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Design of endoperoxides with anti-*Candida* activity

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Abstract—Broad antifungal structure–activity relationships governing epoxy-endoperoxides **2** and **3** and their parent endoperoxides **1** are reported. Their inhibitory activity against *Candida albicans* in conjunction with hemolytic activity and/or growth inhibition of cultured mammalian cells are reported. This information provided guidance for the further development of endoperoxide and epoxy-endoperoxides as topical antifungal agents.
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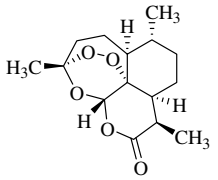
1. Introduction

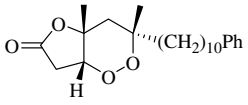
There is an ever-increasing number of peroxide-containing natural products being isolated, with nearly all showing interesting and important biological activities worthy of investigation, Figure 1.[1]

None has had more interest than leading antimalarial natural product artemisinin:[2] the importance of this compound and its expensive production has been the driving force behind an incredible number of analogue syntheses.[3] More recently endoperoxides exhibiting antifungal activity have been isolated from marine sponges of the species *Plakortis*, Figure 1,[4] however a general synthesis of endoperoxides of this structure is yet to be reported.[5] Fungal infections in humans due to aging populations and an increased number of medically induced immunodepressed patients, coupled with relatively low activities of current drugs, are becoming an issue requiring urgent attention. Primarily the source of infection is *Candida* yeast strains and current drugs are limited in their spectrum of efficacy and in many cases display host toxicity and adverse drug–drug interactions.[6] Drug resistance is an emerging trend in virtually all treatments of disease and *Candida* is no exception.[7] New antifungals addressing these issues are needed. One avenue is to explore novel chemical moieties with action through divergent mechanisms; high potency and broad-spectrum activity are also of paramount importance.

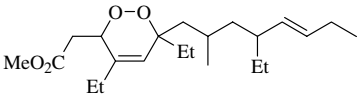
We were inspired, whilst evaluating epoxy-endoperoxides as antimalarials,[8] to investigate the potential of these compounds as antifungals.[9] Our study highlighted a robust, concise and inexpensive synthesis of endoperoxides which in some cases demonstrated better broad-spectrum antifungal activity than two of the current drugs on the market. Importantly, some endoperoxide candidates showed good activity against *Candida krusei*,



Artemisinin: potent commercial antimalarial drug



6-epiplakortolide E: potent cytotoxicity to various cancer lines.



***Plakortis* isolate**: micromolar active antifungal.

Figure 1. Bioactive endoperoxide natural products.

Keywords: Antifungal; Endoperoxide; *Candida*; Hemolytic activity; Artemisinin; Growth inhibition.
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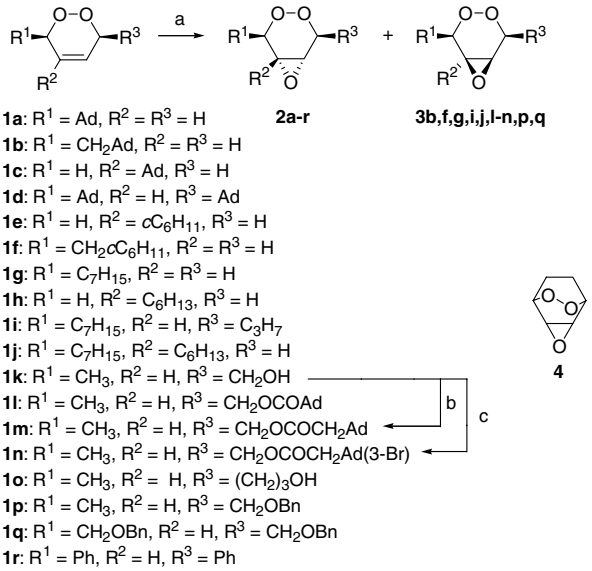
a strain resistant to Fluconazole and other commercial antifungal agents.[10] Prompted by the promising results of this limited study, we now present the synthesis and antifungal activity of a large range of epoxy-endoperoxides and their parent endoperoxides.

2. Results and discussion

Endoperoxides **1a**–**r** were synthesized from the appropriate 1,3-butadiene through photosensitized cycloaddition of singlet oxygen.[11] Epoxidation was achieved in good yield through treatment of endoperoxides with *m*-chloroperbenzoic acid,[12] generating isomers **2** and **3**. Endoperoxides **1m** and **1n** were synthesized from **1k** through DCC coupling with the appropriate carboxylic acid.[13] Tricyclic endoperoxide **4** was synthesized[14] and evaluated for antifungal activities as a mixture of isomers Scheme 1.

Generally a mono-substituted alkyl chain or cycloalkyl grouping at the R^1 position resulted in good antifungal activity (**1a**, **1b**, **1f**, and **1g**), with individual series (parent endoperoxide and epoxy-endoperoxides) **1b**, **2b**, **3b**, etc., appearing in the same IC_{50} brackets, Table 1. Notably *cis* or *trans* orientation of the epoxide moiety with respect to the R^1 and R^3 groups made no difference to anti-*Candida* activity in these series.

