

# *De novo* identification and stability of the artemisinin pharmacophore: Studies of the reductive decomposition of deoxyartemisinins and deoxyarteethers and the implications for the mode of antimalarial action

Michael G.B. Drew<sup>a,\*</sup>, John Metcalfe<sup>a</sup>, Michael J. Dascombe<sup>b</sup>, Fyaz M.D. Ismail<sup>c,\*</sup>

<sup>a</sup> Department of Chemistry, University of Reading, Faculty of Life Sciences, Reading RG6 6AD, UK

<sup>b</sup> Faculty of Life Sciences, The University of Manchester, Stopford Building 1.124, Oxford Road, Manchester M13 9PT, UK

<sup>c</sup> The Medicinal Chemistry Research Group, The School of Pharmacy and Chemistry, Liverpool John Moores University, Liverpool L3 3AF, UK

Received 17 May 2007; received in revised form 13 August 2007; accepted 13 August 2007

Available online 21 August 2007

## Abstract

Reactions of *seco* and *carba* analogues of artemisinin **1** and arteether **16** have been investigated using DFT (B3LYP/6-31+G\* level). The data presented here show that O5 is favoured as a protonated site for **1** and its deoxy compounds, whereas protonation at O1 and O2 is unlikely. In contrast, O3 and O4 deoxy compounds of **16** can be protonated at O2, with subsequent scission of the C4–O2 bond, thereby prompting reaction with the antimalarial receptor by a Lewis acid mechanism. In all cases, addition of an electron leads to scission of the O1–O2 bond if present. The O4 deoxy compounds in this study display similar electron affinities to their respective parent compound, while O3 and O5 deoxy compounds show less negative values (about 6–8 kcal mol<sup>-1</sup>). Formation of the distonic radical anion, with the anion on O1 and the radical on O2, is slightly preferred in all our compounds, but only in the O3 deoxy compounds is this assignment definitive. The energy barrier to subsequent 1,5-intramolecular hydrogen abstraction to produce a carbon-centred radical is similar to that for the parent compounds. Calculations confirm that the generation and subsequent stability of both oxygen and carbon-centred radicals, which contributes to parasitidal action, is influenced most strongly by bioisosteric substitution of O3.

© 2007 Elsevier B.V. All rights reserved.

**Keywords:** Pharmacophore; Artemisinin; Arteether; Density functional theory; Protonation

## 1. Introduction

Malaria, human immunodeficiency virus (HIV) and tuberculosis (TB) comprise a triad of concurrent morbid infections presently causing global concern, especially in communities further compromised by malnutrition [1,2]. The additional health care problems associated with concurrent infections is illustrated by the observation that the antimalarial efficacy of artemisinin is reduced in HIV infected patients co-infected with malaria [3]. Hence there is an urgent need for new affordable therapeutic agents to treat concurrent infections simultaneously, a task made

more difficult by the increase in drug-resistant infections [4,5]. Certain trioxanes and tetroxanes are uniquely suited to simultaneously target malaria, HIV and TB [6–8] although their exact mechanism of action in the later two cases is undefined. Artemisinin is an antimalarial sesquiterpene endoperoxide lactone, and is the active antimalarial component in the herbal drug, qinghaosu isolated from either *Artemisia annua* or *Artemisia apiacea* Hance [9,10]. Entry of artemisinin into malaria parasitized erythrocytes may involve active transport [11]. Artemisinin and its analogues possess a broad spectrum of biological activities that also includes antiviral and anticancer properties [12–24]. The currently favoured mechanism of antimalarial action for 1,2,4-trioxanes, such as artemisinin (**1**, Fig. 1) and arteether (**16**, Fig. 2), involves radical formation [25,26] and this mechanism could be involved in their other

\* Corresponding authors. Tel.: +44 118 378 8789; fax: +44 118 378 6331.  
E-mail addresses: m.g.b.drew@reading.ac.uk (M.G.B. Drew), f.m.ismail@ljmu.ac.uk (F.M.D. Ismail).

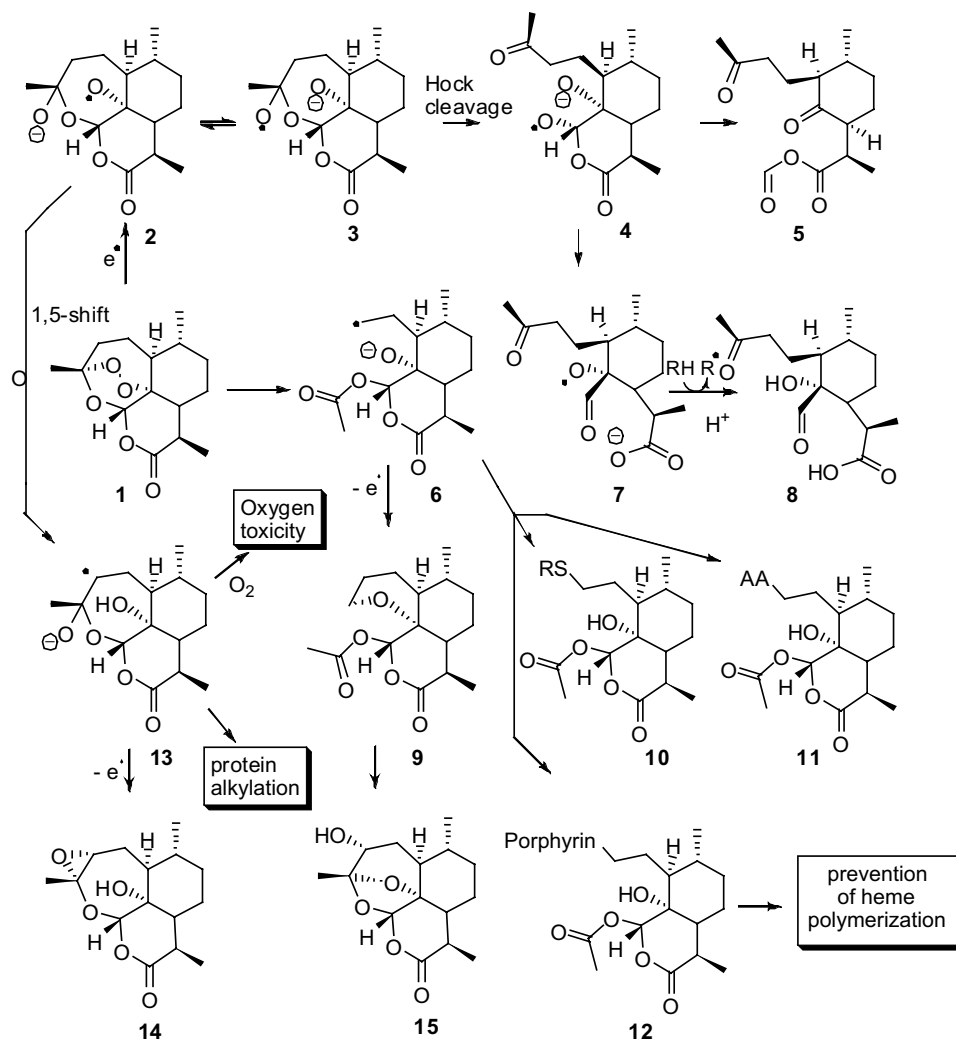


Fig. 1. Putative routes involving reductive decomposition and subsequent fragmentation, rearrangement and termination reactions of artemisinin and subsequent rearrangement. Certain intermediates have been detected spectrometrically [28] or by fast reaction techniques: **2/3**: [29,30]; **8**: as the methyl ester [31–33]; **9**: [34–37]; **11**: where AA represents a free or protein bound amino acid: [38–40]; **12**: [25,26]; **13**: [38–40]; **10**: where RS represents a thiol e.g. cysteine or glutathione; **14**: [36,41,42]; **15**: arteannuin D (qinghaosu IV) [43,44].

actions. Artemether and arteether (**16**) are, respectively, metabolized to release methanol and ethanol *in vivo*. Although **16** is safer, it has been poorly investigated and therefore we chose to investigate its lability to hydrolytic attack [27] and the influence of *carba* substitution on radical generation.

A recognized strategy for (a) understanding the mechanism of drug action and (b) producing inexpensive new compounds is to find *seco*- analogues of natural product leads that retain the warhead or pharmacophore portion, whilst removing or replacing superfluous sections with bioisosteric substitutions [45–51]. Therefore in this study, we have investigated *seco* and *carba* analogues of **1** and **16** using DFT (B3LYP/6-31+G\* level) to ascertain the influence of both through-space and through-bond interactions between remote oxygen atoms and the artemisinin pharmacophore. This may clarify the mode of action in terms of radicals and carbocations and uncover novel centres of reactivity for this class of antimalarials.

