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8. CQ-R P. falciparum IC_{50} = 160 nM
Active in vivo against P. berghei
Synthetic Spirocyclic Endoperoxides: New Antimalarial Scaffolds

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Here we report the development of a straightforward synthetic procedure for the preparation of spirocyclic endoperoxides as synthetic analogues of the natural product dihydroplakortin. Peroxides here presented are more potent antimalplasmodials than dihydroplakortin itself and we proved for the first time their antimalarial activity in vivo.

Cyclic peroxides such as 1,2-dioxolanes, 1,2,4-trioxanes and 1,2-dioxanes are a class of organic compounds with interesting pharmacological properties and widely represented in nature. Artemisinin (ART) is an endoperoxide-based natural product, which is highly effective against clinically relevant P. falciparum strains responsible for human malaria. Currently, the so-called artemisinin-based combination therapies (ACT) are employed as first line treatment in most malaria endemic countries, adhering to WHO recommendations.1-3 However, lower susceptibility to artesimins is being reported from high malaria endemic regions.4 So, novel peroxides characterized by different structural features could delay the potential selection of P. falciparum resistant strains.5 Moreover, cost associated to the extraction of this drug or to synthetic precursors from natural sources, prompted researcher at developing synthetic peroxides as low-cost alternatives to ART.6

Since several years we are engaged in the design of suitable synthetic strategies for the preparation of antimalarial endoperoxides.7-10 In particular we performed the first stereoselective synthesis of 9,10-dihydroplakortin (DHP, Figure 1),10 a natural product endowed with interesting antimalplasmodial properties.11 We also reported the development of novel endoperoxides as synthetic analogues of DHP characterized by a bicyclic tetrahydrofuro[2,3-c][1,2]dioxane core. The synthetic peroxide 2 (Figure 1) was the most potent compound of the series and showed higher antimalplasmodial potency than dihydroplakortin. The major limitation of these analogues was their synthesis as an inseparable mixture of diastereoisomers.7 The structure-activity relationships (SARs) for this class of compounds were rationalized on the basis of the mechanism of action proposed for ART.

![Figure 1 Structure of reference (1-2) and title (3-11) endoperoxides](https://example.com/figure1.png)

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7 Electronic Supplementary Information (ESI) available: [Scheme S1, Figure S1, Experimental details]. See DOI: 10.1039/c000000x/
Although the mechanism of action of ART is still debated, it seems that the peroxide bond is activated within the food vacuole of the parasite, a specialized organelle accommodating the hydrolysis of hemoglobin, a necessary source of amino acids for the parasites. During this process, free Fe(II)-heme is released and reacts with the peroxide bond of ART to form an O-centered radical, that probably undergoes a 1,5-H shift to form a highly toxic C-centered radical, ultimately responsible of the antiparasitodal activity. The formation of C-centered radicals has also been proposed for several synthetic endoperoxides, comprising DHP. For our synthetic endoperoxides, we found that subtle modifications of the substituent at C3, affecting the distance between the C5 of the lateral chain and the O2 of the peroxide bridge (C1–O2 distance ≤ 3 Å for optimal activity), had strong impact on the antiparasitodal potency, in line with the mode of action of ART and DHP. As a continuation of our previous work, we were interested in developing suitable synthetic strategies aimed at modifying the lateral chain at C3 of the bicyclic skeleton (3-8) and the size of the rings forming the bicyclic system (9-11) reducing the number of chiral centers. In particular, we were interested in the synthesis of spiro-derivatives 6-8 that lack the stereogenic center at C3 and represent a further simplification of this class of compounds.