Deviation from the general trend (where epoxides and parent endoperoxides have similar activity) was observed with endoperoxides containing tethered hydroxyl (**1k** and **1o**), ester (**1l–n**) or ether groups (**1p** and **1q**) at the R^3 position, with the epoxides generally showing much poorer activity than the parent endoperoxides, compare compounds **1k** and **2k**, for example. Additionally it was noted that within each series the *cis* epoxides showed equal to or better activity than the corresponding *trans* epoxide. However, as with the series of the alkyl and cycloalkyl-substituted endoperoxides, their activities varied greatly, for example **1q** is in the 16–31 μM range, while **1p** was in the 250–500 μM range.



Scheme 1. Reagents: (a) MCPBA; (b) DCC, $AdCH_2CO_2H$, DMAP; (c) DCC, 3-$BrAdCH_2CO_2H$, DMAP.

Table 1. Growth inhibition of *Candida albicans* exposed to endoperoxide compounds and commercial drugs

Compound	IC_{50} (μM)
Amphotericin B	<0.5
Ketoconazole	250–500
Nystatin	250–500
1a	31–63
2a	31–63
1b	125–250
2b	125–250
3b	125–250
1c	63–125
2c	125–250
1d	>1000
2d	>1000
1e	63–125
2e	500–1000
1f	125–250
2f	125–250
3f	125–250
1g	63–125
2g	63–125
3g	63–125
1h	250–500
2h	500–1000
1i	250–500
2i	500–1000
3i	500–1000
1j	250–500
2j	>1000
3j	>1000
1k	16–31
2k	>1000
1l	125–250
2l	250–500
3l	125–250
1m	125–250
2m	>1000
3m	250–500
1n	250–500
2n	>1000
3n	>1000
1o	250–500
2o	>1000
1p	250–500
2p	125–250
3p	63–125
1q	16–31
2q	250–500
3q	63–125
1r	31–63
2r	500–1000
4	16–31

In order to broaden the scope of functionality, phenyl-substituted endoperoxide **1r** and tricyclic epoxy-endoperoxide **4** were also evaluated as antifungal agents. With their activity in the low-micromolar range, they also showed potential as building blocks for derivatisation, in order to improve antifungal activity.

Table 2. Hemolytic activities of selected novel endoperoxides

Compound	IC_{50} (μM)	Hemolysis [a] (%)
1a	31–63	100
2a	31–63	41
1g	63–125	60
2g	63–125	33
1h	250–500	61
2h	500–1000	31
1i	250–500	86
2i	500–1000	45
3i	500–1000	25
1k	16–31	0
1q	16–31	2
3q	63–125	1
4	16–31	0

[a] Percent hemolysis of erythrocytes induced by 1 mM compound.

Compounds **1a**, **2a**, **1c**, **1e**, **1g**, **2g**, **3g**, **1k**, **3p**, **1q**, **3q**, **1r**, and **4** showed promising growth inhibition activity against *C. albicans* when compared to those of commercially available antifungal drugs, Ketoconazole and Nystatin. The activity of these endoperoxides is still however an order of magnitude higher than that of Amphotericin B.

A selection of the more promising compounds were evaluated for hemolytic activity at the upper concentration limit of the IC_{50} assay (1 mM) to ascertain their potential as serum administered drugs, Table 2. As previously observed the epoxides showed much less hemolysis activity than their parent endoperoxides, however,[8] the epoxides showed poorer antifungal activity than their parent endoperoxide (vide supra). Evaluating the hemolysis data against the IC_{50} values, it appeared that compounds **1a**, **2a**, **1g**, **2g**, **1k**, **1q**, **3q**, and **4** would be suitable for further investigation as their IC_{50} values are much lower than the 1 mM concentration used during the hemolysis assay. Compounds **1k**, **1q**, and **4** were particularly promising, showing no hemolysis at this concentration in conjunction with a low IC_{50} value. It is suspected that high hemolytic activity is associated with the extended hydrocarbon chains, which are able to insert into the lipid bilayer.[8]

A mammalian cell-toxicity assay was performed on compounds **1k**, **1q**, **3q**, **1r**, and **4**, which demonstrated relatively low IC_{50} values. This test was used to evaluate mammalian cell growth inhibition characteristics of potential lead compounds, which were compared to the commercial drugs Fluconazole and Amphotericin B, Figure 2.

Compounds **1q**, **1r**, and **4** showed significant growth inhibitory activity levels at 0.1 mM and 1.0 mM while compounds **1k** and **3q** were inhibitory at 1.0 mM but only moderately toxic at 0.1 mM with **1k** showing the best profile. The solvent (ethanol) was not inhibitory at levels of up to 5% and fluconazole was not inhibitory at 1 mM. Amphotericin B was also used for comparison and was not growth inhibitory at 0.1 mM. For the testing of Amphotericin B at a level of 1.0 mM, the cells were exposed to 5% DMSO, a level that caused about 50% inhibition of cell growth. The Amphotericin B caused no additional growth inhibition compared to solvent alone.

In conclusion, a range of novel endoperoxides and their epoxy derivatives were assessed for antifungal activity on *C. albicans*. A range of activities were observed with some very promising results enabling determination of broad structure activity relationships. Some of the more promising candidates were evaluated for hemolytic activity and mammalian cell toxicity. Compounds containing alkyl substituents with corresponding good IC_{50} values tended to be hemolytic. Diaryl endoperoxide **1r** and tricyclic epoxy endoperoxide **4** were also evaluated to expand our knowledge of antifungal activities of peroxides. Although good IC_{50} levels were observed, both compounds were found to be toxic in the mammalian cell toxicity assay.

