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Exploration of potential prodrug approach of the bis-thiazolium salts T3 and T4 for orally delivered antimalarials

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ABSTRACT

We report here the synthesis and biological evaluation of a series of 37 compounds as precursors of potent antimalarial bis-thiazolium salts (**T3** and **T4**). These prodrugs were either thioester, thiocarbonate or thiocarbamate type and were synthesized in one step by reaction of an alkaline solution of the parent drug with the appropriate activated acyl group. Structural variations affecting physicochemical properties were made in order to improve oral activity. Twenty-five of them exhibited potent antimalarial activity with IC_{50} lower than 7 nM against *Plasmodium falciparum* in vitro. Notably, **3** and **22** showed IC_{50} = 2.2 and 1.8 nM, respectively. After oral administration **22** was the most potent compound clearing the parasitemia in *Plasmodium vinckei* infected mice with a dose of 1.3 mg/kg.

Today, approximately 40% of the world's population is at risk of malaria and 109 countries are endemic for malaria. Every year, 300 million malaria cases cause at least a million deaths, mostly of children under the age of 5.[1] Malaria is caused by protozoan parasites of the genus *Plasmodium*. It is nowadays well described that parasite resistance of *Plasmodium falciparum*, the most severe form of malaria, is responsible for malaria-related mortality and morbidity,[2–4] and constitute a major obstacle for an eradication of malaria.[5] Because of the lack of a vaccine, it is thus clear that the discovery of new treatments based on innovative mechanisms of action is really needed.[6,7]

In the past decade, our group has developed a new approach that targets the lipid peculiarities of *P. falciparum*. During its intra-erythrocytic development, the parasite synthesizes considerable amounts of membranes, from phospholipid precursors scavenged from the human host. Among those, phosphatidylcholine (PC) is the most abundant and its content increases sixfold after infection.[8–10] Consequently, the phospholipid biosynthesis has been viewed as an ideal target for the development of new antimalarial drugs.[11] The essential role of PC for the parasite survival has been demonstrated by using choline analogues, which had been rationally designed and optimised for their ability to inhibit PC biosynthesis in *Plasmodium* and to block parasite proliferation in the low nanomolar range. In this context, compounds containing a duplication of the cationic group (i.e., bisquaternary ammonium salts) separated by a long lipophilic alkyl chain had been designed and evaluated.[12–16] We reported the synthesis and antimalarial activities of a series of bis-thiazolium salts (Fig. 1).[17,18] **T3** and **T4**, the lead compounds in this series, showed powerful in vitro and in vivo biological activities (IC_{50} = 2.2 and 0.65 nM on *P. falciparum*, and ED_{50} = 0.2 and 0.14 mg/kg in mice infected by *Plasmodium vinckei* by intraperitoneal mode). Compound **T3** (SAR97276; 1,12-bis[5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-3-ium]dodecane dibromide) is a new antimalarial drug, which is currently being evaluated in clinical trials (phase II) for severe malaria.

However, the presence of permanently charged cationic groups, a crucial requirement for antimalarial activity of choline analogues,

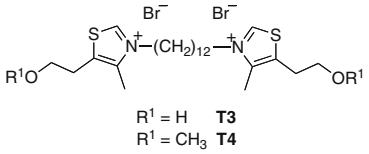

Figure 1. **T3** and **T4**, lead choline analogues.

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is detrimental to oral absorption. As oral administration is essential for treatment in dispensaries in endemic countries and for prophylactic or curative treatment for travellers, this led us to develop neutral precursor of bis-thiazolium salts.[19] These prodrugs incorporate thioester, thiocarbonate or thiocarbamate pro-moieties (**TE** series), which in vivo are expected to revert back to thiazolium salts after enzymatic transformation by plasmatic esterases (Fig. 2a). A similar prodrug approach has been applied to improve oral delivery of Thiamin (vitamin B1), a thiazolium salt which is also poorly absorbed.[20–23]

Preliminary studies on **TE** prodrugs **1**–**3** (Fig. 2b) demonstrated that such molecules lead to a complete protection in murine models using short-course treatment. Furthermore, in Rhesus monkeys prodrug **3** was responsible for a complete cure of *Plasmodium cynomolgi* infection without recrudescence, showing valuable pharmacokinetic properties.[19] Thus, bioprecursor **3** appeared to be stable in saline medium, whereas a rapid conversion into **T3** with an initial conversion half-life of ~5 min and nearly complete bioconversion (recovery >85%) was observed in plasma after 8 h.[24] From these encouraging results, we developed a program to improve the delivery of our thiazolium drugs based on neutral bio- precursors and we synthesized a library of 37 neutral prodrugs of the parent drugs **T3** and **T4**.[17] Structural variations of the pro-moieties were designed to affect lipophilicity, molecular weight and stability towards enzymes. Ideally, prodrugs should be quantitatively and rapidly converted in vivo to the active parent drug after crossing the gastrointestinal barrier.

