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Glycosyl hydroperoxides: A new class of potential antimalarial agents

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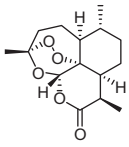
ABSTRACT

Motivated by the antimalarial properties observed in organic peroxides, an extensive series of glycosyl hydroperoxides was prepared with the aim of identifying new bioactive molecules. Selected compounds were tested against a *Plasmodium falciparum* culture (chloroquine-susceptible strain D10 and chloroquine-resistant strain W2). Screening results indicated that the factors critical for antimalarial activity were the presence of a hydroperoxide moiety and solubility in water at pH 5.0. Moreover, the ability to inhibit β-hematin formation in vitro has been evaluated (BHIA Assay).

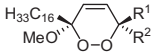
1. Introduction

Malaria is infamous for being one of the most medically and economically damaging diseases present in the world today. It affects more than 200 million people and causes 600,000 deaths every year, particularly of children under five years of age.[1] Malaria caused by *Plasmodium falciparum* (*Pf*) is the most severe with highest rates of complications and mortality. The parasite exhibits a complex life cycle and during the intraerythrocytic stage, it degrades the host's hemoglobin within acidic (pH ≈ 5.5) food vacuoles where heme molecules are detoxified by conversion into crystalline hemozoin (malaria pigment). 4-Aminoquinolines such as chloroquine (CQ), the most popular antimalarials, act by inhibiting hemozoin formation.

At present, artemisinin-based combination therapy (ACT) is recommended as first-line treatment for uncomplicated malaria.[1] Artemisinin (**1**) and its derivatives are the most rapidly effective antimalarials available. However, signs of resistance to this class of drugs have been recently observed in Southeastern Asian countries.[2] There is indeed an urgent need for new, safe and inexpensive drugs for the treatment of malaria. Chemically, artemisinin (**1**) is a sesquiterpene lactone containing an unusual peroxide bridge critical for its antimalarial activity. Other natural peroxides of acetals, such as chondrillin (**2**) and plakorin (**3**), members of a larger family of alkoxydioxines isolated from marine organisms, exhibit antimalarial and antitumor activity.[3] A number of other natural compounds with such a peroxide bridge is known. Nevertheless, their activity is not equal to artemisinin derivatives.[4] Peroxides of acetals have been found to be relatively stable.[5]



1



2: R^1=CO_2Me; R^2=H
3: R^1=H; R^2=CO_2Me

Recently, we reported that the oxidation of 2-deoxysugars or their glycosides to corresponding glycosyl hydroperoxides can be performed with hydrogen peroxide in the presence of an acid catalyst using a range of reaction conditions.[6] Glycosyl hydroperoxides, derived from 2-deoxysugars in a similar way as those derived from 2,3-unsaturated sugars, are relatively stable. They can be purified by silica gel chromatography and stored for weeks in the refrigerator without visible decomposition. The facility of the synthesis of stable hydroperoxides from 2-deoxy- and 2,3-unsaturated sugars has been rationalized as stemming from the propensity of these sugars to form a glycosyl cation to which the hydrogen peroxide molecule can add. In contrast to 2-deoxysugars, the elimination of an alcohol or water molecule from the anomeric center of their fully

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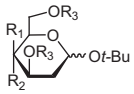
Table 1

Substrate 	Method A	Method B
12 $R_1 = OC_7H_{15}$, $R_2 = H$, $R_3 = C_7H_{15}$	**18α**:**18β** **84**:**16**	**18α**:**18β** **95**:**5**
13 $R_1 = H$, $R_2 = OC_7H_{15}$, $R_3 = C_7H_{15}$	**19α**:**19β** **55**:**45**	**19α**:**19β** **65**:**35**
14 $R_1 = OC_{12}H_{25}$, $R_2 = H$, $R_3 = C_{12}H_{25}$	**20α**:**20β** **85**:**15**[*]	**20α**:**20β** **93**:**7**
15 $R_1 = H$, $OR_2 = OC_{12}H_{25}$, $R_3 = C_{12}H_{25}$	**21α**:**21β** **58**:**42**	**21α**:**21β** **67**:**33**
16 $R_1 = OC_{18}H_{37}$, $R_2 = H$, $R_3 = C_{18}H_{37}$	**22α**:**22β** **87**:**13**[*]	**22α**:**22β** **98**:**2**
17 $R_1 = H$, $OR_2 = OC_{18}H_{37}$, $R_3 = C_{18}H_{37}$	**23α**:**23β** **56**:**44**[**]	**23α**:**23β** **68**:**32**

[*] Toluene was added to homogenize the reaction mixture.
[**] Carbon tetrachloride was added to homogenize the reaction mixture.

hydroxylated congeners to form a glycosyl cation proceeds under conditions which cause decomposition of the hydroperoxide group. Therefore, the synthesis of fully hydroxylated glycosyl hydroperoxides requires a different method.[7]

In the present work the in vitro antiplasmodial activity against both CQ-resistant and CQ-sensitive strains of *P. falciparum* has been evaluated. Moreover, in order to obtain basic information on the mechanism of their action, the ability of these compounds to inhibit hemozoin formation was tested using the β-Hematin Inhibitory Assay (BHIA).

