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Novel endoperoxides: Synthesis and activity against *Candida* species

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Abstract—Fifteen new endoperoxides have been synthesised and tested for activity against pathogenic *Candida* species. These endoperoxides can be prepared in high yields, in one to three steps, from inexpensive starting materials. Despite chemical and structural similarities, their inhibitory activity against *Candida* growth varied greatly from one endoperoxide to another, and one species to another. This study of susceptibility to endoperoxide compounds presented here may lead to the development of potent new antifungal agents.

Endoperoxides are a remarkable class of organic peroxides, having significant chemical and biochemical properties.[1] One endoperoxide of particular importance is the plant-derived drug, artemisinin (Fig. 1), which has been shown to be a potent and fast-acting antimalarial drug.[2,3] More recently, the activity of artemisinin and derivatives against other tropical parasites[4] and various cancer cells[5,6] has been demonstrated. The high cost of artemisinin has led to searches for synthetic endoperoxides with antimalarial activity.[7] We have made similar searches and produced a series of endoperoxides with weak antimalarial activity.[8] In this study, we investigated these compounds for activity against three clinically important *Candida* species.

The occurrence of life-threatening fungal infections is increasing worldwide.[9–12] The most common fungal infections of humans are caused by *Candida* yeast.[13] Of the antifungals currently available to suppress *Candida* growth, none satisfy the medical world completely.[14] Their limitations include weak potency, high cost of development, host toxicity, limited spectrum of efficacy and deleterious drug–drug interactions.[15] In addition, the emergence of drug-resistant isolates continues to increase.[16] Therefore, there is an acute need for the development of novel antifungal compounds with divergent mechanisms of action, appropriate pharmaceutical properties, with high potency and broad spectrum of activity.

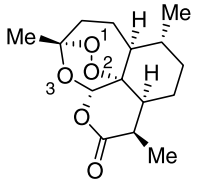

Figure 1. Artemisinin.

A robust synthetic procedure for the ready construction of endoperoxides of types **4** and epoxy-endoperoxides (**5** and **6**) has been developed according to the generalised scheme shown in Figure 2.[8] Key features include a cycloaddition of a singlet oxygen with the appropriate 1,3-butadiene (**3a**–**e**) to generate the core of the endoperoxide ring and form compounds of type **4a**–**e**. Further oxidation with *m*-CPBA at ambient temperature produces the epoxy-endoperoxides **5** and **6**.[8,17] This latter conversion produces, from a single precursor, both epoxy-endoperoxides **5** and **6**, which have the epoxide oxygen atom either on the opposite face (compound **5**) or the same face (compound **6**) to the alkyl or aryl substituents. Consequently, two unique versions of these new pro-drugs are readily available for examining structure–activity relationships. Furthermore, our previous studies[17,18] reported that exposure

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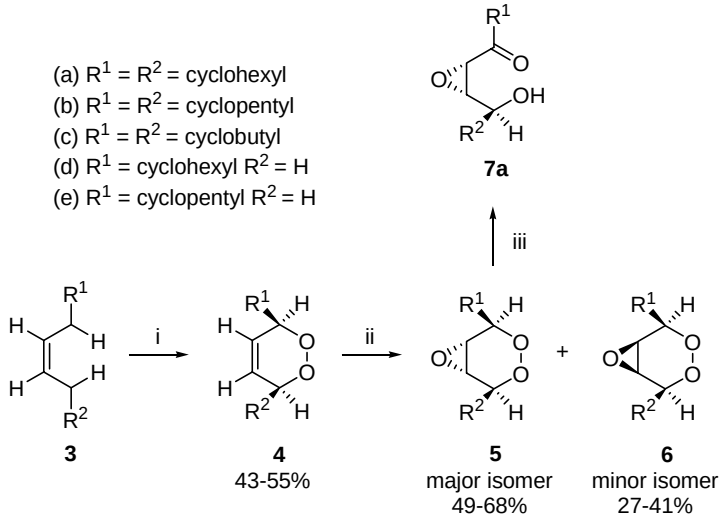

Figure 2. Scheme for synthesis of novel endoperoxides. Reagents and conditions: (i) O_2, Rose Bengal bis(triethylammonium) salt, *hv*, CH_2Cl_2, 7 h, 5 °C; (ii) *m*-CPBA, CH_2Cl_2, 25 °C; (iii) Co(II)salen or NEt_3, CH_2Cl_2, 25 °C.

of epoxy-endoperoxides (**5** and **6**) to catalytic amounts of cobalt(II) salen complexes resulted in a clean free radical rearrangement resulting in the formation of the potential downstream product (**7**). Subsequently, one of these ring-opened products (**7a**) was purified in order to evaluate its activity.