Compound **1k** was found to have good antifungal activity coupled with 0% hemolysis and only moderate toxicity to mammalian cells. We feel that this compound and possibly derivatives thereof may be good candidates for further evaluation. Moreover other compounds tested may represent good candidates for topical applications as hemolytic activity is not necessarily detrimental to being a good candidate in this case.

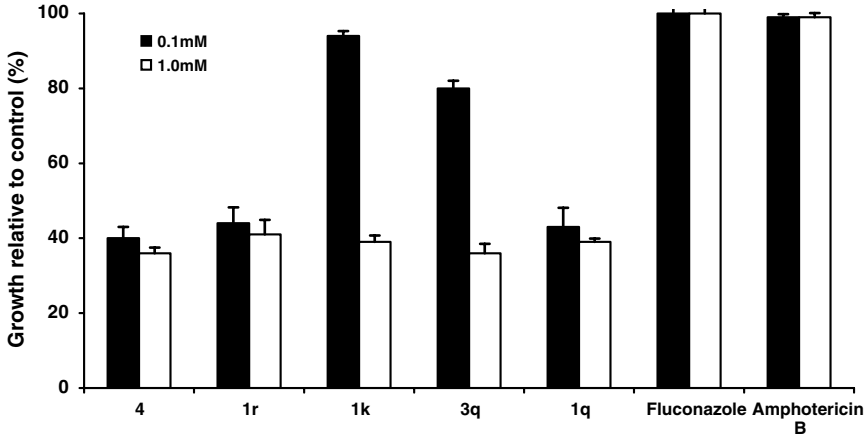


Figure 2. Mammalian cell toxicity of selected endoperoxides (means ± SE).

3. Experimental

3.1. General methods

Solvents were dried by appropriate methods wherever needed. Thin-layer chromatography (TLC) used aluminium sheets coated with silica gel 60 F_{254} (40 × 80 mm) and visualized under 254 nm light, or developed in vanillin or permanganate dip. Flash chromatography was conducted using silica gel 60 of particle size 0.040–0.063 mm. Melting points were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution at either 300 or 600 MHz instrument, TMS (0 ppm) and $CDCl_3$ (77.0 ppm) were used as internal standards. All yields reported refer to isolated material judged to be homogeneous by TLC and NMR spectroscopy.

3.2. General procedure for the synthesis of endoperoxides 1a-k, 1n-r[11]

A solution of the appropriate parent 1,3-butadiene in CH_2Cl_2 (30 mL/g) was photolysed with 3 × 500 W halogen lamps in the presence of Rose Bengal bis(triethylammonium) salt (30 mg/g of 1,2-butadiene) through which was bubbled a constant stream of oxygen for 6 h. The reaction was performed in a Pyrex flask fitted with an external cooling jacket. The solution was concentrated in vacuo and the resulting residue purified by flash chromatography. For known endoperoxides see the following: compounds **1a**,[8] **1c**,[15] **1g**,[16] **1h**,[15] **1i**,[8] **1k**,[16] **1l**,[8] **1r**[11], **1e**,[15] **1f**,[8] and **1o**.[8]

3.3. ± 3-(1-Adamantylmethyl)-3,6-dihydro-1,2-dioxine (1b)

Yield: 234 mg, 37%; colourless oil; R_f 0.49 (1:19 ether/hexane); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.23 (dd, J = 15.0, 3.2 Hz, 1H), 1.43 (dd, J = 15.0, 8.4 Hz, 1H), 1.74–1.56 (m, 12H), 1.96 (br s, 3H), 4.42 (dddd, J = 16.4, 2.2, 2.2, 2.2 Hz, 1H), 4.62 (dddd, J = 16.4, 2.2, 2.2, 2.2 Hz, 1H), 4.85–4.76 (m, 1H), 5.81 (dddd, J = 10.4, 2.2, 2.2, 2.2 Hz, 1H), 5.92 (dddd, J = 10.4, 2.2, 2.2, 2.2 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.6, 32.0, 36.9, 42.7, 47.0, 69.5, 75.3, 123.4, 129.8; IR (neat): 1451, 1106, 1039, 996, 731 cm^{-1}; MS m/z (+EI): 234 (M^+, 8), 204 (16), 203 (53),177 (45), 149 (33), 135 (100); HRMS (+EI) $(M)^+$ found 234.16097; $(M)^+$ calcd for $C_{15}H_{22}O_2$ 234.16198.

3.4. ± (3*R*,6*S*)-3,6-Diadamantyl-3,6-dihydro-1,2-dioxine (1d)

Yield: 43 mg, 74%; colourless solid; mp 109–111 °C; R_f 0.57 (1:19 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60–1.73 (m, 24), 1.98 (br s, 6H), 3.92 (s, 2H), 6.07 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.4, 37.1 (masked carbon), 38.7, 85.8, 125.3; IR (nujol): 1344, 1072, 1027, 995, 756 cm^{-1}; MS m/z (+EI): 354 (M^+, 5), 336 (56), 279 (16), 219 (24), 135 (100); HRMS (+EI) $(M+Na)^+$ found 377.2458; $(M+Na)^+$ calcd for $C_{24}H_{34}O_2Na$ 377.2457.