The synthetic pathway developed takes advantage of the particular reactivity of thiazolium ring (**T**) which may be opened in basic aqueous solution and re-closed upon acidification (Fig. 3). Thus, thiazolium ions show a pH-dependent equilibrium which favours the quaternary cation **T** at low pH and a ring-opened amido enethiolate ($\mathbf{ET}^-$) at high pH, with the hemithioacetal **T°** being the most plausible intermediate. Trapping of the $\mathbf{ET}^-$ intermediate with an acylating agent leads to a neutral thioester, thiocarbonate or thiocarbamate (**TE**) structure according to the method reported by Matsukawa et al.[20] for S-acylated thiamine (Fig. 3).

Thioester derivatives (Table 1) were obtained upon treatment of **T4** with an excess of sodium hydroxide aqueous solution under ice cooling, followed by slow addition (to the in situ formed thiolate) of the appropriate commercial or freshly prepared (from the corresponding acid and thionyl chloride) acyloyl, aryloyl chloride or activated carboxylic acid in dichloromethane. Compound **18** was prepared from levulinic acid activated by isobutyl chloroformate (IBCF) and derivative **2** required the prior preparation of the corresponding substituted-benzoyl chloride.[25] Derivative **20** was obtained in two steps from Boc-proline-OH activated with IBCF followed by cleavage of the *tert*butyloxycarbonyl amino-protecting group with TFA. Thiocarbonate compounds (Table 2) were synthesized from commercially available alkyl chloroformates. Fluorinated derivative **30** required the previous preparation of 2-fluoroethylchloroformate by reacting 2-fluoroethanol with phosgene. Thiocarbamate **32** was synthesized from 4-nitrophenyl 2-oxopyrrolidine-1-carboxylate prepared beforehand from pyrrolidin-2-one, chlorotrimethylsilane and 4-nitrophenyl chloroformate.

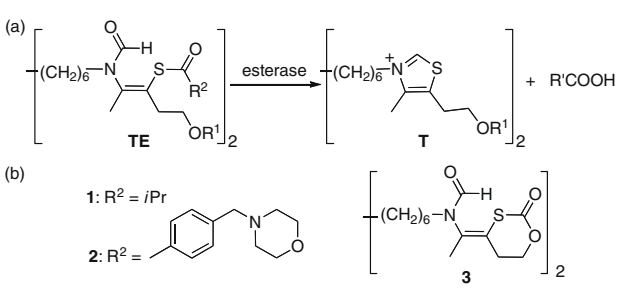


Figure 2. Bioprecursors (**TE**) of thiazolium salts (**T**).

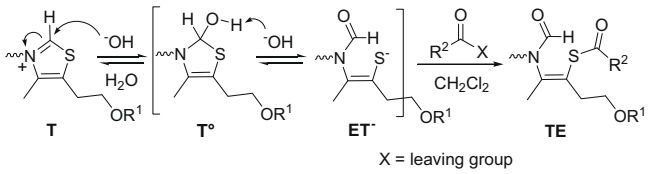
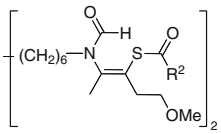
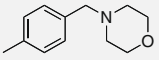


Figure 3. Drug–prodrug conversion mechanism.

