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1,4-Dihydroxy-2,3-dioxatricyclo[8.4.0.0^{4,9}]tetradecane and Derivatives with In Vitro Activity Against *Plasmodium falciparum*, *Trypanasoma b brucei*, *Trypanasoma cruzi*, and *Leishmaniasis infantum*

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Abstract—1,4-Dihydroxy-2,3-dioxatricyclo[8.4.0.0^{4,9}]tetradecane and derivatives have been synthesised and their in vitro activity against *Plasmodium falciparum* (malaria) Ghana, *Trypanasoma b brucei* (sleeping sickness) TB-1, and *Trypanasoma cruzi* (Chagas' disease) TC-1, and *Leishmaniasis infantum* (leishmaniasis) L1 parasite strains has been assessed.

There are several human protozoal parasites that cause devastating diseases in the regions of the World where they are found. Four of these parasites are *Plasmodium falciparum*, *Trypanasoma b brucei*, *Trypanasoma cruzi,* and *Leishmaniasis infantum*, causative agents of malaria, sleeping sickness, Chagas' disease, and Leishmaniasis, respectively. Over the last decade intense research efforts have been directed towards the nonalkaloidal trioxane, naturally occuring lactone, artemisinin **1**, its semisynthetic ether **2** and ester derivatives, and synthetic analogues, for example BO7 **3**[1,2] (Fig. 1). The reason behind this fervent investigation is that these trioxane systems offer a very real chemotherapeutic alternative to standard quinoline (e.g., chloroquine) and antifolate antimalarial drugs to which the *P. falciparum* parasite has become largely resistant.[3]

We are investigating the possibility of using the fact that infected red blood cells are under a high degree of endogenous oxidative stress, stemming from the parasitic presence, to combat erythrocyte parasites. Various physiological processes come into play to relieve this stress, primarily in the provision of substrates for reduction in a process known as the hexose monophosphate shunt (HMS).[4,5] In infected cells HMS activity increases 24-fold and our hypothesis is that the introduction of an alternative reductive target compound (ARTC) into the system will prompt the uptake of the compound by the infected red blood cell. Should this ARTC also contain the chemical composition required to kill the parasite, then it may be possible to produce a drug that is effective against malarial erythrocyte parasites. We have shown the plausibility of this hypothesis through the use of quinine and azure A or proflavin hybrids.[6]

One can view the nonalkaloidal trioxane drugs and other peroxide systems as ARTCs, as the peroxide component may be readily reduced. Mechanistic studies on the action of trioxane systems both in vitro and in vivo, and on how this action results in their potent antimalarial activity have shown that the chemical composition required to kill the parasite is produced on the reduction of the peroxide by Fe(II) in iron-porphyrins.[7] We believe that it is possible that the same hypothesis for the HMS within erythrocytes, with respect to ARTCs, outlined above, could be applied to all protozoal parasites that at some stage of their life cycle occupy cells of the host. If we are correct a peroxide based molecule that acts against the malarial parasite *P. falciparum* could feasibly act against the *T. b brucei, T. cruzi,* and *L. infantum* parasites. These parasites all fulfil the above criteria in so much as they occupy cells of the host during their life cycle.[8]

In pursuit of evidence for this theory we synthesised a series of simple compounds containing a peroxide unit (loosely

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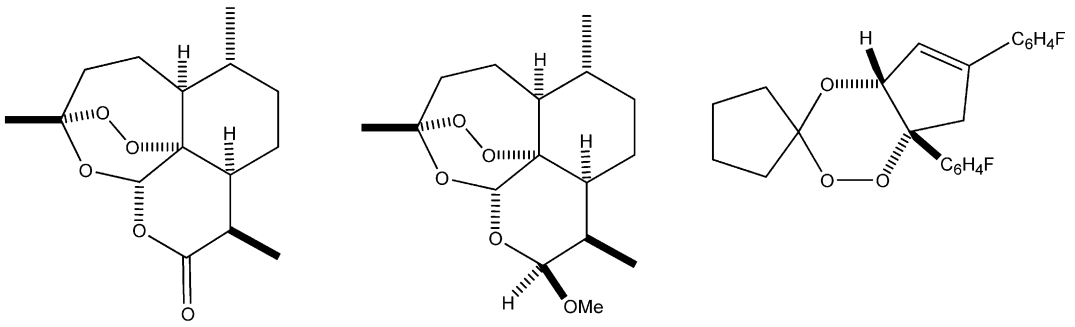
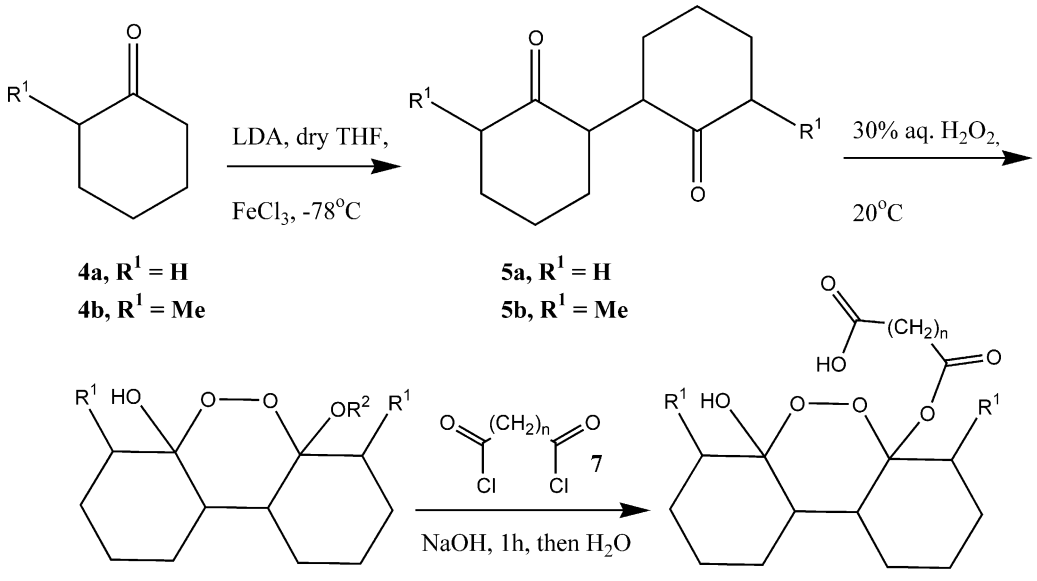