3.5. 3-Heptyl-4-hexyl-3,6-dihydro-1,2-dioxine (1j)

Yield: 584 mg, 34%; colourless oil; R_f 0.25 (1:19 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86–0.90 (m, 6H), 1.28–1.72, (m, 20H), 2.00 (t, J = 7.2 Hz, 2H), 4.27–4.31 (m, 1H), 4.40–4.47 (m, 1H), 4.56–4.63 (m, 1H), 5.59 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 22.59, 22.63, 25.6, 27.3, 29.0, 29.2, 29.5, 31.1, 31.7, 31.8, 32.2, 70.0, 81.1, 117.3, 139.0; IR (neat): 1671, 1348, 1059 cm^{-1}; MS m/z (+EI): 268 (M^+, 5), 251 (44), 236 (16), 153 (100), 127 (27), 57 (56); HRMS (+EI) $(M+Na)^+$ found 291.2296; $(M+Na)^+$ calcd for $C_{17}H_{32}O_2Na$ 291.2300.

3.6. ± (3*R*,6*R*)-3-[(Benzyloxy)methyl]-6-methyl-3,6-dihydro- 1,2-dioxine (1p)

Yield: 2.27 g, 65%; colourless oil; R_f 0.4 (1:9 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (d, J = 6.6 Hz, 3H), 3.57 (dd, J = 10.8, 3.9 Hz, 1H), 3.78 (dd, J = 10.8, 7.5 Hz, 1H), 4.54–4.70 (m, 3H), 4.76 (qq, J = 6.6, 1.5 Hz, 1H), 5.87 (ddd, J = 10.5, 3.0, 1.5 Hz, 1H), 5.94 (ddd, J = 10.5, 1.5, 1.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.8, 70.3, 73.5, 74.3, 78.3, 123.9, 127.6, 127.7, 128.3, 131.0, 138.2; IR (neat): 1496, 1453, 1366, 1091, 737, 720, 698 cm^{-1}; MS m/z (+EI): 221 ($[M+H]^+$, 17), 122 (23), 105 (57), 91 (100); HRMS (+EI) $(M)^+$ found 220.1096; $(M)^+$ calcd for $C_{13}H_{16}O_3$ 220.1099.

3.7. ± (3*R*,6*S*)-3,6-Di[(benzyloxy)methyl]-3,6-dihydro-1,2- dioxine (1q)

Yield: 908 mg, 28%; colourless oil; R_f 0.48 (1:4 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.45 (dd, J = 10.8, 6.6 Hz, 2H), 3.68 (dd, J = 10.8, 7.2 Hz, 2H), 4.53 (d, J = 12.0 Hz, 2H), 4.62 (d, J = 12.0 Hz, 2H), 4.76–4.79 (m, 2H), 4.97 (m, 2H), 7.25–7.34 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 69.9, 73.4, 78.4, 126.1, 127.6, 128.3, 137.9; IR (neat): 1496, 1453, 1363, 1090 cm^{-1}; MS m/z (+EI): 327 [$(M+H)^+$, 20], 181 (100); HRMS (+EI) $(M+H)^+$ found 327.1603.; $(M+H)^+$ calcd for $C_{20}H_{23}O_4$ 327.1596.

3.8. General procedure for DCC coupling of carboxylic acids to endoperoxide 1k[13]

To a solution of endoperoxide **1k** (1 equiv) and DMAP (cat.) in dichloromethane (30 mL/g of endoperoxide) at 0 °C under nitrogen was added carboxylic acid (1 equiv) followed by DCC (1 equiv). The mixture was stirred for 5 h after which time the precipitate was filtered off and washed with cold dichloromethane. The volatiles were removed in vacuo and the crude residue purified by column chromatography.

3.9. ± [(3*R*,6*R*)-6-Methyl-3,6-dihydro-1,2-dioxin-3-yl]methyl 2-adamantylacetate (1m)

Yield: 379 mg, 81%; colourless oil; R_f 0.53 (1:4 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1. 22 (d, J = 6.9 Hz, 3H), 1.61–1.72 (m, 12H), 1.97 (br s, 3H), 2.12 (s, 2H), 4.20–4.31 (m, 2H), 4.54–4.60 (m, 1H),

4.79 (qq, J = 6.9, 1.8 Hz, 1H), 5.87 (ddd, J = 9.9, 3.6, 1.8 Hz, 1H), 6.00 (ddd, J = 9.9, 1.8, 1.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.5, 28.6, 32.8, 36.7, 42.4, 48.8, 63.3, 74.3, 76.7, 122.9, 131.9, 171.6; IR (neat): 1734, 1450, 1257, 1137, 1020, 725 cm^{-1}; MS m/z (+EI): 306 (M^+, 3), 289 (22), 177 (19), 135 (100), 95 (29); HRMS (+EI) $(M+Na)^+$ found 329.1740; $(M+Na)^+$ calcd for $C_{18}H_{26}O_4Na$ 329.1729.