Table 1
In vitro (against *P. falciparum*) and in vivo (against *P. vinckei*) antimalarial activities of the thioester prodrugs and the corresponding drug **T4**



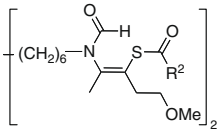
Compd	R^2	Yield (%)	IC_{50} (nM)	ED_{50} (mg/kg)	
				ip	po
T4[a]	—	—	0.65	0.14	8.1
4[c]	-Me	69	1.1	nd	nd
5	-Et	74	4.3	≪0.1	12
1[c]	-*i*Pr	91	1.1	0.12	11
6	-*cyclo*Pr	77	2	nd	16
7	-*i*Bu	96	2.4	nd	16
8[c]	-*t*Bu	72	6.7	nd	nd
9	-*cyclo*Bu	88	3.6	nd	nd
10	-*cyclo*Pentyl	90	8.2	0.65	17
11		17	3.3	nd	16
12[c]	-Ph	70	1.7	<0.5	15
13	-Ph-2-Me	76	110 (7.5)[b]	0.25	24
14	-Ph-2-CF_3	86	2200 (1600)[b]	>2.5	>90
15	$-CH_2OMe$	15	2.3	1.9	80
16	2-Furfuryl	76	1.6	nd	14
17	$(CH_2)_2CO_2Me$	78	5.9	0.4	10
18	$(CH_2)_2COMe$	43	1.6	0.15	3
19	$C(CH_3)_2OCOMe$		15.6	⩽0.3	57
2[c]		80	1.5	3.4	90
20		46[d]	2.8	nd	17
21		22	2.1	nd	<30

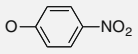
[a] Activity of this compound has already been reported.[17]
[b] After pre-incubation.
[c] Activity of these compounds has already been reported.[18,19]
[d] Total yield over two steps.

Similarly, addition of an acyloyl chloride to an alkaline aqueous solution of **T3** led to the chemically unstable thioester which underwent an intramolecular trans-acylation with the free hydroxyl group, yielding after re-closing of thiazolium ring to an O-acylated **T3** analogue (Fig. 4). Thus, intramolecular acyl migration was avoided by trapping the free hydroxyl group of the thioester intermediate with an excess of acyloyl chloride. This protocol provided O- and S-diacylated **T3** prodrugs **33**–**36** (Fig. 5a, Table 3). Finally, **T3** cyclic thiocarbonate prodrug **3** (**TE3**)[19] and its thio-analogue **37** were obtained by treating an alkaline solution of **T3** with either 4-nitrophenyl chloroformate (Fig. 5b, Table 3) or thiocarbonyldiimidazole.

All new compounds were characterised by ^1H NMR (purity ⩾95%) and MS (ESI) and the data were consistent with the structures.

Table 2
In vitro (against *P. falciparum*) and in vivo (against *P. vinckei*) antimalarial activities of thiocarbonate and thiocarbamate prodrugs and the corresponding drug **T4**



Compd	R^2	Yield (%)	IC_{50} (nM)	ED_{50} (mg/kg)	
				ip	po
T4[a]	–	–	0.65	0.14	8.1
22	$-OCH_3$	88	1.85	0.072	1.3
23	–OEt	71	2.9	0.21	6.3
24	–O*i*Pr	70	6.2	<0.1	6.8
25	OBu	82	5.6	nd	16
26	–OPh	82	3.5	0.3	24
27		50	3.1	nd	>>30
28		95	10.6	0.11	13
29		81	2.7	nd	13
30		77	2.2	0.1	3.8
31		15	310	>5	>90
32		40	29.5	0.9	12

[a] Activity of this compound has already been reported.[17]

The powerful in vitro activities of the bis-thiazolium salts **T3** and **T4**[17] and prodrugs **1**–**3**[18,19] (Tables 1 and 3) have already been reported. IC_{50} values are 2.2 and 0.65 nM for **T3** and **T4**, respectively. Compounds **1**, **2** and **3** exhibited IC_{50} in the same low nanomolar range values as the parent drugs of 1.1, 1.5 and 2.2 nM, respectively, indicating a quantitative conversion.

In vivo **T3**, **T4** and their respective bioprecursors (**1** and **3**) showed outstanding antimalarial activities after intraperitoneal administration (ED_{50} of 0.1–0.25 mg/kg; Tables 1 and 3). Compound **2** had somewhat lower activity (ED_{50} of 3.4 mg/kg). After oral administration, **T3** and **T4** showed 13 and 8.1 mg/kg ED_{50} values and neutral bioprecursors **1**, **2** and **3** exhibited 11, 90 and 5 mg/kg respectively.