Current evidence favours artemisinin generation of free radicals within or adjacent to susceptible sites within *Plasmodia* [52–54]. Single electron transfer from either a metalated macrocycle or, less likely, non-complexed transition metals e.g. iron or copper [41,55] to the trioxane warhead generates one of two primary distonic radical anions, which can then degrade or rearrange to form further reactive species [56]. Several theoretical studies of this reductive activation process have been published, particularly for **1** (Fig. 1) [57–59]. These earlier studies commonly initiated the reductive activation process by single electron transfer from heme or free Fe(II) ion to the endoperoxide bond [34,35,41,55,60–63]. Supporting evidence from electrochemical [64,65], pulsed radiolytic [29,30], electron paramagnetic resonance [66,67] as well as product distribution analysis strongly supports a radical based mechanism rather than one involving Lewis acid or ionic intermediates [68]. Similarly, malignant cells with distorted iron metabolism [23,24] may facilitate their

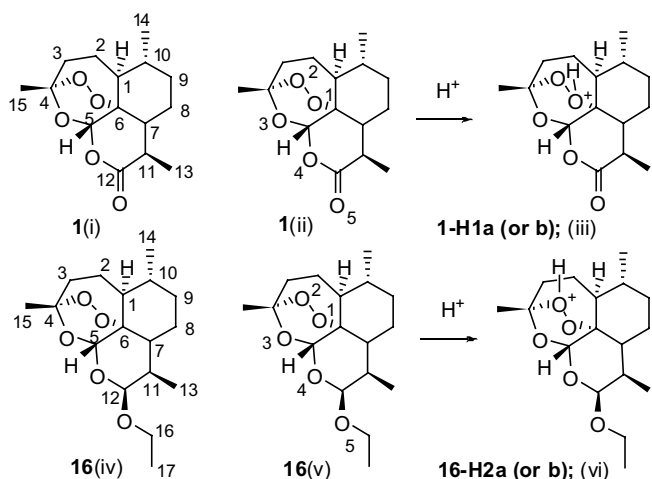


Fig. 2. The structures of artemisinin (**1**) and the ethylether analogue arteether (**16**): (i) numbering system used for artemisinin carbon skeleton; (ii) numbering system used for artemisinin oxygen skeleton; (iii) diagram indicating artemisinin protonated at O1; (iv) numbering system used for arteether carbon skeleton; (v) numbering system used for arteether oxygen skeleton and (vi) diagram indicating arteether protonated at O2.

own destruction by the peroxide warhead [69]. The peroxide bond is necessary for antimalarial efficacy, providing the compounds are stable to formulation and *per oral* delivery to patients [48–51].

Products of such reductions predicted using *ab initio* methods [70,71] were consistent with experimental observations [30]. In previous work, we have also shown that when a bond is formed between Fe(II) in various environments and either O1 or O2, then in all cases, geometry optimisation with Gaussian03 at the B3LYP/6-31+G\* level, leads to scission of the peroxide bond [58,59]. Complementary results were obtained by Taranto et al. [57], who added one electron to artemisinin without specifying its site. They found on geometry optimisation at the B3LYP/6-31G\* level that scission of the O1–O2 bond occurred without any activation barrier and generated an O1–O2 distance of 2.185 Å.

An alternative hypothesis for the antimalarial mechanism of action of artemisinin-like compounds involves ionic Lewis acid mediated scission of the peroxide group with the consequent generation of a carbocation at C-4 (Fig. 3) [49]. We previously investigated this mechanism and established the preferred Lewis acid protonation sites to be: **1** O5 >> O4 ≈ O3 > O2 > O1; **16** O4 ≥ O3 > O5 >> O2 > O1 (atom numbering scheme as shown in Fig. 2), and the consequent decomposition pathways and hydrolysis sites [68]. These effects are considered important not only for the hydrolytic stability of a substance but also during protonation at an active site or loci of drug action. Since protonation is unlikely to occur on the peroxide bond O1–O2 in either molecule we concluded in this instance that: (a) Lewis acid induced ionic scission is unlikely to contribute to the antimalarial action and (b) the alternative radical pathway, where the initial step was the addition of an electron, remained the most likely explanation for the

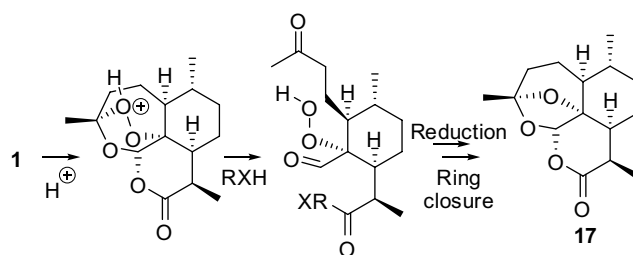


Fig. 3. Lewis acid induced decomposition of **1** to give the urinary metabolite **17**. The nucleophile is denoted by RXH, where X=O, NH, S; adapted from Haynes et al. [49].

antimalarial action of artemisinin, arteether and related trioxanes [68].

In this study, attention was focused on various carbaartemisinins and carbaarteethers (aka deoxyartemisinins and deoxyarteethers) [72–81,20,82] to assess whether the pathways for antimalarial action remain the same as for the parent artemisinins. There has been considerable interest in the deoxyartemisinins to determine whether functionality, especially antimalarial activity, remains after removal of an oxygen atom and replacement by a methylene (CH<sub>2</sub>) group i.e. rational identification of the antimalarial pharmacophore in artemisinins [73,74,79]. Antimalarial activity in non-peroxidic parthenin which has been shown to possess oxygen atoms and hydrocarbon frameworks in common with artemisinin using molecular modelling [83] leads to the suggestion that mechanisms not involving radicals may also be important. From an organic synthesis point of view, not all the deoxyartemisinins can be made easily. Consequently, we decided to conduct a systematic survey of the five possible deoxyartemisinins and their deoxyarteether analogues to identify reactive portions in addition to the peroxidic moiety and to eventually clarify the role of through bond and through space interactions between peroxidic and non-peroxidic oxygen atoms. Application of such methodology to poorly investigated or new peroxides e.g. hexacyclinol [84,85] could then be used to prioritise synthetic efforts to improve productivity and/or pharmacological activity.

## 2. Methods

Individual compounds are numbered **1-n-ma** and **1-n-mb** for the deoxyartemisinins and **16-n-ma**, **16-n-mb**, for the deoxyarteethers, where in both cases n is the number of the oxygen atom replaced by a methylene (CH<sub>2</sub>) group, and m is the number of the oxygen atom that is subsequently protonated. The letters **a** and **b** denote the two possible tetrahedral sites around each oxygen atom that could be protonated, the one exception being O5 in the deoxyartemisinins where the two trigonal positions were alternately protonated.

The crystal structure of artemisinin has been obtained several times with insignificant variations [86–88] and the common structure was used as the initial model for **1** in

our calculations. Structures of other molecules were built using the Cerius<sup>2</sup> software package [89] and approximate structures obtained using molecular mechanics minimisation with the default Universal Force Field. These structures were geometry optimised using the Gaussian03 program [90] using the B3LYP/6-31+G\* methodology, which has been shown to reproduce the structures well [58,59], although the transition state calculations shown in Tables 5 and 6 were carried out at the B3LYP/6-31G\* level.

### 3. Results and discussion

#### 3.1. Deoxy compounds

Each set of five deoxyartemisinins or deoxyarteethers has the same molecular formula (stoichiometry) therefore the relative energies of the molecules can be meaningfully compared [91], because Hess's Law is not violated and subsequently their relative thermodynamic stabilities can be obtained. Table 1 indicates that the heat of formation of the artemisinin and arteether pair of compounds, in which one of the peroxide oxygen atoms is replaced by CH<sub>2</sub>, is significantly less (by more than 49 kcal mol<sup>-1</sup>) than the other three deoxyartemisinins or deoxyarteethers. The optimised structures of **1** and **16** are shown in Fig. 4.

#### 3.2. Effect of reduction by electron addition

Taranto et al. [57] found that addition of an electron to artemisinin led to scission of the O1–O2 bond, with the extra electron spin density concentrated on these two oxygen atoms [57]. We repeated the calculations of Taranto and co-workers not only on **1**, but also applied the same analysis to **16** and all the deoxy analogues in this study. Details of the converged structures are given in Table 2 and the optimised structures for **1** and **16** after addition of an electron are depicted in Fig. 5.

The electron affinity of **1** is more negative than that of **16** by 9.01 kcal mol<sup>-1</sup>. This difference is maintained in the deoxy analogues studied here, although the electronic properties are very similar, the electron affinities of the deoxy compounds based on **1** or **16** are very different. When O1 or O2 in **1** or **16** is replaced by CH<sub>2</sub> the electron affinity is positive so that an electron will not be added, whereas the electron affinity is negative when O3, O4 or

O5 is similarly replaced. The peroxide bond thus appears essential for the addition of an electron in order to generate the parasitidal species [92,93]. This is corroborative evidence supporting other work including bioassays [41,47–54,94] that points to the peroxide as the essential pharmacophore.