3.10. ± [(3*R*,6*R*)-6-Methyl-3,6-dihydro-1,2-dioxin-3-yl]methyl 2-(3-bromoadamantyl)acetate (1n)

Yield: 214 mg, 72%; colourless oil; R_f 0.6 (1:4 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (d, J = 6.6 Hz, 3H), 1.60–1.69 (m, 6H), 2.16–2.33 (m, 10H), 4.21 (dd, J = 12.0, 3.3 Hz, 1H), 4.31 (dd, J = 12.0, 8.1 Hz, 1H), 4.56 (ddddd, J = 8.1, 3.3, 3.6, 1.8, 1.8 Hz, 1H), 4.80 (qq, J = 6.6, 1.8 Hz, 1H), 5.87 (ddd, J = 9.9, 3.6, 1.8 Hz, 1H), 6.00 (ddd, J = 9.9, 1.8, 1.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.5, 32.3, 34.6, 37.2, 40.1, 47.4, 48.3, 53.5, 63.4, 65.0, 74.2, 76.5, 122.6, 132.1, 170.7; IR (neat): 1732, 1681, 1654, 1636, 1449, 1240, 1135, 1022, 815 cm^{-1}; MS m/z (+EI): 385 (M^+, 9), 366 (55), 305 (100), 257 (26), 215 (28), 133 (36), 95 (77); HRMS (+EI) $(M)^+$ found 385.1014; $(M)^+$ calcd for $C_{18}H_{26}O_4Br$ 385.1014.

3.11. General procedure for the synthesis of epoxyendoperoxides 2a–r and 3b, f, g, i, j, l–n, p, q[12]

To a solution of endoperoxide **1a**–**r** (1 equiv) in CH_2Cl_2 (20 mL/g of endoperoxide) was added 70% *m*-chloroperbenzoic acid (2 equiv), the reaction mixture was stirred at ambient temperature until complete by TLC. Dichloromethane was then added and the solution extracted with satd $Na_2S_2O_3$ followed by satd $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered and volatiles removed in vacuo. The crude epoxides were purified by column chromatography. For known epoxyendoperoxides see the following: compounds **2a**,[8] **2c**,[15] **2h**,[15] **2i/3i**,[8] **2l/3l**,[8] **2r**,[12] **2e**,[15] **2f/3f**,[8] **2o**,[8] and **4**.[14]

3.12. ± (1a(*R*/*S*),2*R*,5a(*R*/*S*)-2-(1- Adamantylmethyl)perhydrooxireno[2,3-d][1, 2]dioxine 2b and 3b

Characterised as a mixture. Yield: 207 mg, 97%; colourless solid; R_f 0.24 (1:1 dichloromethane/hexane); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.00–0.88 (m, 2H), 1.48–1.35 (m, 2H), 1.73–1.54 (m, 24H), 1.94 (br s, 6H), 3.21–3.17 (m, 2H), 3.32–3.30 (m, 1H), 3.49–3.46 (m, 1H), 4.28–4.20 (m, 2H), 4.40–433 (m, 2H), 4.52–4.48 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.4, 28.4, 31.4, 31.9, 36.7, 36.7, 42.4, 42.5, 44.1, 45.0, 48.5, 50.8, 52.0, 54.4, 68.9, 69.5, 74.2, 76.0; IR (nujol): 1363, 1106, 1048, 1032, 906, 842 cm^{-1}; MS m/z (+EI): 250 (M^+, 5), 249 (16), 218 (1), 177 (4), 149 (9), 135 (100); Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86; found: C, 72.02; H, 8.64.

3.13. ± (1a*R*,2*S*,5*R*,5a*S*)-2,5-Diadamantylperhydrooxireno[2,3- d][1,2]dioxine (2d)

Yield: 26 mg, 93%; colourless solid; mp 193–194 °C; R_f 0.5 (1:19 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.66–1.77 (m, 24H), 2.02 (br s, 6H), 3.44 (s, 2H), 3.66 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.1, 36.4, 36.9, 38.7, 50.2, 84.6; IR (nujol): 1344, 1268, 1101, 1022, 991, 852 cm^{-1}; MS m/z (+EI): 370 (M^+, 3), 338 (20), 235 (14), 207 (50), 178 (40), 135 (100); HRMS (+EI) $(M+Na)^+$ found 393.2418; $(M+Na)^+$ calcd for $C_{24}H_{24}O_3Na$ 383.1623.

3.14. ± (1a*S*,2*R*,5a*S*)-2-Heptylperhydrooxireno[2,3-d][1, 2]dioxine (2g) and ± (1a*R*,2*R*,5a*R*)-2-heptylperhydrooxireno[2,3-d][1,2]dioxine (3g)

Compound **2g**: yield: 68 mg, 39%; colourless oil; R_f 0.5 (1:19 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J = 6.9 Hz, 3H), 1.28–.73 (m, 12H), 3.26 (dd, J = 4.5, 1.2 Hz, 1H), 3.37 (d t J = 4.5, 0.6 Hz, 1H), 4.28–4.42 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0, 22.6, 24.9, 29.0, 29.4, 30.6, 31.7, 48.7, 53.1, 69.2, 79.2; IR (neat): 11465, 1248, 1042, 914, 733 cm^{-1}; MS m/z (+EI): 200 (M^+, 3), 183 (45), 109 (33), 81 (42), 69 (71), 57 (88), 43 (100); HRMS (+EI) $(M)^+$ found 200.1412; $(M)^+$ calcd for $C_{11}H_{20}O_3$ 200.1412.