On the basis of these results new neutral thioester prodrugs of **T4** bis-thiazolium salt (compounds **4**–**21**, Table 1) were tested in vitro and in vivo for their antimalarial activity. In comparison with prodrugs **1**–**3** and the parent drug, the introduction of lipophilic alkyl or cycloalkyl chain as acyl substituents (R^2, compounds **4**–**11**, Table 1) led to high in vitro active compounds (IC_{50} of 1.1–8.2 nM) probably due to a fast prodrug conversion. However, the oral effective dose was in all cases slightly higher than for parent drug **T4** (ED_{50} values ranging from 11 to 17 mg/kg). A phenyl group as R^2 residue was tolerated (**12**) but the presence of hindered substituents contiguous to the thioester function dramatically increased IC_{50}. Thus, compounds **13** and **14** were, respectively, 65 and 1300-fold less active than **12**. As these compounds showed lower IC_{50} values after incubation, this lack of activity was attributed to a lower prodrug–drug conversion rate. Moreover, in vivo activities correlate with in vitro ones (ED_{50} of 24 and >90 mg/kg, respectively). We then examined the effect of decreasing the pro-moiety lipophilicity. For this purpose, polar functional groups were firstly introduced as ether, ketone or ester (see Table 1, compounds **15** to **19**). The best result was obtained with compound **18** which showed an ED_{50} value of 3 mg/kg after oral administration. Secondly, we introduced a protonatable nitrogen in the R^2 substituent. Thus, compounds **2**, **20** and **21** containing a morpholino, pyrrolidine and pyridine moieties, respectively, exhibited powerful IC_{50} (1.5–2.8 nM). However, oral activities were lower than **T4** parent drug (ED_{50} >17 mg/kg).

The introduction of thiocarbonate and thiocarbamate as enzymo-labile pro-moieties was also studied (Table 2). Alkyl (**22**–**25**), aromatic (**26** and **27**) and halo alkyl (**28**–**30**) derivatives presented IC_{50} values ranging from 1.85 to 10.6 nM. The most potent in vivo

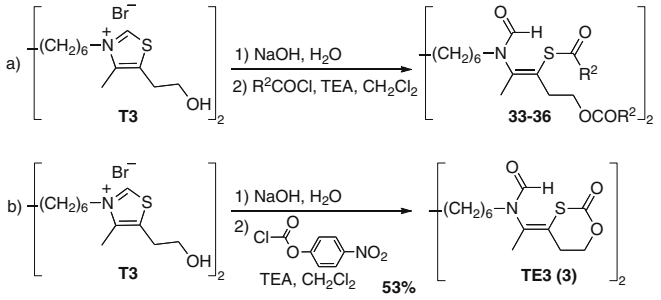
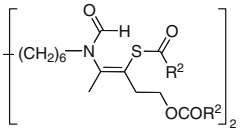
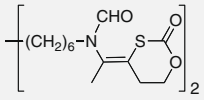
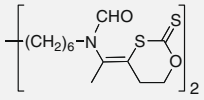


Figure 5. TE prodrug synthesis of bis thiazolium salts **T3**.

Table 3
In vitro (against *P. falciparum*) and *in vivo* (against *P. vinckei*) antimalarial activities of the thioester prodrugs and the corresponding parent drug **T3**



Compd	R^2	Yield (%)	IC_{50} (nM)	ED_{50} (mg/kg)	
				ip	po
T3[a]	–	–	2.2	0.2	13
33	-*i*Pr	–	7	1.2	60
34	$(CH_2)_2CO_2Me$	74	48	0.5	13
35	Ph-2,3,4-$(OCH_3)_3$	44	16.5 (6.5)[b]	1.8	>60
36	Ph-2-Me	52	3500 (570)[b]	>20	>180
3[c]		45	2.2	0.25	5
37		22	4400		>30

[a] Activity of these compounds has already been reported.[17]
[b] After pre-incubation.
[c] Activity of this compound has already been reported.[18,19]

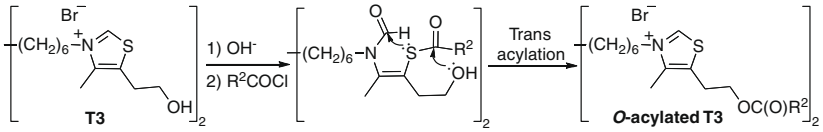


Figure 4. Trans-acylation of **T3** thioester prodrugs.

activities were obtained for the methylthiocarbonate prodrug **22** and for the 2-fluoroethyl derivative **30**. Irrespective of the mode of administration, both derivatives were more potent in vivo than the parent drug **T4** (oral ED_{50} = 1.3 and 3.8 mg/kg, respectively). Pyrrolidine thiocarbamate derivatives **31** and **32** were also evaluated. **31** showed low in vitro and in vivo activities. The presence of the electron withdrawing carbonyl group in **32** probably enhances the rate of the thiocarbonate bond hydrolysis, resulting in better IC_{50} and quite good in vivo activity.