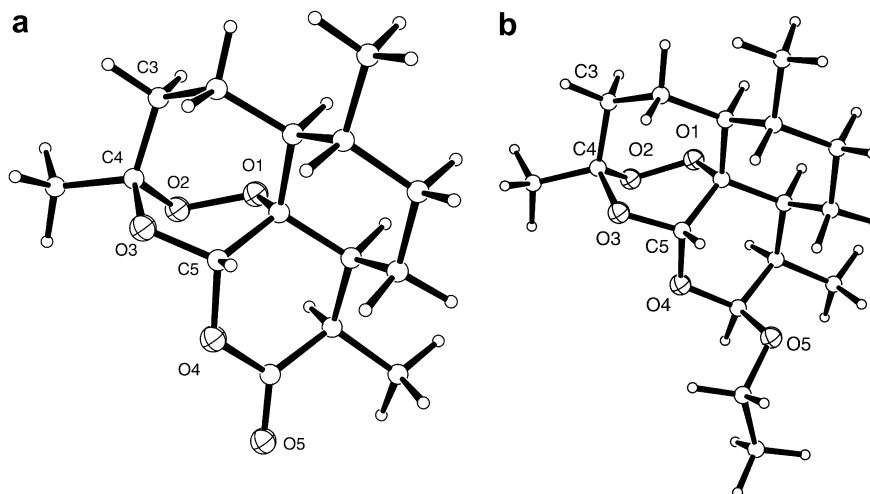
Compounds formed by CH<sub>2</sub> bioisosteric substitution of O1 or O2 show the excess spin and charge delocalised over the entire molecule. In contrast, when O3, O4 or O5 is replaced, whilst the O1–O2 bond is retained within the starting model, the excess spin is located over O1 and O2 after convergence of the structure with the additional electron. The O4 deoxy compounds display similar electron affinity to their parent compound, but O3 and O5 deoxy compounds show less negative values by about 6–8 kcal mol<sup>-1</sup>. The resulting structures (Table 2) are very similar to those for **1** and **16** (Fig. 5) in that the O1–O2 bond breaks to give a distance of about 2.21 Å. Upon O1–O2 bond scission, one of the oxygens can be considered as a radical and the other as anionic, as separation of spin and charge forms distonic radical anions. However, Table 2 indicates differentiation between the two oxygen atoms in this way is perhaps too simplistic. For **1**, O2 has slightly more (0.075) spin density than O1, and O1 has slightly more negative charge (0.093) than O2. This pattern of differences is repeated in **16** and in nearly all the deoxy derivatives of both **1** and **16**. This difference is greater in 3-deoxy compounds where the excess spin density is 0.239 for **1–3** and 0.322 for **16–3**. This is noteworthy as it clearly demonstrates that the replacement of O3 by CH<sub>2</sub> has a significant effect on the electron properties of the peroxide bond presumably because of the close proximity of O3–O2 (2.32 Å in **1**). Despite O4 and O5 being remote from the peroxide bond, their bioisosteric substitution has an effect. The 5-deoxy compounds follow the same pattern as the 3-deoxy compounds though to a lesser extent. However for the 4-deoxy compounds, the differences between O1 and O2 are much less and indeed **16–4** is the only compound in which the spin density is greater for O1 than for O2, albeit by only 0.011. Note that the results obtained for **1** can be compared to those obtained by Taranto et al. [57] using the 6-31G\* basis set. The spin densities are similar (O1: 0.448; O2: 0.509) but the Mulliken charges are significantly larger (O1: –0.561; O2: –0.489) though the trend is the same. The distribution of spin density and charge has implications on the next step in the reactive decomposition of the molecules. This is considered in the next section where results indicate the reductive decomposition mechanism shown in Fig. 1 would be followed readily by the 3, 4 and 5 deoxy derivatives of artemisinin and arteether.

#### 3.3. Protonation studies

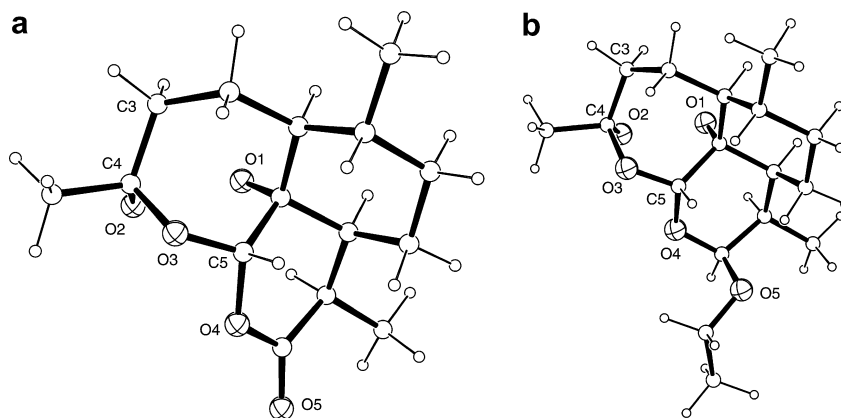
We have previously studied the effect of protonation on **1** and **16** [68] and here employ the same strategy and methodology described previously to study the effect of protonation on the ten deoxy compounds. In the absence of a

Table 1  
Relative energies (kcal mol<sup>-1</sup>) of the deoxyartemisinins and the deoxyarteethers with one oxygen atom replaced by a methylene group

Oxygen replaced	<b>1</b>	<b>16</b>
1	1.63	1.03
2	0.00	0.00
3	50.80	51.30
4	55.52	50.89
5	74.17	51.38

Fig. 4. Optimised structures of (a) artemisinin, **1**, and (b) arteether **16**.Table 2  
Selected structural properties of artemisinin, arteether and their deoxy derivatives obtained after electron addition

Structure	Relative energy kcal mol <sup>-1</sup>	Electron affinity kcal mol <sup>-1</sup>	Spin density		Partial charge		O–O distance (Å)
			O1	O2	O1	O2	
<b>1</b>	n/a	–44.66	0.428	0.503	–0.397	–0.304	2.322
<b>1–1</b>	0.0	12.77	n/a	n/a	n/a	n/a	n/a
<b>1–2</b>	0.76	15.17	n/a	n/a	n/a	n/a	n/a
<b>1–3</b>	–0.61	–36.38	0.348	0.587	–0.435	–0.323	2.230
<b>1–4</b>	–1.81	–42.92	0.454	0.478	–0.391	–0.361	2.251
<b>1–5</b>	21.31	–38.46	0.439	0.491	–0.399	–0.315	2.215
<b>16</b>	n/a	–35.65	0.453	0.477	–0.396	–0.320	2.323
<b>16–1</b>	0.00	18.15	n/a	n/a	n/a	n/a	n/a
<b>16–2</b>	1.18	20.35	n/a	n/a	n/a	n/a	n/a
<b>16–3</b>	4.28	–27.83	0.375	0.562	–0.433	–0.339	2.265
<b>16–4</b>	–4.46	–35.92	0.471	0.460	–0.397	–0.367	2.269
<b>16–5</b>	–2.55	–28.55	0.448	0.481	–0.397	–0.316	2.234

Fig. 5. The structures of (a) artemisinin, **1** and (b) arteether, **16** after the addition of an electron, showing the scission of the O–O bond.

peroxide function, this could reveal sites of carbocationic reactivity [49]. Each of the deoxy compounds has four remaining oxygen atoms after one had been replaced by a CH<sub>2</sub> group. Calculations were made with each oxygen

protonated in each of the two alternative tetrahedral lone-pair positions in turn (Table 3).

The relative energies for the deoxyartemisinins show distinct trends. Protonation at O5 in **1** is highly favoured (by

Table 3  
Relative energies (kcal mol<sup>-1</sup>) of the protonated deoxyartemisinins

Protonated position	Structure						
	1	1-1	1-2	1-3	1-4	1-5	
1a	31.41	n/a	23.61	22.71	17.54	14.76	
1b	=1a	n/a	23.41	22.89	=1a	16.68	
2a	17.83	24.55	n/a	22.61	10.21	5.90	
2b	26.33	20.09	n/a	18.00	=2a	9.14	
3a	13.11	8.41	22.48	n/a	3.54	2.82	
3b	=3a	=3a	19.48	n/a	2.41	=3a	
4a	11.96	7.08	8.64	20.24	n/a	0.00	
4b	20.33	=4a	=4a	18.15	n/a	0.90	
5a	0.00	0.00	0.00	0.00	0.82	n/a	
5b	4.00	5.23	4.14	=5a	0.00	n/a	
Protonation energy/kcal mol <sup>-1</sup> <sup>a</sup>	-216.60	-224.14	-222.51	-219.75	-211.63	-213.62	

<sup>a</sup> Calculated as the difference in energy between that of the protonated species with the lowest energy and the parent molecule.

about 12 kcal mol<sup>-1</sup>) over protonation at any of the other four oxygen atoms to give the structure shown in Fig. 6. This observation provides rationalisation for the production of artemisinin congeners derived by structure–activity observations [72,73].

These data also indicate that protonation is not likely to occur on either O1 or O2 hence scission of the O1–O2 bond may also not occur. These observations are consistent with our earlier conclusion that the protonation route is not the likely basis of the biological activity of **1** [68]. Replacement of O1 or O2 by CH<sub>2</sub> makes little difference to the protonation pattern compared to the values for **1**. The carbonyl oxygen O5 remains favoured, but the energy of protonation at O3 and O4 is only greater than that for O5 by 7–8 kcal mol<sup>-1</sup> compared to a value of 12 kcal mol<sup>-1</sup> for **1**. However, replacement of O3, O4 or O5 by CH<sub>2</sub> has a much greater effect; replacement of O3 to give **1-3** makes protonation at O4 much less favourable (~19 kcal mol<sup>-1</sup> more than that for protonation at O5) thereby decreasing hemiacetal formation. In contrast, the relative energy of protonation at O3 in **1-4** is reduced (only ~3 kcal mol<sup>-1</sup>

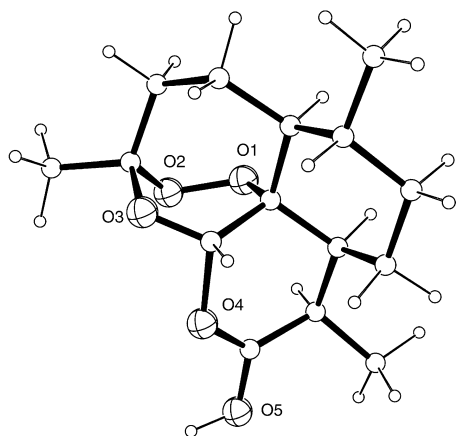


Fig. 6. Lowest energy structure obtained after protonation of **1**. All deoxyartemisinins gave a similar lowest energy structure with O5 protonated.

greater than that for O5). The optimised structure of **1-4-3b** (Fig. 7a) shows breaking of the C4–O3 bond gives rise to a reactive carbon-centred radical. Diverting the site of preferred radical formation as found in **1** may be one explanation for the observed loss of antimalarial activity *in vitro*.