Compound **3g**: yield: 30 mg, 17%; colourless oil; R_f 0.45 (1:19 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J = 6.9 Hz, 3H), 1.27–1.51 (m, 10H), 1.69 (q, J = 7.2 Hz, 2H), 3.31 (dd, J = 4.5, 1.8 Hz, 1H), 3.52 (ddd, J = 4.5, 3.6, 1.5 Hz, 1H), 4.27–4.44 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0, 22.6, 25.2, 29.1, 29.5, 29.6, 31.7, 50.6, 50.8, 69.9, 77.9; IR (neat): 1466, 1378, 1247, 1044, 913, 734 cm^{-1}; MS m/z (+EI): 200 (M^+, 1), 183 (100), 127 (98), 109 (47), 83 (52), 57 (83); HRMS (+EI) $(M)^+$ found 200.1413; $(M)^+$ calcd for $C_{11}H_{20}O_3$ 200.1412.

3.15. ± (1a*S*,2*R*,5a*S*)-2-Heptyl-1a-hexylperhydrooxireno[2,3- d][1,2]dioxine (2j) and ± (1a*R*,2*R*,5a*R*)-2-heptyl-1a-hexylperhydrooxireno[2,3-d][1,2]dioxine (3j)

Compound **2j**: yield: 221 mg, 42%; colourless oil; R_f 0.45 (1:9 ether/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86–0.90 (m, 6H), 1.29–1.90 (m, 22H), 3.26 (s, 1H), 4.18 (dd, J = 9.0, 3.6 Hz, 1H), 4.27–4.38 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0, 14.0, 22.5, 22.6, 24.4, 25.7, 28.2, 29.1, 29.2, 29.5, 31.6, 31.8, 32.7, 55.1, 59.5, 70.0, 80.2; IR (neat): 1467, 1378, 1045, 911, 726 cm^{-1}; MS m/z (+EI): 284 (M^+, 4), 267 (25), 157 (63), 113 (100), 109 (80), 85 (90); HRMS (+EI) $(M)^+$ found 284.2351; $(M)^+$ calcd for $C_{17}H_{32}O_3$ 284.2351.

Compound **3j**: yield: 128 mg, 24%; colourless oil; R_f 0.4 (1:9 ether/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86–0.91 (m, 6H), 1.21–1.45 (m, 18H), 1.50–1.63 (m, 2H), 1.84–1.96 (m, 2H), 3.27 (d, J = 3.6 Hz, 1H), 4.08 (br d, J = 10.2 Hz, 1H), 4.21 (ddd, J = 13.5, 3.9, 1.5 Hz, 1H), 4.51 (dd, J = 13.5, 0.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.97, 14.03, 22.5, 22.6, 23.4, 25.8, 29.1, 29.2, 29.3, 29.4, 31.6, 31.76, 31.83, 55.7, 59.0, 69.9, 80.2; IR (neat): 1467, 1378, 1044, 968, 903 cm^{-1}; MS m/z (+EI): 284 (M^+, 4), 267 (21), 253 (66), 237 (51), 199 (36), 185 (46), 157 (100), 139 (33); HRMS (+EI) $(M)^+$ found 284.2352; $(M)^+$ calcd for $C_{17}H_{32}O_3$ 284.2351.

3.16. ± [(1a*R*,2*S*,5*R*,5a*S*)-5-Methylperhydrooxireno[2,3-d][1,2]dioxin-2-yl]methanol (2k)

Yield: 173 mg 66%; colourless oil; R_f 0.2 (2:3 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (d, J = 6.6 Hz, 3H), 2.22 (br s, 1H), 3.28 (dd, J = 4.2, 0.9 Hz, 1H), 3.59 (t, J = 4.2 Hz, 1H), 3.89 (dd, J = 12.0, 4.2 Hz, 1H), 4.11 (dd, J = 12.0, 8.1 Hz, 1H), 4.36 (ddd, J = 8.1, 4.2, 4.2 Hz, 1H), 4.53 (d q, J = 6.6, 0.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.9, 51.1, 51.3, 60.0, 73.6, 77.9; IR (neat): 3419, 1454, 1374, 1252, 1004, 926, 886, 727 cm^{-1}; MS *m*/*z* (+EI): 147 ([M+H]$^+$, 2), 146 (M$^+$, 2), 115 (60), 45 (100); HRMS (+EI) (M)$^+$ found 146.0581; (M)$^+$calcd for $C_6H_{10}O_4$ 146.0579.