As described in Scheme 1, a three-step reaction protocol was employed for the assembly of the bicyclic core starting from olefin intermediates 12a-d and 13a-c. The first step of the protocol consisted in the Mukaiyama peroxysilylation reaction of the double bond of intermediates 12a-d and 13a-c to afford peroxides 14a-d and 15a-c, respectively. The Mukaiyama reaction proceeds regioselectively at the more substituted carbon of the double bond. Subsequently the lactone functionality was reduced to the corresponding lactol by using disobutylaluminum hydride (DIBAL). Finally, trimethylsilyl triflate (TMSOTf)-promoted simultaneous deprotection and cyclization of the resulting lactols afforded the cyclised endoperoxides 3-5, isolated as inseparable mixtures of diastereoisomers, and spiroperoxides 6-9 obtained as racemates. Following a slightly modified protocol, the synthesis of endoperoxide 10 is reported as Supplementary Information (SI). The synthesis of intermediates 12a,d and 13c is described in Scheme 2. 1-Adamantylaldehyde 16a and cyclohexane 16b were submitted to the Horner–Wadsworth–Emmons olefination reaction to afford the α,β-unsaturated esters 17a,b. DIBAL-mediated reduction of a bove esters 17a,b and of commercially available ester 17c afforded the corresponding primary alcohols that were
subsequently converted into the iodides 18a-c. While iodides 18a,c were obtained by treatment of the alcohols with iodine, triphenylphosphine, and imidazole, iodide 18b could not be obtained by using the same protocol. 18b was instead obtained by treatment of the alcohol precursor with potassium iodide in the presence of boron trifluoride. Iodides 18a-c were next used to alkylate lactone 19 using lithium hexamethyldisilazide (LiHMDS) as the base to afford 12a,d and 13c. Intermediates 12b,c, bearing cyclohexyl and cyclopentyl substituents, were prepared as reported in Scheme 3. The synthesis of an analogue bearing an adamantyl-radical at the same position was also attempted without success. Accordingly, cyclohexyl and 1-adamantyl derivatives 20a,b were treated with cerium chloride as a Lewis acid and sodium hypochlorite as the oxidizing agent to afford chloromethyl derivatives 21a,b in reasonable yields. Intermediates 21a,b were in turn converted into the corresponding iodides 22a,b. Intermediate 22c was synthesized starting from 25, in turn prepared following a different synthetic approach by reacting the Grignard reagent 23, formed in situ, with propargyl alcohol 24. The resulting primary alcohol 25 was then converted to 22c via a mesyl ester intermediate that was sonicated in the presence of lithium iodide to afford the desired iodide 22c in good overall yield. Iodides 22a-c were used as alkylating agents for the enolate of lactone 19. While 22a,c smoothly furnished the corresponding alkylation products, the same reaction performed with the adamantyl derivative 22b failed, probably due to its higher steric hindrance. For the synthesis of intermediates 13a,b (Scheme 4), alcohol intermediates 27a,b, in turn obtained by aldol condensation of aldehydes 26a,26b with 19 in the presence lithium diisopropylamide (LDA), were dehydrated to the corresponding olefins. Finally, dioxolane rac-11 was synthesized as described in Scheme 5. The carbonyl group of the commercially available ketone 28 was reduced using sodium borohydride and the double bond

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<th>DC0 (µM)</th>
<th>D20 (µM)</th>
<th>D20 (µM)</th>
<th>D21 (µM)</th>
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*IC0 values are the mean of at least three determinations in duplicate. Standard errors were all within 10% of the mean. The distances were calculated and reported in Å by means of measurement tool implemented in Maestro graphical interface from the poses obtained by docking calculation; The binding energy was calculated by means of Prime MM-GSBA2; E1 = epimer 1: 3R*,4aS*,7aR*; E2 = epimer 2: 3S*,4aS*,7aR*. |
was hydroperoxylated to 29 employing Mukaiyama reaction conditions. Treatment of 29 with methanesulfonyl chloride in the presence of triethylamine directly afforded the cyclization product rac-11.

The antiplasmodial activity of the novel endoperoxides 3-11 was tested against two laboratory P. falciparum strains, the chloroquine-sensitive (CQ-S) D10 and the chloroquine-resistant (CQ-R) W2, accordingly to described procedures. The resulting IC₅₀ are reported in Table 1.