The preferred site of protonation in **1-5** is O4 giving the structure **1-5-4a** (Fig. 7b) where the distance from O4 to C5 is 2.016 Å, so the bond can be considered broken. In artemisinin **1**, protonation at O4 leads to a much smaller lengthening of the C5–O4 bond, 1.658 Å. Another low energy structure **1-5-3a** with a relative energy of 2.82 kcal mol<sup>-1</sup> is found with protonation at O3 (Fig. 7c). Protonation at O2 gives an energy only 5.90 kcal mol<sup>-1</sup> higher than the minimum, which suggests that protonation of **1-5** could lead to the scission of the O1–O2 bond, thereby accessing reductive decomposition pathways. However, when the protonation energies are calculated from the differences in energies between the lowest energy protonated structure and that of the parent compound, they show that protonation is significantly less favoured for **1-4** and **1-5**.

The protonation patterns of **16**, given in Table 4, are very different from those of **1**. The energies of protonation for (**16**) at O3, O4 and O5 are within a range 2 kcal mol<sup>-1</sup>, although protonation at O1 and O2 remains less favourable. The lowest energy structure for **16-4a** with protonation at O4 has a C–O4 bond lengthened to 1.65 Å but unbroken (Fig. 8a). Protonation of **16** has previously been concluded, as for (**1**), to be an unlikely trigger for scission of the O1–O2 bond [56]. However, the pattern of energies for the deoxyartemisinins is significantly different (Table 4). In **16-1**, protonation at O3 becomes favoured by >3.5 kcal mol<sup>-1</sup> to give the structure shown in Fig. 8b. Replacement of O2 has a very different effect; protonation at O5 favours the structure illustrated in Fig. 8c.

In this latter structure (Fig. 8c), protonation at O5 leads to the formation of ethanol by breaking of the C12–O15 bond as in the parent structure **16** [27] confirming observa-

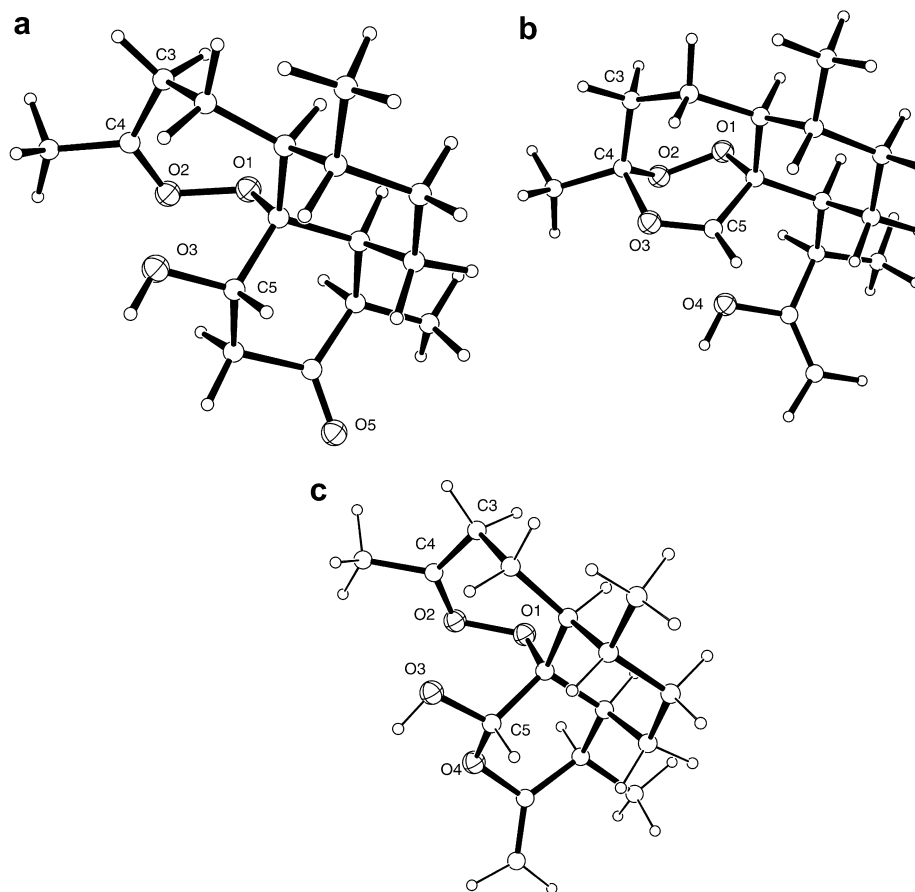


Fig. 7. The optimised structures of (a) **1-4-3b** showing the scission of the C4–O3 bond, (b) **1-5-4a** showing the scission of the C5–O4 bond and (c) **1-5-3a** showing the scission of the C4–O3 bond.

Table 4  
Relative energies (kcal mol<sup>-1</sup>) of the protonated deoxyarteteethers

Protonated position	Structure					
	<b>16</b>	<b>16-1</b>	<b>16-2</b>	<b>16-3</b>	<b>16-4</b>	<b>16-5</b>
1a	16.70	n/a	6.33	10.78	12.14	17.81
1b	18.52	n/a	7.11	13.11	12.54	20.25
2a	10.40	8.49	n/a	5.18	6.57	10.64
2b	10.84	15.54	n/a	14.05	=2a	12.24
3a	1.79	0.00	9.20	n/a	0.00	3.94
3b	=3a	0.00	5.67	n/a	=3a	=3a
4a	0.00	3.52	3.12	0.00	n/a	0.00
4b	5.02	4.09	1.08	8.92	n/a	4.16
5a	2.39	5.69	0.00	4.55	6.49	n/a
5b	=5a	5.68	=5a	3.21	=5a	n/a
Protonation energy/kcal mol <sup>-1</sup> <sup>a</sup>	-219.57	-224.77	-218.16	-221.58	-216.18	-221.43

<sup>a</sup> Calculated as the difference in energy between that of the protonated species with the lowest energy and the parent molecule.

tions of its formation during metabolism. Protonation at O3 in **16-2** is now much less likely, but protonation at O4 in **16-3** and at O3 in **16-4** is favoured. It can be concluded for **16-3** and **16-4** that protonation could occur on O2 with relative energies of 5.18 and 6.57 kcal mol<sup>-1</sup>, respectively (Fig. 8d and e) [32]. It is interesting that the C4–O2 bond is broken in **16-4-2b** to give a *carba* radical, equivalent to that portrayed in Fig. 3. Neither the C4–O2

nor the O1–O2 bond is broken in **16-3-2b**, because presumably both are stabilised by the presence of CH<sub>2</sub> at the 3 position stabilising the O–O bond. These observations indicate it could be possible for **16-4** to follow the protonation route suggested by Haynes et al. [49,95,96], which provides an alternative mechanism to radical attack at the target site. This suggestion is supported by the relative loss of antimalarial potency in these *carba* analogues of artemisi-

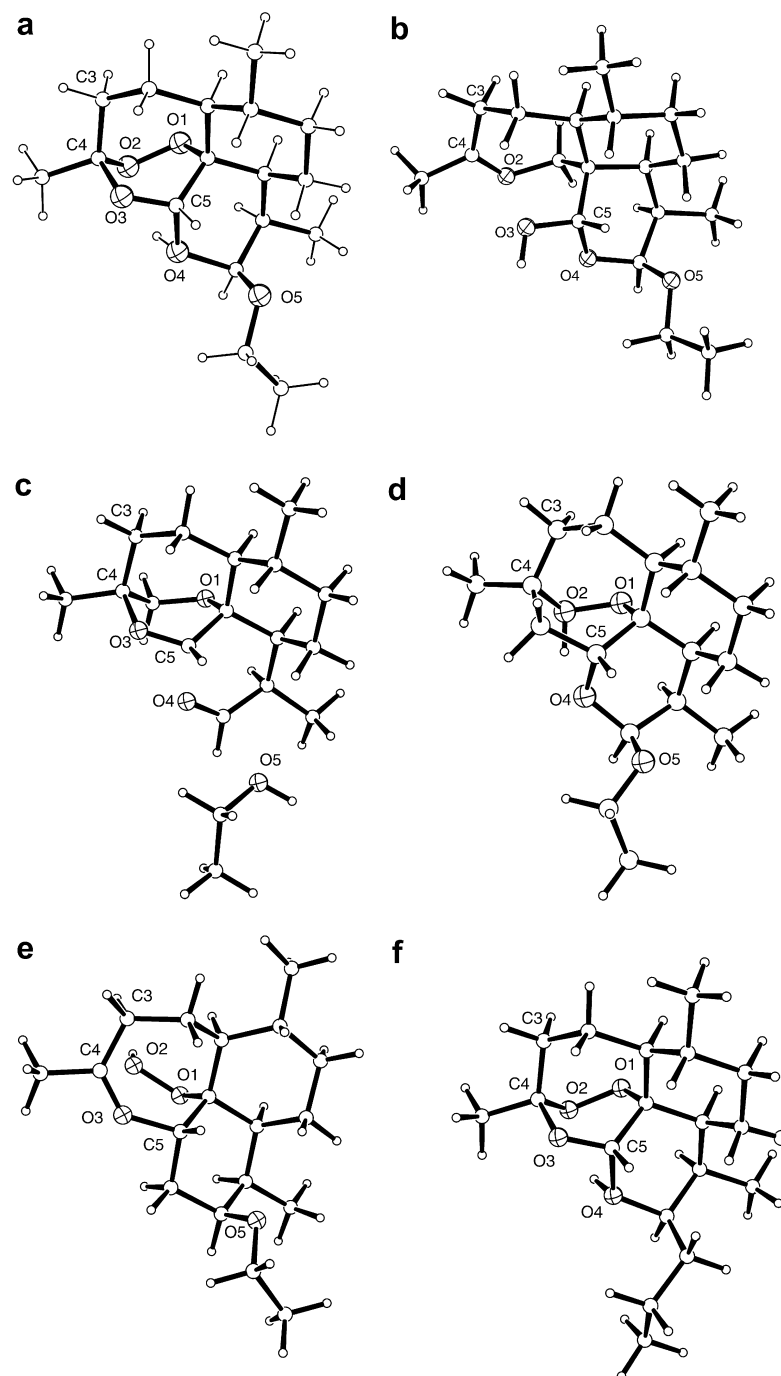


Fig. 8. The lowest energy structures of arteether **16** and deoxyartethers after protonation (a) **16-4a**, (b) **16-1-3a**, (c) **16-2-5a**, (d) **16-3-2a**, (e) **16-4-2a**, (f) **16-5-4a**.

nin [73–79]. In **16-5**, protonation at O4 is favoured to give the structure **16-5-4a** shown (Fig. 8f) in which all bonds remain intact.