Figure 1.

based on the artemisinin trioxane substructure),[9,10] (Scheme 1). In choosing our peroxide containing structures we also took into account current theories on the mode of action of endoperoxides, that is the ability of active endoperoxides to undergo cleavage to form an Fe(IV)=O species and an oxygen radical that subsequently undergoes a 1,5-H shift to produce a carbon-centred radical.[11] Our choice of peroxide theoretically had the potential to satisfy these requirements. Using LDA and $FeCl_3$ we dimerised cyclohexanone **4a** to form the bis(cyclohexyl)-2,2′-dione **5a**, similarly we coupled 2-methylcyclohexanone **4b** to form bis(3,3′-dimethylcyclohexyl)-2,2′-dione **5b**. On subsequent reaction with 30% aqueous hydrogen peroxide the diones **5a** and **5b** gave 1,4-dihydroxy-2,3-dioxatricyclo[8.4.0.0^{4,9}]tetradecane **6a**, and 5,14-dimethyl-1,4-dihydroxy-2,3-dioxatricyclo[8.4.0.0^{4,9}]tetradecane **6b**, respectively. Reaction of **5a** with 30% aqueous hydrogen peroxide in the presence of methanol gave 1-methoxy-4-hydroxy-2,3-dioxatricyclo[8.4.0.0^{4,9}]tetradecane **6c**. The 1,4-dihydroxy-2,3-dioxatricyclo[8.4.0.0^{4,9}]tetradecane **6a** was then reacted with four diacid chlorides **7** to form the esters **8a–d**.[12] Compounds **6a–c** and **8a–d** were then assayed in vitro against the *P. falciparum* Ghana, *T. b brucei* TB-1, *T. cruzi* TC-1, and *L. infantum* L1 strains, and cytoxicity was tested on the cell line MRC-5. The results of the biological in vitro testing are given in Table 1.

As can be seen from the results given in Table 1 for the initial in vitro biological assessment of compounds **6a–c** and **8a–d** they are all active against the protozoal para-



Scheme 1.

Table 1.

Compd	IC_{50} (μM) Ghana	IC_{50} (μM) TB-1	IC_{50} (μM) TC-1	IC_{50} (μM) L1	IC_{50} (μM) MRC-5
6a	0.1	13.0	24.0	> 32.0	10.0
6b	> 32.0	< 64.0	> 32.0	> 32.0	> 32.0
6c	5.0	16.0	8.0	> 32.0	8.0
8a	6.0	10.0	> 32.0	> 32.0	> 32.0
8b	1.0	4.0	13.0	> 32.0	13.0
8c	19.0	16.0	15.0	> 32.0	> 32.0
8d	> 32	17.0	19.0	> 32.0	> 32.0

sites for which they were assayed. The anti-protazoal parasite activities of these molecules are not potent in comparison to other compounds currently used to treat the corresponding disease. For example artemether has an activity of 0.025 μM against *P. falciparum* Ghana strain, Suramin 0.043 μM against *T. b brucei* TB-1 strain, Nifurtimox 0.39 μM against *T. cruzi* TC-1 strain, and PX-6518 0.019 μM against *L. infantum* L1 strain.[13] However, the fact that they are active against all of these parasites, that at some point in their life cycle invade cells of the host, indicates that the HMS and the use of ARTCs may provide a route to delivery of active anti-protazoal parasite compounds. This approach may lead to a pan-anti-protazoal drug.

The synthesis of the peroxide systems **6** that we have assayed for biological activity is relatively simple and open to adaptation to form numerous analogues that may show greater efficacy against the aforementioned parasites. We are currently investigating this option.

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13. Data supplied by the Tropical Disease Research unit of the World Health Organisation, Geneva, Switzerland.