3.17. ± [(1a*R*,2*S*,5*R*,5a*S*)-5-Methylperhydrooxireno[2,3-d][1,2]dioxin-2yl]methyl 2-adamantylacetate (2m) and ± [(1a*S*,2*S*,5*R*,5a*R*)-5-methylperhydrooxireno[2,3-d][1,2]dioxin-2yl]methyl 2-adamantylacetate (3m)

Compound **2m**: yield: 133 mg, 47%; colourless oil; R_f 0.35 (dichloromethane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (d, J = 6.6 Hz, 3H), 1.61–1.68 (m, 12H), 1.97 (br s, 3H), 2.12 (s, 2H), 3.25 (dd, J = 4.2, 1.2 Hz, 1H), 3.57 (t, J = 4.2 Hz, 1H), 4.36–4.53 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.9, 28.6, 32.8, 36.7, 42.3, 48.7, 51.30, 51.32, 60.8, 73.6, 75.2, 171.6; IR (neat): 1733, 1450, 1255, 1137, 1026 cm^{-1}; MS *m*/*z* (+EI): 322 (M$^+$, 9), 305 (39), 279 (41), 206 (26), 135 (100); Anal. Calcd for $C_{18}H_{26}O_5$: C, 67.06; H, 8.13; found: C, 66.97; H, 8.16.

Compound **3m**: yield: 57 mg, 20%; colourless oil; R_f 0.29 (dichloromethane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (d, J = 6.9 Hz, 3H), 1.59–1.73 (m, 12H), 1.98 (br s, 3H), 2.13 (s, 2H), 3.24 (dd, J = 4.2, 0.9 Hz, 1H), 3.33 (d, J = 4.2 Hz, 1H), 4.31–4.54 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.9, 28.6, 32.9, 36.7, 42.4, 48.7, 49.4, 53.5, 61.7, 74.8, 76.1, 171.4; IR (neat): 1732, 1452, 1257, 1198, 1137, 1019, 915 cm^{-1}; MS *m*/*z* (+EI): 322 (M$^+$, 19), 405 (30), 279 (34), 220 (82), 135 (100); HRMS (+EI) (M+Na)$^+$ found 345.1669; (M+ Na)$^+$ calcd for $C_{18}H_{26}O_5Na$ 345.1678.

3.18. ± [(1a*R*,2*S*,5*R*,5a*S*)-5-Methylperhydrooxireno[2,3-d][1,2]dioxin-2yl]methyl 2-(3-bromoadamantyl)acetate (2n) and ± [(1a*S*,2*S*,5*R*,5a*R*)-5-methylperhydrooxireno[2,3-d][1,2]dioxin-2yl]methyl 2-(3-bromoadamantyl)acetate (3n)

Compound **2n**: yield: 40 mg, 26%; colourless oil; R_f 0.44 (dichloromethane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (d, J = 6.3 Hz, 3H), 1.59–1.72 (m, 6H), 2.16–2.34 (m, 10H), 3.25 (dd, J = 4.2, 1.2 Hz, 1H), 3.57 (t, J = 4.2 Hz, 1H), 4.42–4.54 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.9, 32.3, 34.6, 37.3, 40.2 (masked carbon), 47.4, 48.3, 51.3, 53.5, 61.0, 65.0, 73.7, 75.0, 170.8; IR (neat): 1735, 1448, 1241, 1164, 1132, 1026 cm^{-1}; MS *m*/*z* (+EI): 401 (M$^+$, <0.1), 321 (100), 303 (12), 175 (32), 147 (66), 133 (47), 91 (74); HRMS (+EI) (M)$^+$ found 401.0963; (M)$^+$ calcd for $C_{18}H_{26}O_5Br$ 401.0964.

Compound **3n**: yield: 18 mg, 12%; colourless oil; R_f 0.39 (dichloromethane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (d, J = 6.9 Hz, 3H), 1.59–1.71 (m, 6H), 2.17–2.34 (m, 10H), 3.26 (dd, J = 4.2, 0.9 Hz, 1H), 3.31 (d, J = 4.2 Hz, 1H), 4.31–4.54 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.9, 32.3, 34.6, 37.3, 40.2, 40.3, 47.4, 48.3, 49.2, 53.49, 53.54, 61.9, 74.8, 76.0, 170.6; IR (neat): 1732, 1454, 1241, 1135, 1017, 923, 815 cm^{-1}; MS *m*/*z* (+EI): 401 (M$^+$, 2), 384 (23), 321 (79), 303 (80), 193 (40), 147 (79), 133 (75), 94 (92), 43 (100); HRMS (+EI) (M)$^+$ found 401.0967; (M)$^+$ calcd for $C_{18}H_{26}O_5Br$ 401.0964.

3.19. ± [(1a*S*,2*R*,5*S*,5a*R*)-5-Methylperhydrooxireno[2,3-d][1,2]dioxin-2-yl]methyl benzyl ether (2p) and ± [(1a*S*, 2*S*,5*R*,5a*R*)-5-methylperhydrooxireno[2,3-d][1,2]dioxin-2-yl]methyl benzyl ether (3p)

Compound **2p**: yield: 585 mg, 55%; colourless oil; R_f 0.15 (1:4 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (d, J = 6.9 Hz, 3H), 3.25 (dd, J = 6.6, 1.8 Hz, 1H), 3.55 (t, J = 6.6 Hz, 1H), 3.81 (dd, J = 15.9, 8.1 Hz, 1H), 3.89 (dd, J = 15.9, 10.2 Hz, 1H), 4.41–4.53 (m, 2H), 4.58 (d, J = 18.0 Hz, 1H), 4.65 (d, J = 18.0 Hz, 1H), 7.26–7.38 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.9, 51.6, 51.7, 67.4, 73.6, 73.7, 76.6, 127.7, 127.8, 128.4, 138.0; IR (neat): 1496, 1453, 1373, 1108, 741 cm^{-1}; MS *m*/*z* (+EI): 236 (M$^+$, 6), 105 (84), 91 (100), 77 (53); HRMS (+EI) (M)$^+$ found 236.1045; (M)$^+$ calcd for $C_{13}H_{16}O_4$ 236.1049.

