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Endoperoxide polyketides from a Chinese *Plakortis simplex*: Further evidence of the impact of stereochemistry on antimalarial activity of simple 1,2-dioxanes

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ABSTRACT

Chemical investigation of the organic extract obtained from the sponge *Plakortis simplex* collected in the South China Sea afforded five new polyketide endoperoxides (**2** and **4**–**7**), along with two known analogues (**1** and **3**). The stereostructures of these metabolites have been deduced on the basis of spectroscopic analysis and chemical conversion. The isolated endoperoxide derivatives have been tested for their in vitro antimalarial activity against *Plasmodium falciparum* strains, showing IC_{50} values in the low micromolar range. The structure–activity relationships were analyzed by means of a detailed computational investigation and rationalized in the light of the mechanism of action proposed for this class of simple antimalarials. The relative orientation of the atoms involved in the putative radical generation and transfer reaction was demonstrated to have a great impact on the antimalarial activity. The resulting 3D pharmacophoric model can be a useful guide to design simple and effective antimalarial lead compounds belonging to the class of 1,2-dioxanes.

1. Introduction

Malaria continues to plague a large part of the world population, with a special incidence in the sub-Saharan Africa, where about 90% of cases and deaths occur.[1] The introduction and nowadays wide availability of artemisinin-based combination therapies (ACTs), together with the mass use of insecticide impregnated bed nets, can be recognized as important reasons for the significant decrease in the number of yearly deaths, about 25–30%, reached over the last decade. However, the current estimated 660,000 deaths and more than 200 millions of infection cases per year still cannot be acceptable. In addition, the emergence of artemisinin-resistant *Plasmodium falciparum* strains,[2] whose presence has been detected in the Greater Mekong sub-region (Cambodia, Myanmar, Thailand and Vietnam), is raising severe concerns. The diffusion of resistance may create a tremendous therapeutic void, especially if resistant strains should extend to the sub-Saharan Africa countries. Thus, there is an urgent need to find, as soon as possible, viable alternatives to the existing therapeutic options.

In the last 10 years we have been carrying out a systematic investigation of marine invertebrates, with particular regard to Porifera, in order to exploit this attractive source of chemodiversity to find new antimalarial lead compounds.[3] In this context, we have demonstrated that marine sponges belonging to the genus *Plakortis* (family Plakinidae) constitute a prolific source of simple endoperoxide derivatives, often endowed with a promising antimalarial activity.[3]

The plausible, although not yet rigorously demonstrated, polyketide biosynthetic origin of this class of compounds offers an efficient rationalization of the large structural variability found. Indeed, the architecture of the several different carbon skeletons can be predicted on the basis of the different ketide elongation units (acetate, propionate or even butyrate) employed in the assembling steps, while an additional degree of structural variability is introduced by the subsequent functionalization, for example,

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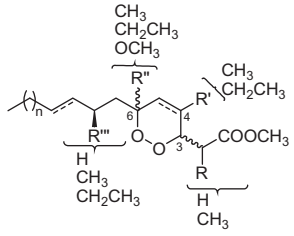

Figure 1. Schematic structure of polyketide endoperoxides from *Plakortis* sponges.

the presence of double bonds within the ring ($\Delta^{4,5}$) or in the alkyl side chain. A general structure for *Plakortis* polyketide 1,2-dioxanes is reported in Figure 1. The configuration at the two stereogenic centers C-3 and C-6 has not been specified because different configurations have been detected at these centers, even in the same organism (see below).

This structural diversity is of considerable interest because, in many cases, it has been strictly correlated to a wide variability in the biological activity. A remarkable example is given by plakortin (R = H, R′ = β-CH_2CH_3, R″ = α-CH_3, R‴ = CH_2CH_3, n = 1, see also Table 3), obtained from a Caribbean *Plakortis simplex*, active against chloroquine-resistant strains of *P. falciparum* in the high nanomolar range, with no cellular toxicity.[4] Evaluation of the antimalarial activity of natural analogues[5,6] and semi-synthetic derivatives[7] of plakortin, interpreted in the light of detailed computational calculations, allowed us to postulate a mechanism for the antimalarial action of this family of simple 1,2-dioxanes.[8] This mechanism, inspired by results obtained with artemisinin and other endoperoxides,[9] is based on the interaction between the endoperoxide oxygen atoms and the iron(II) of heme proteins. This results in the generation of toxic radical species (ROS), starting with the formation of an O-centered radical, immediately followed by an intramolecular rearrangement yielding a C-centered radical, located on the 'western' alkyl side chain (Fig. 2).[8] We have also demonstrated that, in the case of the peroxyketal derivatives manadoperoxides (R″ = OCH_3), obtained from an Indonesian *Plakortis* specimen,[10] the different relative configuration and the presence of a 6-methoxy substitution hinder the accessibility of the reactive iron species to the endoperoxide oxygen atoms, resulting in a very moderate antimalarial activity.[10] Conversely, manadoperoxides exhibited a very potent activity against another protozoan species (*Trypanosoma brucei rhodesiense*),[11] an activity that proved to be strongly dependent from the structure of R and R′ (R = CH_3, R′ = H: no activity; R = H, R′ = CH_3: potent activity).