### 3.4. The subsequent step in reductive decomposition of oxy and deoxycompounds

These calculations show that the addition of an electron leads to O–O bond scission in both **1** and **16** and their 3, 4 or 5-deoxy derivatives. The next step in the reductive decomposition of these compounds was investigated. Two

mechanisms are considered possible (Fig. 9) and these are dependent upon the structure obtained after scission, which will contain one oxyradical and one negatively charged oxygen.

It is generally accepted that when O1 in **1** is protonated to give **O1H-r**, then the next step is the scission of the C4–C3 bond to give the carbonyl species **O1H-p**. In contrast, when O2 is protonated to give **O2H-r**, the next step is a 1,5-hydrogen shift to give a C-centred radical species **O2H-p** [28–30]. However, our results (Table 2) demonstrate that scission of the O–O bond does not clearly lead

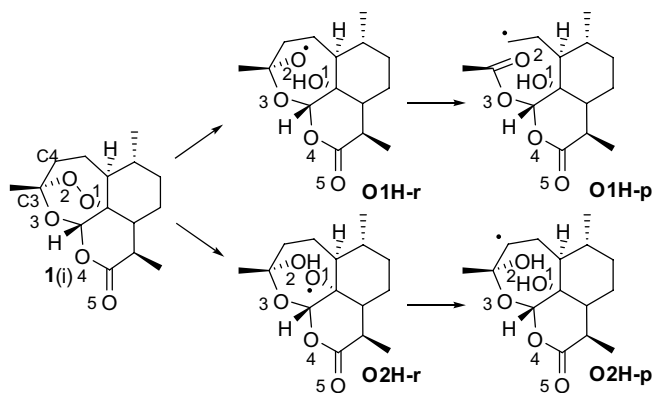


Fig. 9. Artemisinin and the two possible products **O1H-r** (Fig. 1, protonated form of 3) and **O2H-s** (Fig. 1, protonated form of 2) obtained after the addition of an electron and subsequent protonation. **O2H-r** undergoes the 1-5-hydrogen shift to give **O2H-p** (Fig. 1, protonated form of 13) and **O1H-r** gives rise to the ring opening product **O1H-p** (Fig. 1, protonated form of 6).

to one oxygen atom being a radical and the other being negatively charged. Formation of the distonic radical anion with the radical on O2 and the anion on O1, rather than the reverse, is slightly preferred in all compounds, because O2 has the highest spin density and O1 the most negative partial charge in all cases. However, this assignment is definitive only in the O3 deoxy compounds therefore we considered both possible assignments in this study. In order to characterise the oxygen atoms clearly and facilitate calculations, one or other of the oxygen atoms (O1, O2) was protonated to leave a neutral species.

Experiments *in vitro* show that the radicals originating from **1** preferentially populate intramolecular pathways (Fig. 1) and show a greater selective toxicity than simple alkyl radicals when they form within cells. This provides one explanation for the low toxicity of artemisinin radical precursors at therapeutic doses [43]. These radical species, **O1H-p** and **O2H-p** can undergo further reactions, which will not be discussed here as the aim of this study was to investigate the effect of replacing the O3, O4 or O5 by CH<sub>2</sub> upon the energetics of the initial reactions. We first calculated the energies of the reactants, **O1H-r** and **O2H-r**, the structures and energies of the transition states **O1H-ts** and **O2H-ts**, all of which had 1 imaginary frequency, and the energies of the products **O1H-p** and **O2H-p** for **1**. The resulting structures for **1** are shown in Fig. 10. In **O1H-ts** the dotted line represents a distance of 2.03 Å between C4 and C3, with the bond completely broken in **O1H-p**. In **O2H-ts**, the C3...H and H...O1 distances are 1.28 and 1.25 Å, respectively. The calculations were repeated for **16** and the 3-deoxy, 4-deoxy and 5-deoxy artemisinins and arteethers. The structures obtained were very similar to those shown in Fig. 10 with relative energies in Table 5 for the O1H pathway and Table 6 for the O2H pathway.

The energies of the reaction for **O1H** leading to scission of C4–C3 deserve comment. In **1** the energy barrier is

8.79 kcal mol<sup>-1</sup>, the final product having a relative energy of -15.90 kcal mol<sup>-1</sup>. These results are different from those obtained for **1** by Taranto et al. [57] who obtained 3.86, 0.02 kcal mol<sup>-1</sup> at the 6-31G\* level and 3.21, -11.56 kcal mol<sup>-1</sup> at the 6-31G\*\*//6-31G\*\* level. These authors also studied the reaction without protonation, thus with anionic radicals and obtained energies of 21.92, -1.94 kcal mol<sup>-1</sup> at the 6-31G\*\*//6-31G\*\* level. Thus the energy barrier is very much reduced by protonation. Consequently, these results suggest that antimalarial drug binding to putative receptors (such as heme) within acidified parasitic vacuoles would be modulated by protonation.

Similar values to those found in this present work for **1** are also found for **16** and the 4-deoxy and 5-deoxy derivatives of both **1** and **16**. However, the relative energies are significantly different for the 3-deoxy derivatives of both **1** and **16**. For **1-3**, the energy barrier is increased to 14.06 kcal mol<sup>-1</sup> and the relative energy of the final product, **O1H-p** has increased by >10 to -5.11 kcal mol<sup>-1</sup>. The equivalent energies for **16-3** are 6.00 and -8.62 kcal mol<sup>-1</sup>, respectively. Replacement of O4 or O5 by CH<sub>2</sub> in **1** or **16** makes little difference to the energetics of the reaction.

The energy barrier for the 1,5-hydrogen shift in **1** is 9.18 kcal mol<sup>-1</sup> with the product **O2H-p** having a relative energy of 0.02 kcal mol<sup>-1</sup> compared to the starting reactant **O2H-r**. In this case these are equivalent to the values obtained by Taranto et al. [57]. They obtained values of 7.11, -2.54 kcal mol<sup>-1</sup> at the 6-31G\*\*//6-31G\*\* level and 23.62, -3.80 kcal mol<sup>-1</sup> for the corresponding anionic radical reaction. As in the previous C3–C4 scission reaction, protonation reduces significantly the energy barrier of the transition state.

Energy values for all deoxy compounds except the 3-deoxy compounds are similar. Although the energy barriers for these compounds remain similar to those in **1** or **16**, the products have lower energies at -1.73 and -7.53 kcal mol<sup>-1</sup>, respectively. The data on spin density and charge distribution after scission (Table 2) indicate that it is more probable that O2 is the radical and O1 is negatively charged. However this is at odds with the relative energies of the **O1H** and **O2H** pairs (Table 7) as the difference in energy ( $\Delta E1$ ), calculated as  $E(\mathbf{O2H-r}) - E(\mathbf{O1H-r})$ , is negative with values for mostly about -6 kcal mol<sup>-1</sup>. By contrast  $\Delta E2$  calculated as  $E(\mathbf{O2H-p}) - E(\mathbf{O1H-p})$  is positive with values of about 8 kcal mol<sup>-1</sup>, showing that the product from the C4–C3 bond scission route via the formation of **O1H** has the lowest energy [97]. The exception to these general trends is the group of 3-deoxy compounds, which has negative  $\Delta E2$  values of -3.51 kcal mol<sup>-1</sup> for **1-3** and -0.94 kcal mol<sup>-1</sup> for **16-3**.

To summarise thus far, it is clear that the 3-deoxy compounds show significant differences in electronic properties from (a) their respective parent compound, **1** or **16** and (b) the 4-deoxy and 5-deoxy derivatives. It seems more likely that these 3-deoxy compounds would follow the **O2H** route rather than the **O1H** route to a greater extent than the par-

