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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.

Keywords:
Bis-C-alkylamidine
Total clearance of parasitemia
Design of prodrug candidates
Convenient route
N-substituted alkylloxadiazolone
Alkylamidoxime
Oral antimalarial agent

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 physio-

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IC50 = 0.3 nM
ED50 i.p. > 10 mg/kg
ED50 p.o. > 100 mg/kg

IC50 = 2.2 nM
ED50 i.p. = 0.2 mg/kg
ED50 p.o. = 13 mg/kg

Figure 1. Bis-cationic antimalarial drugs.

Figure 2. Bis-C-alkylloxadiazolone and bis-C-alkylamidoxime antimalarial prodrugs.
rarely mask the basic character allowing oral delivery of the bis-C-
alkylamide drug M34.

It is noteworthy that the M34 alkylamide 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 vinckeii 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}, and, above all, the lipophilicity of amides
pharmacokinetics. Thus, the application of the amidoxime-based prodrug strategy
may improve the oral activity of the resulting drugs. The oxadiazole derivatization was one of the O-substituents needed to
obtain molecules with a relevant oral antimalarial activity in alkyl-
amidine series. In the same way, Kitamura et al. described oxadiazole derivatives of platelet aggregator inhibitors.

The derivatization of the benzamido group resulted in prodrugs with
improved oral bioavailability.

The aim of this study was to develop a convenient route to N-
substituted bis-C-alkylamide drugs, the corresponding C-alkylami-
doximes 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-alkylamide drugs and the oral activity
of the amidoxime and oxadiazole prodrug candidates.

The target compounds were synthesized as outlined in Scheme 1.

The bis-alkyloxadiazole 1a was prepared in three steps. 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 oxadiazole alkylation did not succeed due to the insolubility of the starting oxadiazolone
in solvents such as acetone or methanol. 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 satisfactory yields (61-70% for
1b-e, Scheme 1). 27

Our first attempts to reach the corresponding N-substituted bis-
alkylaminodoximes 2b-e using classical basic conditions (NaOH 5%)
failed. However, the N-substituted bis-alkylaminodoxime derivatives
2b-e were obtained from 1b-e using sodium methoxide in
anhydrous methanol with acceptable yields (58-83%). To prepare
the N-substituted bis-alkylamine 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 debenzy-
lation, the derivative 3b was generated as an hydrochlorate salt
using a Zn powder suspension in a methanolic solution of acetic
acid, 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 with Pinner's conditions. 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. The reduction of oxygenated
derivatives was used several times to generate amidines, and
oxadiazolones have been reported as suitable amidines protections.
Thus, Moormann et al. described a versatile oxadiazole synthon for the synthesis of acetamidine derivatives.
The compounds were characterized by 1H and 13C NMR, MS (ESI),
FTIR and the data were consistent with the structure.

The in vitro antimalarial activities were evaluated against a
dilute chloroquine-sensitive strain of P. falciparum (Nigerian strain).
Results are given in Table 2. All C-alkylamine 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 antimal-
arial potency of alkylamide drugs. On the other hand, their amidino-
doxime 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 investi-
gated against the Plasmodium vinckeii petteri strain (279BV) in
female Swiss mice. 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 parasit-
eemia 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 antimal-
arial activities. On the other hand, the N-substituted C-alkylami-
dine 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 methoxymethyl N-monosubstitutions of
alkylamide drugs lead to improved antimalarial in vivo activity.

The amidoxime derivative of compound 2b possessed no signif-
ificant in vivo antimalarial activity. On the other hand, the N-substi-
tuted C-alkylaminodoximes 2c, 2d and 2e revealed significant ip
antimalarial activity with 5 mg/kg of 2c, 2d and 2e reducing para-
sitemia 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 ob-
served was a 20% and 16% reduction of parasitemia with 180 mg of
compounds 2d and 2e respectively. Thus, N-substituted amidoxi-
ne 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-alkylami-
doximes, due to the formation of insoluble compounds. The only
soluble N-substituted C-alkyloxadiazolone was 1e and oral admin-

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/acetone, 60°C, 3 h (yield 3b: 83%).
Table 1
Optimisation of oxadiazolone 1a N-alkylation