In the frame of this screening, we recently had the opportunity to investigate the organic extract of a *Plakortis simplex* specimen collected in the South China Sea. Analysis of the endoperoxide fraction of this extract afforded five new polyketide endoperoxides (**2** and **4**–**7**), along with two known analogues (**1** and **3**) (Fig. 3), and in this paper we report details about their isolation, stereostructural characterization, and antimalarial activity. Since our pharmacophoric model for antimalarial 1,2-dioxanes had been designed utilizing compounds embedding a saturated heterocyclic ring, the isolation of 1,2-diox-4-enes (**1**–**4**) offered us an interesting opportunity to further extend the structure–activity relationships and refine our knowledge on the mechanism of action of this class of simple antimalarials.

2. Results and discussion

2.1. Chemistry

The sponge *Plakortis simplex*, collected off the Xisha Islands in the South China Sea, was exhaustively extracted with methanol and the obtained residue was successively extracted with *n*-hexane, CH_2Cl_2, EtOAc and BuOH. The CH_2Cl_2 phase was subjected to repeated column and HPLC chromatography over silica gel to afford two known polyketide 1,2-dioxanes (**1** and **3**) along with five new analogues (**2** and **4**–**7**) in the pure state.

Table 1
^{1}H (500 MHz) NMR data of compounds **4**–**7** in $CDCl_3$

Pos.	**4** δ_H, mult. (J in Hz)	**5** δ_H, mult. (J in Hz)	**6** δ_H, mult. (J in Hz)	**7** δ_H, mult. (J in Hz)
2a	2.90, dd (15.9, 9.5)	3.01, dd (15.6, 9.5)	2.65, dd (15.7, 3.0)	2.65, dd (15.7, 3.0)
2b	2.57, dd (15.9, 3.0)	2.38, dd (15.6, 3.8)	2.37, dd (15.7, 9.3)	2.37, dd (15.7, 9.3)
3	4.62, dd (9.5, 3.0)	4.50, ddd (9.5, 4.0, 3.8)	4.13, ddd (10.4, 9.3, 3.0)	4.13, ddd (10.4, 9.3, 3.0)
4	—	2.19, m	1.69, m	1.69, m
5a	5.46, s	1.96, dd (14.5, 6.3)	1.71, dd (14.4, 4.4)	1.71, dd (14.4, 4.4)
5b		1.38, dd (14.5, 4.2)	1.27[a]	1.27[a]
7a	1.46[a]	2.18[a]	1.36[a]	1.36[a]
7b		1.27[a]	1.31[a]	1.31[a]
8	1.17[a]	1.50[a]	1.50[a]	1.52[a]
9	1.32[a]	1.27[a]	1.27[a]	1.07[a]
10	1.27[a]	1.27[a]	1.27[a]	1.39
11	0.88, t (7.0)	1.28[a]	1.28[a]	1.27[a]
				1.08
12	2.01, m	0.89, t (7.0)	0.89, t (7.0)	1.28[a]
13	1.07, t (7.0)	1.34 [a]	1.34 [a]	1.28 [a]
		1.58 [a]	1.58 [a]	
14	1.33, s	0.82, t (7.0)	0.82, t (7.0)	0.89, t (7.0)
15a	1.48[a]	1.40[a]	1.40[a]	1.46[a]
15b	1.28[a]	1.52[a]	1.52[a]	1.09[a]
16	0.90, t	0.87, t (7.0)	0.87, t (7.0)	0.90, t (7.0)
17	—	1.21[a]	1.21[a]	1.33, s
		1.15[a]	1.15[a]	
18	—	0.92, t (7.0)	0.92, t (7.0)	1.45[a]
				1.21[a]
19				0.81, t (7.0)
20				0.83, d (6.8)
1-OMe	3.73, s	3.71, s	3.71, s	3.69, s

[a] Overlapped with other signals.