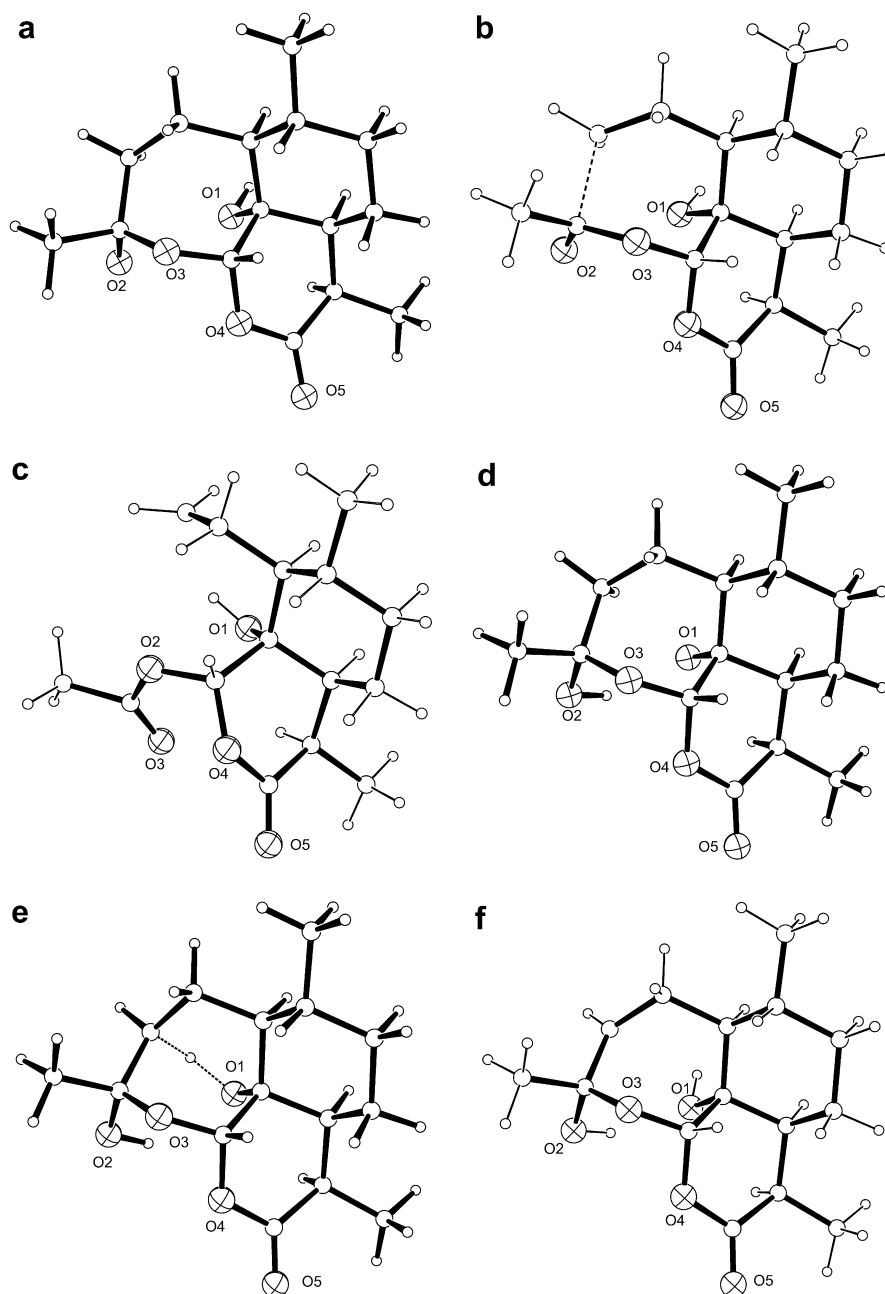


Fig. 10. The optimised structures based on artemisinin (a) **O1H-r** and (d) **O2H-r** obtained after adding an electron (reduction) and, subsequently, a proton to **1**. **O1H-r** then reacts to give (c) **O1H-p** via transition state (b) **O1H-ts** and **O2H-r** reacts to give (f) **O2H-p** via transition state (e) **O2H-ts**.

ent compounds. This may explain the reported decreases in antimalarial activity *in vitro* [73,75]. These results suggest that the presence of O3 to give the trioxane ring modulates not only electronic properties influencing binding to antimalarial drug receptors (heme [25] or the SERCA pump [39,40,99]) but also influences the stability and decomposition pathways accessed by both **1** and **16** and hence antimalarial activity. Furthermore, our findings suggest that the **O1H** route favours attack on heme to prevent biomineralisation in the parasitic food vacuole. The **O2H** route could result in direct alkylation of critical proteins [98] or con-

tribute to oxidative stress. Since current evidence supports two major antimalarial drug targets, namely heme and the SERCA pump, it may be that following selective accumulation of the peroxidic species, multiple targets may be available, but only those that have the necessary activating components (e.g. metalated co-factors) or transition metal binding sites will initiate distonic radical anion formation.

Only some of these compounds have been constructed by total synthesis, therefore it is pertinent to discuss the results presented and correlate the data with published experimental observations where possible. Compounds in

Table 5

Relative energies compared to reactant **O1H-r** of the transition state **O1H-ts** and product **O1H-p** for the formation of a carbon radical on C3 from C3–C4 bond breaking (Figs. 9 and 10)

Compound	Energies (kcal mol <sup>-1</sup> ) relative to the reactant <b>O1H-r</b>	
	Transition state <b>O1H-ts</b>	Product <b>O1H-p</b>
<b>1</b>	8.79	-15.90
<b>1-3</b>	14.06	-5.11
<b>1-4</b>	6.91	-14.43
<b>1-5</b>	8.75	-13.25
<b>16</b>	8.40	-14.67
<b>16-3</b>	6.00	-8.62
<b>16-4</b>	7.41	-17.32
<b>16-5</b>	8.42	-15.12

Table 6

Relative energies compared to **O2H-r** of the transition state **O2H-ts** and product **O2H-p** for the reaction involving a 1–5 hydrogen shift from C4 to O1 creating a carboradical on C3 (Figs. 9 and 10)

Compound	Energies (kcal mol <sup>-1</sup> ) relative to the reactant <b>O2H-r</b>	
	Transition state <b>O2H-ts</b>	Product <b>O2H-p</b>
<b>1</b>	9.18	0.02
<b>1-3</b>	7.72	-1.73
<b>1-4</b>	10.14	0.13
<b>1-5</b>	9.78	1.33
<b>16</b>	9.78	3.60
<b>16-3</b>	8.70	-7.53
<b>16-4</b>	10.79	0.27
<b>16-5</b>	9.59	1.23

Table 7

Comparison of the energy differences (kcal mol<sup>-1</sup>) for the two reactions illustrated in Fig. 9, following either O1 or O2 protonation

Compound	Differences in energy	
	$\Delta E1^a$	$\Delta E2^b$
<b>1</b>	-6.25	9.66
<b>1-3</b>	-6.89	-3.51
<b>1-4</b>	-4.57	9.73
<b>1-5</b>	-7.69	6.90
<b>16</b>	-7.95	10.32
<b>16-3</b>	-2.03	-0.94
<b>16-4</b>	-5.16	9.11
<b>16-5</b>	-8.04	7.98

<sup>a</sup>  $\Delta E1$  is the difference in energy calculated from  $E(\mathbf{O2H-r}) - E(\mathbf{O1H-r})$ .

<sup>b</sup>  $\Delta E2$  is the difference in energy calculated from  $E(\mathbf{O2H-p}) - E(\mathbf{O1H-p})$ .

which O1 has been bioisosterically replaced by CH<sub>2</sub> have been described [72]. The antimalarial activity of such compounds has not been fully reported to our knowledge, but our observations suggest that they have no significant antimalarial activity. Analogues in which O3 has been replaced by CH<sub>2</sub> i.e. of (+)-13-carbaartemisinin show a reduction in antimalarial potency (IC<sub>50</sub> = 38.8 ng/ml; D-6 clone) when compared to **1** (IC<sub>50</sub> = 0.97 ng/ml) when tested against the D-6 clone (chloroquine sensitive and mefloquine resistant) of *Plasmodium falciparum* *in vitro* [74–76]. Avery and co-workers have suggested that the inactivity of C-4ct substituted artemisinin analogs could be correlated to their inability to undergo C-4 hydrogen atom abstraction [74–

76]. While C-4 radical stability would be predicted to have an impact on antimalarial potency, these results contradict the notion that increased stability of the C-4 radical is related *a priori* to improved antimalarial activity, because the C-4 radical derived from (+)-13-carbaartemisinin should be more stable than the radical analogous to **13** (Fig. 11) [72]. Interestingly, a product with the corresponding inversion of the stereochemistry surrounding the trioxane scaffold, namely the diastereomeric peroxide tetracycle (1a*S*,3*S*, 5a*S*,6*R*,8a*S*,9*R*,12*S*)-10-deoxy-13-carbaartemisinin possesses antimalarial activity (IC<sub>50</sub> = 1.72 ng/ml) [72].

An alternative explanation is that molecular recognition during drug absorption and distribution e.g. *via* an active transporter [11] is affected and compounds fail to reach their site(s) of antimalarial action in adequate concentrations. It would appear that compounds resembling the stereoelectronic profile of artemisinin **1**, such as parthenin [83] and possibly thapsigargin [38] may allow importation into parasites but it is likely that these non-peroxidic compounds act by Michael addition rather than free radical pathways. Further bioisosteric substitution of **1** to provide (+)-10-deoxy-13-carbaartemisinin, in which the non-peroxidic oxygen within the 1,2,4- trioxane scaffold as well as the carbonyl group has been replaced by CH<sub>2</sub>, restores antimalarial activity (IC<sub>50</sub> = 2.87 ng/ml) perhaps due to increased stability towards hydrolysis in acid media (such as the parasite food vacuole). A fine balance between stability towards premature hydrolytic attack, especially during per oral delivery [78,79] and/or within the parasite food vacuole with the desired reactivity at the target site is modulated by oxygen atoms, especially O3 that exist outside the pharmacophore.

#### 4. Conclusions

This study investigated the changes in reactivity of selected deoxyartemisinins and deoxyarteethers when compared to the parent compounds by considering both (i) the free radical mechanism generated by the addition of an electron and (ii) subsequent protonation. With regard to the free radical mechanism, replacement of O3, O4 or O5 for both **1** and **16** made little difference to the first step in the mechanism, the scission of the O1–O2 bond. Only the O4 deoxy compounds displayed electron affinities similar to the parent compounds; the O3 and O5 deoxy compounds both had less electron affinity (>5 kcal mol<sup>-1</sup>). The generation and stability of these carbon-centred radi-

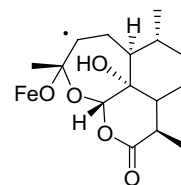


Fig. 11. Putative carbon-centred radical produced by reaction of **1** by Fe(II) from the intramolecular 1,5-abstraction pathway [99].

cals, which contributes to parasitidal action, is influenced most strongly therefore by bioisosteric substitution of O3. The importance and geometry of such carbon-centred radicals have been highlighted [28,100] and their direct detection is in process [30].