The inhibitory activity of amphotericin B, ketoconazole and our endoperoxide compounds was assayed against *Candida albicans*, *Candida krusei* and *Candida tropicalis* (Table 1).[19] Growth of *Candida* species was inhibited by all our compounds to greater or lesser degrees. The activity of individual compounds generally varied with species. Amphotericin B was the most potent compound tested, but some of our endoperoxides had activities comparable with that of ketoconazole. In particular, compound **4d** displayed high growth inhibitory activity to *C. albicans*, *C. krusei* and *C. tropicalis*.

Table 1. Growth inhibition of some *Candida* species by endoperoxide compounds and reference drugs

Compound	IC_{50} (μM)		
	Candida albicans	*Candida tropicalis*	*Candida krusei*
Amphotericin B	<0.5	0.1–0.4	0.2–0.4
Ketoconazole	250–500	12–47	100–200
Nystatin	250–500	100–200	100–200
4a	>1000	200–400	400–800
5a	100–200	200–400	>1000
6a	100–200	25–50	400–800
7a	250–500	63–125	500–1000
4b	250–500	125–250	>1000
5b	125–250	125–250	63–125
6b	250–500	125–250	125–250
4c	125–250	125–250	250–500
5c	250–500	500–1000	500–1000
6c	250–500	250–500	500–1000
4d	9.5–37	40–80	75–150
5d	250–500	125–250	500–1000
4e	500–1000	250–500	>1000
5e	500–1000	250–500	500–1000
6e	250–500	75–150	300–600

The work presented here describes the examination of a series of related endoperoxide compounds plus a downstream derivative for their potential activity as antifungal compounds.

For all compounds, the route for construction was robust, safe, inexpensive and could be performed on a large scale. Many of the endoperoxides in this series not only had the endoperoxide linkage but also had a third oxygen atom (forming the epoxide group), which is positioned in an environment similar to that of the ether oxygen atom within artemisinin. In addition, cobalt catalysis was used to induce ring-opening of the endoperoxides, providing a synthetic route to the construction of isomeric ring-opened compounds with potential use as pro-drugs. This free radical mechanism to open the ring is not an unlikely fate of endoperoxides in the body due to the presence of a relatively weak and easily cleaved oxygen–oxygen bond. For example, the prostaglandin endoperoxide exists as a short-lived intermediate in the body.[20] The synthesis of ring-opened analogues allowed the possible downstream products to be tested for activity. For example, it appears that epoxide **7a**, a downstream product for epoxy-endoperoxide **5a**, has a higher activity against *C. krusei* and *C. tropicalis* than its parent compound. While the apparent minimum pharmacophore for biological activity of most of the endoperoxides is the endoperoxide ring, activity was slightly increased for compound **7a** (ring-open structure).

A number of endoperoxide compounds showed inhibitory activity against the pathogenic yeast *C. krusei*. This is of notable importance since *C. krusei* is resistant to fluconazole and many other antifungal agents.[21] It has been hypothesised that the extensive use of fluconazole is responsible for the changing epidemiology of fungal infections, leading to the emergence of drug-resistant

strains such as *C. krusei*; however, a causal relationship has not yet been established and the theory remains controversial.[22] Nevertheless, the activity of the endoperoxides described here against *C. krusei* demonstrates their potential utility as novel antifungals for drug-resistant non-albicans *Candida* species.

Research aimed at assessing the clinical usefulness of novel drugs depends critically on in vitro toxicity assays. Some of the endoperoxides described here have high haemolytic activity,[8] suggesting that potential problems with haemolysis may result from internal applications. However, the high haemolytic potential of some endoperoxides may not be a major toxic liability when they are to be used to treat superficial fungal infections. A distinct pattern implicating the presence or absence of certain structural features in haemolytic activity has been reported.[8] Modification of these haemolysis-causing structural features should allow an improvement to the overall therapeutic profile of these endoperoxides by reducing their haemolytic activity.

As part of continuing studies, we propose to further improve the efficacy of these endoperoxides by adding substituents onto the cyclic ring systems. In the case of the endoperoxide artemisinin, addition of an amino side chain has been shown to improve efficacy,[23] presumably by enhancing drug uptake. If the efficacy of the endoperoxides described here can be improved and effective in vivo, it would provide a much-needed alternative for treating fungal infections. The endoperoxide compounds described in this work are structurally unrelated to any other antifungals in clinical use and provide an account of the utility of endoperoxides as biologically active agents.

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