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Solvent</th>
<th>Equivalents of EtBr</th>
<th>Conversion rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.4 K₂CO₃</td>
<td>Acetone/MeOH 4/1</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2.4 K₂CO₃</td>
<td>DMF</td>
<td>2.4</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td>2 BuOK</td>
<td>DMF</td>
<td>2.4</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>2 BuOK</td>
<td>DMF</td>
<td>5</td>
<td>81</td>
</tr>
<tr>
<td>5</td>
<td>3 Cl₂CO₂</td>
<td>DMF</td>
<td>5</td>
<td>91</td>
</tr>
<tr>
<td>6</td>
<td>3 MeOH</td>
<td>DMF</td>
<td>5</td>
<td>99</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R</th>
<th>P. falciparum (IC₅₀, nM)*</th>
<th>P. vinckei ED₅₀, mg/kga</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ip</td>
<td>po</td>
</tr>
<tr>
<td>Amidine</td>
<td>M34b</td>
<td>0.3</td>
<td>&gt;10</td>
</tr>
<tr>
<td>3b</td>
<td>Bn</td>
<td>4.6</td>
<td>&gt;20</td>
</tr>
<tr>
<td>3c</td>
<td>Me</td>
<td>14.4</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3d</td>
<td>Et</td>
<td>9.4</td>
<td>&gt;180</td>
</tr>
<tr>
<td>3e</td>
<td>(CH₂)₂OMe</td>
<td>9.2</td>
<td>6.3</td>
</tr>
<tr>
<td>2a</td>
<td>H</td>
<td>3500</td>
<td>nd</td>
</tr>
<tr>
<td>2b</td>
<td>Bn</td>
<td>1045</td>
<td>&gt;20</td>
</tr>
<tr>
<td>2c</td>
<td>Me</td>
<td>10,100</td>
<td>&gt;90</td>
</tr>
<tr>
<td>2d</td>
<td>Et</td>
<td>6200</td>
<td>&gt;100</td>
</tr>
<tr>
<td>2e</td>
<td>(CH₂)₂OMe</td>
<td>2900</td>
<td>3.1</td>
</tr>
<tr>
<td>Oxadiazolone</td>
<td>1a</td>
<td>9200</td>
<td>&gt;20</td>
</tr>
<tr>
<td>1b</td>
<td>Bn</td>
<td>4890</td>
<td>&gt;100</td>
</tr>
<tr>
<td>1c</td>
<td>Me</td>
<td>nd</td>
<td>&gt;45</td>
</tr>
<tr>
<td>1d</td>
<td>Et</td>
<td>10,000</td>
<td>&gt;20</td>
</tr>
<tr>
<td>1e</td>
<td>(CH₂)₂OMe</td>
<td>6450</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

* IC₅₀ are means of at least two independent experiments conducted in duplicate.

Antimalarial activities (efficacy dose 50, ED₅₀) 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).

Compounds M34, 2a and 1a were previously described.⁵⁷

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 (IC₅₀ = 9.2 nM and ED₅₀ 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,⁵⁵ yet it is more convenient since it gives access in a few steps to the three targeted compounds that are N-substituted alkylamidines drugs and the corresponding amidoxime and oxadiazolone prodrug candidates. The key-step consists of an N-alkylation of oxadiazolone 1a to provide the N-substituted alkylloxadiazolones 1b–e, which constitute efficient intermediates to generate the corresponding C-alkylamines 3b–e and C-alkylamidoximes 2b–e that were obtained in only one step from 1b–e.⁵⁰,⁶⁴

Acknowledgements

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Supplementary data

Supplementary data (¹H and ¹³C 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.

References and notes

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-(4H)-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-methoxyethane 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. 1H (CDCl3, 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). 13C (CDCl3, 75 MHz) δ: 24.6; 24.7; 29.1; 29.2; 29.5; 42.6; 59.1; 69.4; 159.5; 160.1. FTIR cm⁻¹: 1593; 2850; 2921. ES⁺ SM: 455.2 ([M+H]+, 100%); 909.5 ([2 M+H]+, 58%). HRMS calcd for C22H39N4O6+ 455.2870; found 455.2889.