Data presented here for the protonation mechanism in deoxyartemisinins, including the high relative energies for the protonation of O1 and O2, are consistent with our earlier report [68]. Protonation of these compounds appears equally unlikely as for the parent **1** to lead to scission of the O1–O2 bond. In contrast, there was a significant shift in the relative energies of deoxyartemisinins. It was possible in both O3 and O4 deoxyartemisinins for protonation to occur on O1 or O2, respectively, leading to scission of the C4–O2 or O1–O2 bond. This indicates that in these compounds at least ionic intermediates could contribute towards the hydrolytic lability of the compounds.

The energetics of further reactions of the protonated compounds following 1,5-hydrogen shift or C–C bond scission show that the 3-deoxy compounds have very different profiles from the other compounds studied here. This difference may account for their lack of biological activity, unlike the 4-deoxy and 5-deoxy compounds, which have similar profiles to those of the parent compounds. Overall our continuing *ab initio* calculations promote confidence in making predictions to aid molecular design leading to new antimalarials.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.theochem.2007.08.007](https://doi.org/10.1016/j.theochem.2007.08.007).

## References

- [1] S. Accorsi, M. Fabiani, B. Nattabi, B. Corrado, R. Iriso, E.O. Ayella, B. Pido, P.A. Onok, M. Ogowang, S. Declich, *Trans. R. Soc. Trop. Med. Hyg.* 99 (2005) 226.
- [2] M. Chopra, I. Darnton-Hill, *Public Health Nutr.* 9 (2006) 544.
- [3] Y. Birku, F. Mekonnen, A. Bjorkman, D. Wolday, *Ethiop. Med. J.* 40 (Suppl 1) (2002) 17.
- [4] M. Mayxay, S. Pukrittayakamee, K. Chotivanich, M. Imwong, S. Looareesuwan, N.J. White, *Am. J. Trop. Med. Hyg.* 65 (2001) 588.
- [5] F. Perandin, N. Manca, G. Piccolo, A. Calderaro, L. Galati, L. Ricci, M.C. Medici, C. Arcangeletti, G. Dettori, C. Chezzi, *Microbiologica* 26 (2003) 91.
- [6] D. Opsenica, D.E. Kyle, W.K. Milhous, B.A. Solaja, *J. Serb. Chem. Soc.* 68 (2003) 291.
- [7] M. Jung, R.F. Schinazi, *Bioorg. Med. Chem. Lett.* 4 (1994) 931.
- [8] F.M.D. Ismail, unpublished observations (2007).
- [9] A.R. Butler, Y.L. Wu, *Chem. Soc. Rev.* 21 (1992) 85.
- [10] E. Hsu, *Trans. R. Soc. Trop. Med. Hyg.* 100 (2006) 505.
- [11] N. Vyas, B.A. Avery, M.A. Avery, C.M. Wyandt, *Antimicrob. Agents Chemother.* 46 (2002) 105.
- [12] A.K. Bhattacharya, R.P. Sharma, *Heterocycles* 51 (1999) 1681.
- [13] M. Frederich, J.M. Dogne, L. Angenot, P. De Mol, *Curr. Med. Chem.* 9 (2002) 1435.
- [14] K. Borstnik, I.H. Paik, T.A. Shapiro, G.H. Posner, *Int. J. Parasitol.* 32 (2002) 1661.
- [15] Y. Li, Y.-L. Wu, *Curr. Med. Chem.* 10 (2003) 2197.
- [16] J. Wiesner, R. Ortmann, H. Jomaa, M. Schlitzer, *Angew. Chem. Int. Ed.* 42 (2003) 5274.
- [17] R.K. Haynes, *Curr. Top. Med. Chem.* 6 (2006) 509.
- [18] M.K. Jung, R.F. Schinazi, *Bioorg. Med. Chem. Lett.* 4 (1994) 931.
- [19] M.R. Romero, T. Efferth, M.A. Serrano, B. Castano, R.I. Macias, O. Briz, J.J.G. Marin, *Antivir. Res.* 68 (2005) 75.
- [20] M. Jung, S. Lee, J. Ham, K. Lee, H. Kim, S.K. Kim, *J. Med. Chem.* 46 (2003) 987.
- [21] M. Jung, K. Lee, H. Kim, M. Park, *Curr. Med. Chem.* 11 (2004) 1265.
- [22] H. Lai, T. Sasaki, N.P. Singh, *Expert Opin. Ther. Targets* 9 (2005) 995.
- [23] H. Lai, T. Sasaki, N.P. Singh, A. Messay, *Life Sci.* 76 (2005) 1267.
- [24] T. Efferth, *Curr. Drug Targets* 7 (2006) 407.
- [25] A. Robert, F. Benoit-Vical, C. Claparols, B. Meunier, *Proc. Natl. Acad. Sci. USA* 102 (2005) 13676.
- [26] A. Robert, F. Benoit-Vical, C. Claparols, B. Meunier, *Proc. Natl. Acad. Sci. USA* 103 (2006) 3943.
- [27] N. Acton, R.J. Roth, *Heterocycles* 41 (1995) 95.
- [28] G.H. Posner, C.H. Oh, D. Wang, L. Gerena, W.K. Milhous, S.R. Meshnick, W. Asawamahasadka, *J. Med. Chem.* 37 (1994) 1256.
- [29] P.G. Fuocho, G. Marconi, Q.G. Mulazzani, *Int. J. Radiat. Biol.* 81 (2005) 319.
- [30] Ongoing nanosecond resonance Raman and pulsed radiolysis study at: (a) the Lasers for Science Facility, Rutherford-Appleton Lab, UK and (b) Free Radical Research Facility, Daresbury Laboratory, UK: S. Alizadeh-Shekalgourabi, M. Ashton, J.P. Bégué, R.H. Bisby, D. Bonnet-Delphon, C. Chollet, I.P. Clark, I.P.A. Crisostomo, M.J. Dascombe, N. Dempster, E.T. Denisov, T. Denisova, M.G.B. Drew, G. Edwards, P.G. Evans, M.J. Frearson, F.M.D. Ismail, J. Metcalf, S. Navaratnam, A.W. Parker, E. Ruth, Unpublished work. Preliminary report: F.M.D. Ismail; M.G.B. Drew, M.J. Dascombe, I.P. Clark, A.W. Parker, <<http://www.clf.rl.ac.uk/Reports/2003-2004/pdf/54.pdf>>.
- [31] J.-M. Liu, M.-Y. Ni, Y.-F. Fan, Y.-Y. Tu, Z.-H. Wu, Y.-L. Wu, W.-S. Chou, *Acta Chim. Sin.* 37 (1979) 129.
- [32] R.K. Haynes, S.C. Vonwiller, *J. Chem. Soc. Chem. Commun.* (1990) 451.
- [33] L.-K. Sy, G.D. Brown, *Tetrahedron* 58 (2002) 897.
- [34] D.-Y. Wang, Y.-K. Wu, Y.-L. Wu, Y. Li, F. Shan, *J. Chem. Soc. Perkin Trans. 1* (1999) 1827.
- [35] Note that in the presence of excess thiols such as cysteine, a catalytic amount of a non-iron transition-metal ion ( $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Ti}^{4+}$ , and  $\text{Mn}^{2+}$ ) may also promote cleavage of artemisinin to generate products synonymous with  $\text{Fe}^{2+}$ -mediated degradation. Y.K. Wu, H.H. Liu, *Helv. Chim. Acta* 86 (2003) 3074.
- [36] W.-M. Wu, Z.-J. Yao, Y.-L. Wu, K. Jiang, Y.-F. Wang, H.-B. Sehn, F. Shan, Y.J. Li, *J. Chem. Soc. Chem. Commun.* (1996) 2213.
- [37] R.K. Haynes, S.C. Vonwiller, *Tetrahedron Lett.* 37 (1996) 287.
- [38] U. Eckstein-Ludwig, R.J. Webb, I.D.A. van Goethem, J.M. East, A.G. Lee, M. Kimura, P.M. O'Neill, P.G. Bray, S.A. Ward, S. Krishna, *Nature* 424 (2003) 957.
- [39] A.C. Uhlemann, A. Cameron, U. Eckstein-Ludwig, J. Fischbarg, P. Iserovich, F.A. Zuniga, M. East, A. Lee, L. Brady, R.K. Haynes, S.A. Krishna, *Nat. Struct. Mol. Biol.* 12 (2005) 628.
- [40] M. Jung, H. Kim, K.Y. Nam, K.T. No, *Bioorg. Med. Chem. Lett.* 15 (2005) 2994.
- [41] W.-M. Wu, Y. Wu, Y.-L. Wu, Z.-J. Yao, C.-M. Zhou, Y. Li, F. Shan, *J. Am. Chem. Soc.* 120 (1998) 3316.
- [42] L. Flohe, H.J. Hecht, P. Steinert, *Free Radic. Biol. Med.* 27 (1999) 966.
- [43] Y. Wu, H.H. Liu, *Chem. Res. Toxicol.* 16 (2003) 1202.
- [44] R.S. Bhakuni, D.C. Jain, R.P. Sharma, S. Kumar, *Curr. Sci.* 80 (2001) 35.
- [45] Y.-L. Wu, J.-L. Zhang, J.-C. Li, *Acta Chim. Sin.* 43 (1985) 901.
- [46] Y. Li, P.-L. Yu, Y.-X. Chen, J.-L. Zhang, Y.-L. Wu, *Ke. Tongbao* 31 (1986) 1038.
- [47] Y.-L. Wu, Y. Li, *Med. Chem. Res.* 5 (1995) 569.