2. Results and discussion

The structural similarity of anomeric hydroperoxides to antimalarial drugs containing a peroxide motif prompted us to examine their antimalarial properties. Bearing in mind that artemisinin and other active peroxides are compounds of low polarity, we synthesized glycosyl hydroperoxides possessing lipophilic protecting groups, which attenuated their inherent hydrophilicity.

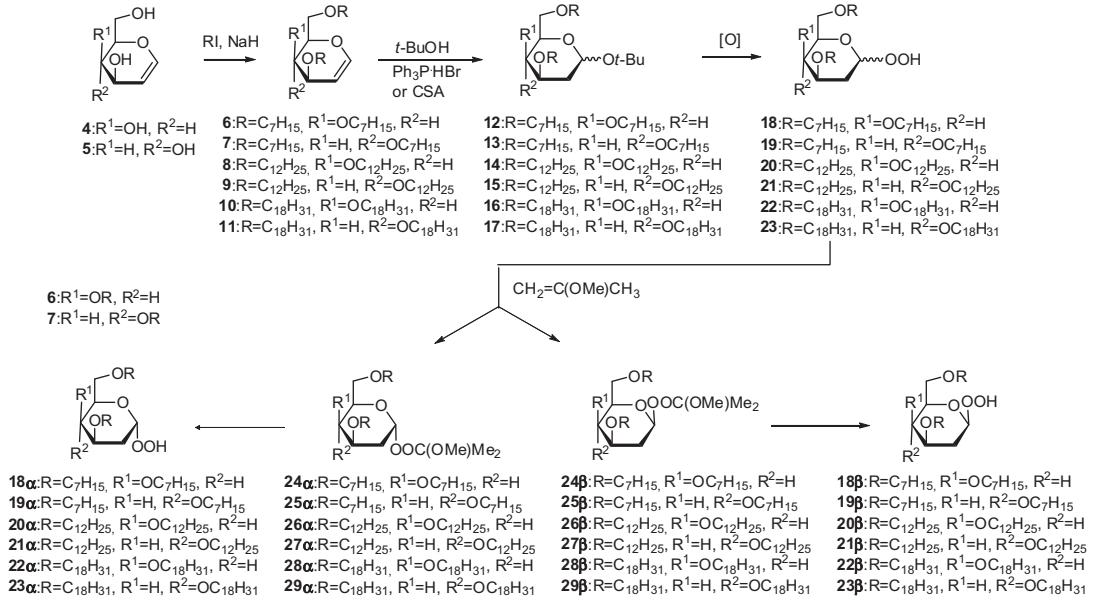
For the present study we set out to synthesize glycosyl hydroperoxides derived from 2-deoxy-D-glucose, 2-deoxy-D-galactose and 2-deoxy-D-ribose using methodologies recently described by us.

2.1. Alkylation

The alkylation of D-galactal **4** and D-glucal **5** was performed using a standard alkylation procedure with appropriate alkyl iodides in the presence of NaH to afford compounds **6**–**11**. These were subsequently transformed into corresponding *tert*-butyl tri-*O*-alkyl-2-deoxy-D-*arabino*- and D-*lyxo*-hexopyranosides (**12**–**17**) by the addition of *tert*-butanol in the presence of a Ph_3P/HBr complex following the methodology of Mioskowski.[8] The oxidation of glycosides **12**–**17** was performed using two procedures described by us previously. In one (Method A) H_2O_2 in *tert*-butanol (ca. 3 M, Milas solution) and in the other (Method B) H_2O_2 in ether (ca. 2 M) was used. As a rule, mixtures of anomeric hydroperoxides **18**–**23** were formed. The ratios of α and β anomers were estimated using the integration of the signals of anomeric protons in the ^1H NMR spectra of the crude reaction products. Overall, the oxidation of glycosides of *lyxo* configuration **12**, **14**, **16** was more selective than that of their *arabino* counterparts **13**, **15**, **17**. Additionally, it was observed that ethereal H_2O_2 gave over 90% α-selectivity in the *lyxo* series and also slightly better selectivity in the *arabino* series. The proportion of the products in the reported reactions is given in Table 1.

Mixtures of anomers which were poorly or not at all separable chromatographically were reacted with 2-methoxypropene to form mixed peroxides, usually possible to fully separate by chromatography. No less important is the fact that peroxides are more stable than hydroperoxides, which should be stored in the cold. Separation of peroxides **24**–**29** into pure anomers followed by acid hydrolysis provided corresponding hydroperoxides **18α**–**23α** and **18β**–**23β** (Scheme 1).

The same methodology was applied to glycosyl hydroperoxides derived from 2-deoxy-D-pyranose. Glycal **30** was alkylated with heptyl bromide using the standard method to afford **31**, which was subsequently transformed into *tert*-butyl glycoside **32** and



Scheme 1.