plasmodium vinckei petteri* strain (279BY) in female Swiss mice.[34] The mice were infected on day 0 and were treated by *ip* or oral (*po*) administration of compounds once daily for four consecutive days (days 1–4 post infection). The parasitemia levels were monitored in mice after *ip* or *po* administration of 3 appropriate doses ($n = 3$ per dose). No antimalarial effect was observed after *ip* administration of 10 mg/kg of **M34**, **3b** and **3c**, even though these compounds possess potent in vitro antimalarial activities. On the other hand, the N-substituted *C*-alkylamidine drugs **3d** and **3e** revealed potent activity after *ip* administration with complete clearance of parasitemia (without recrudescence in the following 28 days) and ED_{50} of 6.3 and 8 mg/kg. Only ethyl and methoxyethyl N-monosubstitutions of alkylamidine drugs lead to improved antimalarial in vivo activity.

The amidoxime derivative of compound **2b** possessed no significant in vivo antimalarial activity. On the other hand, the N-substituted *C*-alkylamidoximes **2c**, **2d** and **2e** revealed significant *ip* antimalarial activity with 5 mg/kg of **2c**, **2d** and **2e** reducing parasitemia by 44%, 76% and 33% respectively, compared to control. When amidoximes were administered orally, no antimalarial activity could be detected for **2c**. The only oral effect that could be observed was a 20% and 16% reduction of parasitemia with 180 mg of compounds **2d** and **2e** respectively. Thus, N-substituted amidoxime derivatives could not be considered as efficient prodrugs.

Except for one compound, the N-substituted oxadiazolone derivatives were not active against the malaria parasite after *ip* or oral administration to mice at the dose indicated in Table 2. Problems of very poor water-solubility were encountered with these very lipophilic derivatives. Indeed, **2b**, **2c** and **2d** could not be solubilised for testing at higher doses than 90, 45 and 20 mg/kg respectively. Among the O-modulations needed to improve the oral antimalarial activity of amidoximes, the oxadiazolone derivatization could not be applied to N-substituted *C*-alkylamidoximes, due to the formation of insoluble compounds. The only soluble N-substituted *C*-alkyloxadiazolone was **1e** and oral admin-

Table 1
Optimisation of oxadiazolone **1a** N-alkylation[a]

Entry	Base	Solvent	Equivalents of EtBr	Conversion rate (%)
1	2.4 K_2CO_3	Acetone/MeOH 4/1	2.4	0
2	2.4 K_2CO_3	DMF	2.4	51
3	2 *t*-BuOK	DMF	2.4	78
4	3 *t*-BuOK	DMF	5	81
5	3 Cs_2CO_3	DMF	5	91
6	3 MeONa	DMF	5	99

[a] Reaction conditions: All assays were performed stirring overnight at room temperature (optimised conditions). Modifying the other parameters did not influence the reaction.

Table 2
In vitro and in vivo antimalarial activities of the compounds

	Compounds	*R*	*P. falciparum* (IC_{50}, nM[a])	*P. vinckei* ED_{50}, mg/kg[b]	
				ip	*po*
Amidine	**M34**[c]	H	0.3	>10	>100
	3b	Bn	4.6	>20	>90
	3c	Me	14.4	>10[d]	>180
	3d	Et	9.4	6.3	>180
	3e	$(CH_2)_2OMe$	9.2	8	>90
Amidoxime	**2a**[c]	H	3500	nd	120
	2b	Bn	1045	>20	>90
	2c	Me	10,100	5[d]	>180
	2d	Et	6200	3.1	>180
	2e	$(CH_2)_2OMe$	2950	>5[d]	>180
Oxadiazolone	**1a**[c]	H	9200	>20	100
	1b	Bn	4950	>20	>90
	1c	Me	nd	>20	>45
	1d	Et	10,000	>10	>20
	1e	$(CH_2)_2OMe$	6450	>20	90

[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 *per os* (*po*) administration of the compounds once daily for four consecutive days to infected mice (3 mice/dose).
[c] Compounds **M34**, **2a** and **1a** were previously described.[17]
[d] Toxicity is observed at higher doses.

istration of 90 mg/kg of **1e** reduced mice parasitemia to 50% compared to control. This oxadiazolone prodrug candidate **1e** appeared more efficient by oral administration at 90 mg/kg than the parent drug **3e**, which did not reveal any effects at that concentration. Since the oxadiazolone **1e** possesses very weak in vitro activity, it is likely that the significant *po* antimalarial activity is linked to the conversion of **1e** into the active drug **3e** (IC_{50} = 9.2 nM and ED_{50} *ip* = 8 mg/kg).

In conclusion, we obtained two N-substituted *C*-alkylamidines drugs **3d** and **3e** with potent in vivo antimalarial activities. The N-substituted *C*-alkyloxadiazolone **1e** revealed significant in vivo antimalarial activity after oral administration. This is the first report describing the synthesis of N-monosubstituted bis-*C*-alkylamidoximes as potent antimalarial agents. This strategy is competitive with classical methodologies,[35] yet it is more convenient since it gives access in a few steps to the three targeted compounds that are N-substituted alkylamidine drugs and the corresponding amidoxime and oxadiazolone prodrug candidates. The key-step consists of a N-alkylation of oxadiazolone **1a** to provide the N-substituted alkyloxadiazolones **1b–e**, which constitute efficient intermediates to generate the corresponding *C*-alkylamidines **3b–e** and *C*-alkylamidoximes **2b–e** that were obtained in only one step from **1b–e**.[20,24]

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Supplementary data

Supplementary data (^1H and ^{13}C NMR, MS (ESI), FTIR and melting point data of new compounds and biological protocol) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.001.

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26. The compounds possessing a C-12 alkyl chain have often revealed to react in harsher conditions (longer time, higher temperature, stronger bases...) than those described for other substrates (unpublished results).
27. For example, 1,12-*bis*-[(4-methoxyethyl-1,2,4-oxadiazol-5-(4*H*)-one)-3-yl]-dodecane **2e**: 5 g (14.53 mmol.) of oxadiazolone 1a and 2.39 g (44.19 mmol) of sodium methoxide are suspended in 75 mL of dimethylformamide. 14 mL (73.84 mmol) of 1-bromo-2-mehoxyethane are added. The reaction is stirred overnight at room temperature. The mixture is poured into water and extracted with ethyl acetate. The yellow solid obtained is purified by recrystallization from ethyl acetate. 4.7 g (70%) of white crystals are obtained. ^{1}H ($CDCl_3$, 300 MHz) δ: 1.28–1.42 (m, 16H); 1.71 (m, 4H); 2.59 (t, 4H, 7.6 Hz); 3.32 (s, 6H); 3.57 (t, 4H, 4.8 Hz); 3.7 (t, 4H, 4.8 Hz). ^{13}C ($CDCl_3$, 75 MHz) δ: 24.6; 24.7; 29.1; 29.2; 29.4; 29.5; 42.6; 59.1; 69.4; 159.5; 160.1. FTIR cm^{-1}: 1593; 1767; 2850; 2921. ES$^+$ SM: 455.2 ([M+H]$^+$, 100%); 909.5 ([2 M+H]$^+$, 58%). HRMS calcd for $C_{22}H_{39}N_4O_6^+$ 455.2870; found 455.2889.
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