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Antiplasmodial activity of alkyl-substituted 1,2-dioxetanes against *Plasmodium falciparum*

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ABSTRACT

This article reports the in vitro antiplasmodial activity of two endoperoxides of the class 1,2-dioxetanes against *Plasmodium falciparum*: bis(adamantyl)-1,2-dioxetane and 3,3,4,4-tetramethyl-1,2-dioxetane. The results reveal that bis(adamantyl)-1,2-dioxetane displays substantial antiplasmodial activity, at least two orders of magnitude higher than that of artemisinin, while 3,3,4,4-tetramethyl-1,2-dioxetane is less active.

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Peroxidic antiplasmodial drugs like artemisinin (**1**) are essential in frontline therapies capable of destroying or inactivating the malaria-causing *Plasmodium* parasite;[1] systematic studies observed that several other peroxides with different structural patterns also displayed significant antiplasmodial activity.[2] The exact mechanism of action of peroxidic drugs remains controversial, however it is well established that redox-active iron(II) species play an important role, supposedly through a mechanistic pathway based on the Fenton-reaction, that produces reactive radical species (Fig. 1).[3]

1,2-Dioxetanes are four-membered cyclic organic peroxides that have the unique ability to produce light upon thermal or chemical activation;[4] therefore, they find extensive applications as chemiluminescent reactants, mainly in bioanalytical assays.[5] The antiplasmodial activity of 1,2-dioxetanes has been originally briefly reported by Sharma and cooworkers;[6] recently, our group conducted a more extensive examination of the activity of a series of 3-methoxy-1,2-dioxetanes, and found a moderate in vitro antiplasmodial activity.[7] This has encouraged us to test other 1,2-dioxetanes against *Plasmodium falciparum* cultures.

The antiplasmodial activity of the 1,2-dioxetanes was evaluated observing their efficiency in deactivating *Plasmodium falciparum* cultures in vitro, using artemisinin as reference; this data is reported as IC_{50} values (Table 1). Schizont forms of the parasite were incubated with a fresh culture of erythrocytes in the presence of the tested compounds in different concentrations.[8] The percentage of parasite invasion[9] (determined by dividing the number of parasites presenting intracellular rings by the total number of parasites after 24 hours of incubation) yielded the parasitemia levels, in the presence or absence of the tested substances. The presence of rings is easily observed and indicates that the parasite was active at the time of measurement.[10]

Also, the hemolytic activity of the substances was evaluated; uninfected erythrocytes (kept in the same conditions used in the invasion assay) were treated with 10^{-8} M of each tested compound at 37 °C for 24 h. After this time, the supernatant was collected and clarified at 900 g for 8 min and the free hemoglobin amount was determined by absorption at 530 nm, using untreated cells as control for non-specific hemolysis. The hemolytic activity was determined calibrating the level of 100% hemolysis treating the cells with distilled water (Table 1).

The therapeutic index (TI) – Minimum Hemolytic Concentration/Minimum Inhibitory Concentration (MHC/MIC) ratio – is a parameter used to represent the specificity of the antiplasmodial

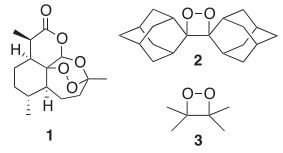

Figure 1. Structure of artemisinin (**1**), bis(adamantyl)-1,2-dioxetane (**2**) and 3,3,4,4-tetramethyl-1,2-dioxetane (**3**).

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Table 1
Antiplasmodial and hemolytic data for artemisinin (**1**), bis(adamantyl)-1,2-dioxetane (**2**) and 3,3,4,4-tetramethyl-1,2-dioxetane (**3**)

Entry	IC_{50} ($\times 10^8$ M)	Relative antiplasmodial activity	Hemolytic activity (%)	Therapeutic index
1	1.0 ± 0.2	1	1.5 ± 0.2	10^8
2	0.019 ± 0.001	105	2.0 ± 0.4	10^8
3	4.5 ± 0.1	0.45	1.4 ± 0.4	10^7

Conditions: IC_{50} assay – compounds were diluted in seven concentrations giving 16–84% inhibition. Hemolytic assay – uninfected human red blood cells were treated with distilled water and kept in the same conditions used in the invasion assay. After incubation, the supernatant was collected, centrifuged and the hemoglobin content was detected in a spectrophotometer.

compound in parasite infection and it is associated with different parasites growth stages in erythrocytes from blood.[11] It was calculated by the ratio of MHC and MIC; higher values in therapeutic index represent greater antiplasmodial specificity. The MHC values for all compounds were calculated and compared to compound **1**. Even if the experiments exhibited different results for MIC and MHC values, the TIs for compounds **1** and **2** are equivalent, and on the other hand, compound **3** presented different specificity that is observed by TI value for compound **3** (Table 1 and Table S2 of Supplementary Data).

Despite all compounds presenting low hemolytic activity, their antiplasmodial activity was remarkably diverse. Surprisingly, in the conditions tested, bis(adamantyl)-1,2-dioxetane (**2**) displayed a remarkable antiplasmodial activity, two orders of magnitude higher then artemisinin on a molar basis. However, 3,3,4,4-tetramethyl-1,2-dioxetane (**3**) had a much lower antiplasmodial activity, about half of the artemisinin. Since **3** is much more sensitive then **2** to thermal cleavage and chemical reactivity, it is possible that it is affected and destroyed before entering the parasite, while **2** is much more robust, it can enter and affect the parasite more effectively.

In summary, this work supports the hypothesis that 1,2-dioxetanes have significant antiplasmodial activity in vitro. The effects of these compounds were studied in schizont forms of *Plasmodium falciparum* of the W2 strain, and the statistical analysis of the obtained data corroborates the existence of a significant effect compared to the control experiment. The discovery of a new class of peroxides with significant antiplasmodial activity can open new horizons for the study of these compounds in several other conditions, such as in different phases of the plasmodium or using different concentrations or structures of 1,2-dioxetane to tune the formation of a specific set of reactive radicals. It may also be important in the development of new therapeutic strategies to address the new artemisinin-resistant strains that are slowly emerging worldwide.[12]

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Supplementary data

Supplementary data (synthesis details, complete data sets regarding the parasite counting and statistical analysis) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.08.096.

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