Then six O-acylated **T3** prodrugs were evaluated (Table 3). Compound **33** (R^2 = isopropyl) exhibited the highest in vitro activity within the series (7 nM). However, this prodrug showed a poor ED_{50} probably due to its high lipophilic character and to a fast prodrug–drug conversion rate. The introduction of a polar function within the R^2 moiety led to derivative **34** which showed lower in vitro activities but interestingly oral activities in the range of the parent **T3** drug. In order to decrease the converting rate, we introduced a 3,4,5-trimethoxyphenyl group (compound **35**), with donor electronic properties or a steric demanding 2-methylphenyl substituents (compound **36**). Both compounds showed high IC_{50} values, which decreased after pre-incubation, and low in vivo activities (ED_{50} >60 mg/kg). These results may be associated to an incomplete prodrug–drug transformation.

In order to get a low molecular weight prodrug the cyclic thiocarbonate prodrug **3** was prepared. This derivative showed an in vitro IC_{50} = 2.2 nM and the oral activity was enhanced (ED_{50} = 5 mg/kg).[19] However, pharmacokinetics data showed that this compound is rapidly converted into **T3** in the gastrointestinal tract. To slow up the conversion rate we prepared a structural analogue of **3**, the dithiocarbonate **37**, which should be more stable towards esterase hydrolysis. Thereby, **37** appeared to be less active (IC_{50} of 4400 nM), probably due to an incomplete conversion and a decreased oral activity was also observed (ED_{50} >30 mg/kg).

Aiming to improve the oral bio-availability of our lead compounds **T3** and **T4**, we focused our efforts on a library of 37 thioester, thiocarbonate and thiocarbamate neutral precursors. These derivatives were obtained in one or two steps with yields ranging from 15% to 96% and using a low cost synthetic pathway. Comparison of compounds **6**–**10** to **4**, **13** and **14** to **12**, and **35** to **36**, showed that the presence of bulky groups contiguous to the thioester moiety leads to lower antimalarial in vitro and in vivo activities. These results suggest that with hindered substituents the conversion does not occur or is incomplete. Thus, a pre-incubation period in the culture environment (before *P. falciparum* infection) allowed in some cases to improve the in vitro activity (**13**, **14** and **36**). Similarly, electron donor substituents decrease the conversion rate (compare **35**–**33**) and electron withdrawing groups accelerate the hydrolysis (**32**–**31**). Lipophilicity of the prodrug derivatives may be modulated by the introduction of a protonatable side chain. Thus, compounds **2**, **20** and **21** exhibited excellent in vitro antimalarial activity (IC_{50} = 1.5–2.8 nM) but displayed limited oral absorption. It is noteworthy that, at the contrary to most of the other TE prodrugs (which are liquids) these three amine-containing derivatives behave as solids, easy-to-handle and appropriate for an oral therapy. Among all prodrugs evaluated in vivo against the lethal rodent malaria, *P. vickei*, derivatives **18**, **22**, **30**, and **3** were more potent (either by intraperitonial and oral administration) than the parent drugs **T4** and **T3**. The common features of these compounds are a low molecular weight (**18**, 713.0 g/mol; **22**, 632.9 g/mol; **30**, 696.9 g/mol and **3**, 540.7 g/mol) and a low *c* log P (**22**, 6.45; **30**, 7.04; **18**, 6.68 and **3**, 6.20)[26] compared to the weaker active ones.

The thiocarbonate derivative **22** appears to be the most powerful. Indeed, it is 3–4 times more potent than previously reported TE3 prodrug (**3**). To our knowledge, this class of antimalarials represents one of the most potent in the rodent model tested yet, and preliminary studies on efficacy and tolerance of these compounds in mice and primates are very promising, indicating that this approach may be applicable to the human infection.[24]

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.001.

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