To understand the modulation of antiplasmodial potency observed for the synthesized compounds, we in depth analyzed the interaction of the peroxide moiety of compounds 4-11 with Fe(II)-heme (which is the first and the key step for the formation of the toxic radical species) by molecular docking analysis employing Glide software, after structure optimization performed by using ab initio calculation employing the Jaguar software. Endoperoxides here reported have been produced and tested as mixtures of diastereoisomers (3-5) or as racemates (6-11), while docking studies were performed for each enantiomer. As expected, heme is unable to discriminate between enantiomers and no differences in the binding mode of each enantiomeric couple were observed. Based on our molecular docking calculations, we found a good correlation between the distance of oxygen atoms from the reactive heme iron center and the antimalarial activity (Table 1). In fact, the compounds that display a large distance of the peroxide moiety from the iron center such as compound 6, 9-11 were found less potent in vitro than compounds 3, 5, 7, 8 exhibiting a shorter distance. For example, peroxide 4 (one of the most active compound in the set) displaying a very short distance between the peroxide oxygen atoms and the iron center (Table 1) and both atoms could be able to coordinate the heme iron. On the contrary, the inactive analogue 9 does not possess the appropriate distance to interact with heme iron.

For comparison, distances calculated for the reference compounds ART and DHP are also in good agreement with a correct interaction with heme (Table 1 and Figure S1 in the Supporting Information). Also the free-binding energy (ΔGbind) calculated for all complexes (Table 1), displayed a good correlation with antimalarial potency, being around -20 kcal/mol for inactive compounds (9), comprised between -20 and -25 kcal/mol for compounds in the micromolar range (6, 10, 11), and < -25 kcal/mol for compounds in the low micromolar range (1, 3, 5, 7, 8). It is worth noticing that ART presents a calculated ΔGbind well below the value found for the spiroperoxides here presented, consistent with its nanomolar antimalarial potency. Compound 8, displaying an estimated ΔGbind of -30.69 kcal/mol, is the most potent plakortin-related endoperoxide described to date. Accordingly, both epimers of compounds 4 and 5 (Figure 2A-D) interact with heme in a similar fashion. However, the larger steric hindrance of the cyclohexyl ring of 4 with respect to the cyclopentyl ring of 5 results in a stronger hydrophobic interaction with heme for 4. For the couple of analogues 6 and 7 (Figure 3AB), the steric hindrance of the cyclohexyl ring physically hampers the accommodation of 6 for a strong interaction with Fe(II)-heme (Figure 2A), while the cyclopentyl ring of 7 (Figure 3B) has a reduced steric hindrance and is well tolerated allowing both O₂ and O₁ of the peroxide system to interact with Fe(II)-heme. Docking studies (Figures 2, 3) highlighted that subtle differences in the structure of the peroxide and in particular the size and shape of their side chains are responsible for a fine-tuning of the antimalarial activity. On the other hand, the bulky adamantane ring (8, Figure 3C) is able to maximize hydrophobic interactions with the planar protoporphyrin-IX ring at the same time maintaining a correct orientation of both the peroxide oxygens for their interaction with Fe(II)-heme, thus resulting in an overall improved ΔGbind and, as a consequence, higher antimalarial potency. Finally, the seven-membered ring of compound 9 (Figure 3D) is not tolerated since it hampers a favourable conformation for reaching the right distance to iron atom contained in the protoporphyrin ring (Figure 3D).
Spiroperoxide 8, the most potent antiplasmodial agent of the series, was submitted to a preliminary in vivo evaluation in the P. berghei mouse model of malaria using the Peters 4-day test. After administration of 8 at a daily dose of 100 mg/kg (i.p.), mice showed a 72.5% reduction in parasitaemia. Notably, compound 8 did not elicit, at the concentration used in the in vivo study, any sign of toxicity in mice consistently with its determined in vitro toxicity against mouse fibroblasts NIH3T3 (IC50 = 150 µM).

In conclusion, we herein presented the development of a straightforward synthetic procedure for the preparation of spiroperoxides as racemic mixtures endowed with antiplasmodial activity against P. falciparum CQ-S and CQ-R strains. Being an achiral target, compounds were tested as racemates since no difference on antimalarial activity for each enantiomer could be expected. Molecular modelling studies highlighted key molecular features responsible for activity and potency, and will be useful for further optimizing their in vitro potency. Moreover, we have herein for the first time provided evidence that plakortin-derived synthetic analogues do have antimalarial activity in vivo. The straightforward synthetic approach to spirocyclic endoperoxides may pave the way to the development of more potent analogues in vivo.

The Authors thank MIUR for financial support. S. B. thanks S-IN Soluzioni Informatiche for technical support.

Notes and references