Compound **3p**: yield: 301 mg, 28%; colourless oil; R_f 0.3 (1:4 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (d, J = 6.9 Hz, 3H), 3.20 (d, J = 4.2 Hz, 1H), 3.41 (d, J = 4.2 Hz, 1H), 3.72 (dd, J = 10.5, 5.4 Hz, 1H), 3.84 (dd, J = 10.5, 5.7 Hz, 1H), 4.43–4.50 (m, 2H), 4.57 (d, J = 12.0 Hz, 1H), 4.61 (d, J = 12.0 Hz, 1H), 7.30–7.37 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.9, 50.0, 53.1, 68.3, 73.6, 74.6, 77.2, 127.6, 127.7, 128.4, 137.6; IR (neat): 1496, 1453, 1370, 1113, 739 cm^{-1}; MS *m*/*z* (+EI): 236 (M$^+$, 1), 105 (48), 91 (100), 77 (24); HRMS (+EI) (M)$^+$ found 236.1047; (M)$^+$ calcd for $C_{13}H_{16}O_4$ 236.1049.

3.20. ± (1a*R*,2*S*,5*R*,5a*S*)-2,5-Di[(benzyloxy)methyl]perhydrooxireno[2,3-d][1, 2]dioxine (2q) and ± (1a*R*,2*R*,5*S*,5a*S*)-2,5-di[(benzyloxy)methyl]perhydrooxireno[2,3-d][1,2]dioxine (3q)

Compound **2q**: yield: 437 mg, 60%; colourless oil; R_f 0.2 (1:4 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.46 (m, 2H), 3.74–3.77 (m, 4H), 4.52–4.67 (m, 6H), 7.26–7.38 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.9, 67.7, 73.7, 77.1, 127.8 (masked carbon), 128.4, 137.8; IR (neat): 1496, 1453, 1365, 1255, 1207, 1114, 1027, 907, 739 cm^{-1}; MS *m*/*z* (+LSI): 342 (M$^+$, 4), 251 (13), 181 (100), 154 (57); HRMS (+EI) (M+H)$^+$ found 343.1545; (M+H)$^+$ calcd for $C_{20}H_{23}O_5$ 343.1545.

Compound **3q**: yield: 169 mg, 23%; colourless oil; R_f 0.3 (1:4 ethylacetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.44 (s, 2H), 3.69 (dd, J = 10.8, 5.4 Hz, 2H), 3.82 (dd, J = 10.8, 5.4 Hz, 2H), 4.51 (t, J = 5.4 Hz, 2H), 4.56 (s,

4H), 7.29–7.39 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.3, 68.2, 73.6, 77.7, 127.7, 127.9, 128.5, 137.6; IR (neat): 1496, 1453, 1364, 1273, 1097, 740 cm^{-1}; MS *m/z* (+EI): 342 (M^+, 1), 251 (48), 91 (100); HRMS (+EI) $(M+H)^+$ found 343.1541; $(M+H)^+$ calcd for $C_{20}H_{23}O_5$ 343.1545.

3.21. Compounds

For testing in vivo stocks of the novel compounds made up in ethanol or dimethyl sulfoxide (DMSO) at concentrations of up to 100 mM. Amphotericin B was purchased from Sigma and fluconazole was a gift from Pfizer. Fluconazole was dissolved in ethanol and Amphotericin B was dissolved in DMSO.

3.22. Yeast assays

Candida albicans strain JRW#5 is a clinical isolate obtained from Dr. J. R. Warmington (Curtin University). It was employed in drug sensitive assays to measure the levels at which compounds caused 50% growth inhibition (IC_{50}) as previously described.[9] The level of ethanol used to dissolve compounds caused no growth inhibition to the yeast, but DMSO at a level of 5%, but not 1%, caused growth inhibition.

3.23. Erythrocyte lysis assay

Washed human erythrocyctes (400 μL, 0.4×10^8 cells) in phosphate-buffered saline were incubated with the compounds to a final concentration of 1 mM and incubated 30 min at 37 °C. Cells were pelleted and the absorbance of the supernatant was compared with that for a sample of freeze-thawed cells to determine the percentage of hemolysis. By themselves, the solvents (DMSO and ethanol) used to dissolve compounds caused no hemolysis.

3.24. Mammalian cell toxicity assay

The toxicity of compounds to mammalian cells was determined using a Chinese Hamster Ovary (CHO) cell line. Compounds were added to final concentrations of 0.1 and 1.0 mM and the cells were cultured for in minimal essential Eagle's medium (MEM) Alpha medium. After 3 days of incubation cells were visually examined and then harvested to estimate the amount of growth which was estimated from protein levels. Protein levels were estimated using the Pierce BCA Protein Assay Reagent kit. Ethanol at levels up to 5% caused no growth inhibition. DMSO caused no inhibition at 1% but significant inhibition at 5%.

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Supplementary data

Experimental details for diene precursors to endoperoxides **1b**, **1c**, **1d** and **1j**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.10.021.

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