- [48] J.A. Vroman, M. Alvim-Gaston, M.A. Avery, *Curr. Pharm. Des.* 5 (1999) 101.
- [49] R.K. Haynes, H.H.O. Pai, A. Voerste, *Tetrahedron Lett.* 40 (1999) 4715.
- [50] G. Bez, B. Kalita, P. Sarmah, N.C. Barua, D.K. Dutta, *Curr. Org. Chem.* 7 (2003) 1231.
- [51] Y. Tang, Y. Dong, J.L. Vennerstrom, *Med. Res. Rev.* 24 (2004) 425.
- [52] D.Y. Wang, Y.L. Wu, Y.K. Wu, J. Liang, Y. Li, *J. Chem. Soc. Perkin Trans. 1* (2001) 605.
- [53] Y.K. Wu, *Acc. Chem. Res.* 35 (2002) 255.
- [54] P.M. O'Neill, G.H. Posner, *J. Med. Chem.* 47 (2004) 2945.
- [55] Y. Wu, Z.-Y. Yue, Y.-L. Wu, *Angew. Chem. Int. Ed.* 38 (1999) 2580.
- [56] D.L.B. Stringle, R.N. Campbell, M.S. Workentin, *Chem. Commun.* (2002) 1246.
- [57] A.G. Taranto, J.W.D. Carneiro, M.T. de Araujo, *Bioorg. Med. Chem.* 14 (2006) 1546.
- [58] M.G.B. Drew, J. Metcalfe, F.M.D. Ismail, *J. Mol. Struct. Theochem.* 711 (2004) 95.
- [59] M.G.B. Drew, J. Metcalfe, F.M.D. Ismail, *J. Mol. Struct. Theochem.* 756 (2005) 87.
- [60] C. Arantes, M.T. de Araujo, A.G. Taranto, J.W.D. Carneiro, *Int. J. Quantum Chem.* 103 (2005) 749.
- [61] M.A. Avery, P. Fan, J.M. Karle, J.D. Bonk, R. Miller, D.K. Goins, *J. Med. Chem.* 39 (1996) 1885.
- [62] Y.-L. Wu, H.-B. Chen, K. Jiang, Y. Li, F. Shan, D.-Y. Wang, Y.-F. Wang, W.-M. Wu, Y. Wu, Z.-J. Yao, Z.-Y. Yue, C.-M. Zhou, *Pure Appl. Chem.* 71 (1999) 1139.
- [63] Y. Wu, Z.-Y. Yue, H.-H. Liu, *Helv. Chim. Acta* 84 (2001) 928.
- [64] W.-M. Wu, Y.-L. Wu, *J. Chem. Soc. Perkin Trans. 1* (2000) 4279.
- [65] F. Najjar, M. Baltas, L. Gorrichon, Y. Moreno, T. Tzedakis, H. Vial, C. Andre-Barres, *Eur. J. Org. Chem.* (2003) 3335.
- [66] A.R. Butler, B.C. Gilbert, P. Hulme, L.R. Irvine, L. Renton, A.C. Whitwood, *Free Radic. Res.* 28 (1998) 471.
- [67] P.M. O'Neill, A. Miller, L.P.D. Bishop, S. Hindley, J.L. Maggs, S.A. Ward, S.M. Roberts, F. Scheinmann, A.V. Stachulski, G.H. Posner, B.K. Park, *J. Med. Chem.* 44 (2001) 58.
- [68] M.G.B. Drew, J. Metcalfe, M.J. Dascombe, F.M.D. Ismail, *J. Med. Chem.* 49 (2006) 6065.
- [69] T. Efferth, A. Benakis, M.R. Romero, M. Tomicic, R. Rauh, D. Steinbach, R. Hafter, T. Stamminger, F. Oesch, B. Kaina, M. Marschall, *Free Radic. Biol. Med.* 37 (2004) 998.
- [70] For a discussion using a semiempirical approach based on intersecting parabolas see E.T. Denisov, T. Denisova, F.M.D. Ismail, *Int. J. Chem. Kinet.* 37 (2005) 554.
- [71] S.L. Solodova, E.T. Denisov, *Russ. Chem. Bull.* 55 (2006) 1557.
- [72] B. Ye, Y.-L. Wu, *Tetrahedron* 45 (1989) 7287.
- [73] B. Ye, Y.-L. Wu, *J. Chem. Soc. Chem. Commun.* (1990) 726.
- [74] M.A. Avery, P.C. Fan, J.M. Karle, R. Miller, K. Goins, *Tetrahedron Lett.* 36 (1995) 3965.
- [75] M.A. Avery, S. Mehrotra, J.D. Bonk, J.A. Vroman, D.K. Goins, R. Miller, *J. Med. Chem.* 39 (1996) 2900.
- [76] M.A. Avery, S. Mehrotra, T.L. Johnson, J.D. Bonk, J.A. Vroman, R. Miller, *J. Med. Chem.* 39 (1996) 4149.
- [77] C.H. Oh, H.J. Kim, S.H. Wu, H.S. Won, *Tetrahedron Lett.* 40 (1999) 8391.
- [78] M. Jung, K. Lee, H. Jung, *Tetrahedron Lett.* 42 (2001) 3997.
- [79] M. Jung, K. Lee, H. Kendrick, B.L. Robinson, S.L. Croft, *J. Med. Chem.* 45 (2002) 4940.
- [80] M.A. Avery, M. Alvim-Gaston, C.R. Rodrigues, E.J. Barreiro, F.E. Cohen, Y.A. Sabnis, J.R. Woolfrey, *J. Med. Chem.* 45 (2002) 292.
- [81] M.A. Avery, K.M. Muraleedharan, P.V. Desai, A.K. Bandyopadhyaya, M.M. Furtado, B.L. Tekwani, *J. Med. Chem.* 46 (2003) 4244.
- [82] B.J. Kim, T. Sasaki, *Org. Prep. Proced. Int.* 38 (2006) 1.
- [83] M. Hooper, G.C. Kirby, M.M. Kulkarni, S.N. Kulkarni, B.A. Nagasampagi, M.J. O'Neill, J.D. Phillipson, S.R. Rojatkhar, D.C. Warhurst, *Eur. J. Med. Chem.* 25 (1990) 717.
- [84] J.F. La Clair, *Angew. Chem. Int. Ed.* 45 (2006) 2769.
- [85] M.G.B. Drew, M.J. Dascombe, J. Metcalfe, F.M.D. Ismail, unpublished ab initio study on the reduction of hexacyclinol [see 84].
- [86] I. Leban, L. Golic, M. Japelj, *Acta Pharm. Jugoslavica* 38 (1988) 71.
- [87] K.L. Chan, K.H. Yuen, H. Takayanagi, S. Janadasa, K.K. Peh, *Phytochemistry* 46 (1997) 1209.
- [88] J.N. Lisgarten, B.S. Potter, C. Bantuzeko, R.A. Palmer, *J. Chem. Crystallogr.* 28 (1998) 539.
- [89] Cerius2, Accelrys: San Diego, CA, 2002.
- [90] M.J. Frisch et al., *Gaussian03*, Revision C.02 ed. (2004).
- [91] A referee has commented that it might have been preferable to replace C=O5 by CH—CH<sub>3</sub>. However such a molecule would not have been isoelectronic with the other molecules studied in this paper and also the effect of reducing this bond is already being investigated by our study of the arteethers.
- [92] C.W. Jefford, *Drug Discov. Today* 12 (2007) 487.
- [93] J.L. Vennerstrom, S. Arbe-Barnes, R. Brun, S.A. Charman, F.C.K. Chiu, J. Chollet, Y.X. Dong, A. Dorn, D. Hunziker, H. Matile, K. McIntosh, M. Padmanilayam, J.S. Tomas, C. Scheurer, B. Scorneaux, Y.Q. Tang, H. Urwyler, S. Wittlin, W.N. Charman, *Nature* 430 (2004) 900.
- [94] Although organic chemists can identify the peroxide function as redox active (by experience), the classical binding and upregulation of receptors can also induce a pharmacological response and it is necessary to identify methods that can do this using QM methods that facilitate *in silico* drug discovery and mechanism of action.
- [95] S. Krishna, A.-C. Uhlemann, R.K. Haynes, *Drug Resist. Updat.* 7 (2004) 233.
- [96] S. Krishna, C.J. Woodrow, H.M. Staines, R.K. Haynes, O. Mercereau-Puijalon, *Trends Mol. Med.* 12 (2006) 200.
- [97] It is interesting to note that these energy differences are very dependent upon the basis set used as Taranto et al. [57] found values of  $-0.72$ ,  $9.46$  kcal mol<sup>-1</sup> with 6-31G\* and  $17.69$ ,  $26.71$  kcal mol<sup>-1</sup> with 6-31G\*\*//6-31G\*\* for  $\Delta E1$ ,  $\Delta E2$ , respectively.
- [98] P.L. Olliaro, R.K. Haynes, B. Meunier, Y. Yuthavong, *Trends Parasitol.* 17 (2001) 122.
- [99] G.H. Posner, C.H. Oh, *J. Am. Chem. Soc.* 114 (1992) 8328.
- [100] G.H. Posner, D. Wang, J.N. Cumming, C.H. Oh, A.N. French, A.L. Bodley, T.A. Shapiro, *J. Med. Chem.* 38 (1995) 2273.