

Evidence for a Common Non-Heme Chelatable-Iron-Dependent Activation Mechanism for Semisynthetic and Synthetic Endoperoxide Antimalarial Drugs**

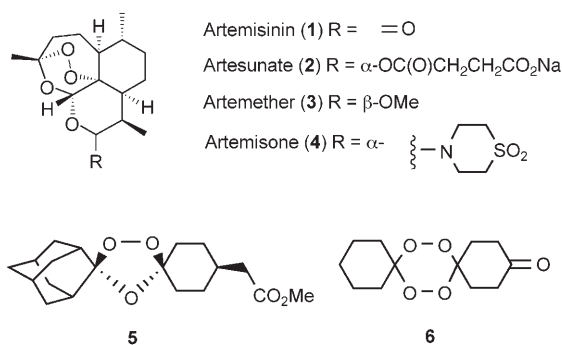
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Herbal extracts of *Artemisia annua* have been used to treat malaria since ancient times. Artemisinin (1), the refined natural product, is extremely potent and displays little or no cross-resistance with other antimalarial compounds.^[1,2] However, it is relatively expensive, has poor bioavailability, and has poor pharmacokinetics, properties that are shared by semisynthetic derivatives such as artesunate (2) and artemether (3).^[3,4] Recently, the development of several structural

intriguing questions about the mechanism of antimalarial selectivity of such diverse chemical structures. Here, we present evidence that all endoperoxide antimalarial compounds share a common free-iron-dependent mechanism of activation in malaria parasites, regardless of their other structural features. None of these drugs are activated in mammalian cell lines, which is consistent with the proposal that selectivity stems from a parasite-specific iron-dependent activation mechanism, rather than recognition of a specific parasite protein target.

The critical common pharmacophore for the entire drug class is an endoperoxide bridge. Analogues without a peroxidic oxygen atom (desoxo derivatives) are essentially devoid of antimalarial activity.^[6,7] This simple observation indicates that all of the endoperoxides must be activated in some way to exert their antimalarial effect. Some investigators have attributed the potency of these compounds to a random "dirty bomb" mechanism centered on the production of oxyl radicals that rearrange to more stable carbon-centered radical (CCR) intermediates.^[8–10] These CCR intermediates are proposed to kill the parasites by alkylating vital parasite macromolecules and heme. In this case, the antimalarial selectivity stems from parasite-specific drug activation.^[11] Other researchers have focused on the interactions of artemisinins with specific target proteins.^[12–14]

An early clue to the involvement of iron in the activation mechanism came from studies which showed that the antimalarial activity of artemisinin is strongly antagonized by iron chelators.^[15] Since the chelators used in this earlier study are selective for non-heme sources of iron, it is apparent that chelatable parasite iron sources may have an important role to play in the mechanism of action of endoperoxide-based antimalarial drugs. Meshnick and co-workers also demonstrated that heme is alkylated by artemisinin,^[16] and elegant work by the Meunier research group has elucidated and described the chemical structures of several drug–heme adducts.^[17–21] Furthermore, metabolites of hemo–artemisinin adducts have been detected in malaria-infected mice treated with artemisinin, thus indicating the potential importance of heme alkylation in the mechanism of action of 1,2,4-trioxanes.^[22] In this study, we set out to demonstrate the importance of non-heme sources of iron for expression of antimalarial activity and irreversible drug accumulation for the three most important classes of endoperoxide antimalarial drugs. In contrast to the recent research published by the Haynes research group, where conclusions on the mechanism



classes of totally synthetic derivatives has raised the real prospect of cheaper alternatives which retain effectiveness and tolerability.^[1,5] This promising work has raised some

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[**] This work was supported by grants from the BBSRC (UK) (P.A.S., P.O.N., P.G.B., V.B., M.J., S.A.W.; BB/C006321/1, BBS/B/05508, BBS/Q/Q/2004/06032, and BBS/S/P/2003/10353), Romark, Florida (R.A.), and in part by the EU (Antimal. FP6 Malaria Drugs Initiative).

Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

of action were drawn based on the reactivity of various C-10 amino analogues with ferrous salts, we have restricted our observations and interpretations to data obtained using living parasites.^[23]

We have employed a combination of isobole analysis and analysis of IC₅₀ values on a series of representative semi-synthetic (**1–4**) and synthetic endoperoxides (**5**, **6**) as well as single-cell confocal imaging of malaria-infected erythrocytes and endoperoxide-insensitive mammalian cell lines with two subsets of purposely designed fluorescent-tagged probe peroxide structures. The latter cell lines were included to demonstrate the exquisite selectivity of malaria parasites for this class of drug.

The isobole technique employed here is accepted by the majority of investigators as the best way of depicting synergistic or antagonistic interactions of a drug combination.^[24,25] A marked antagonism was observed for all the endoperoxide compounds in combination with the iron chelator DFO (**7**, Figure 1). For example Figure 1A shows

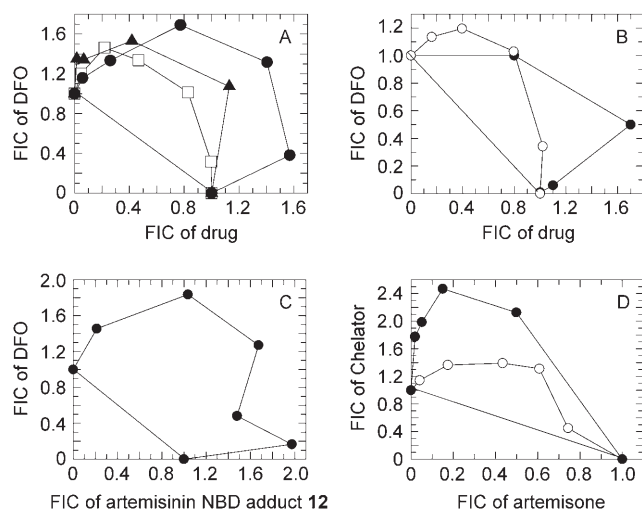


Figure 1. Isobolograms for: A) artemether (**1**; □), the ozonide ester (**5**; ●), and the tetraoxane (**6**; ▲) in combination with DFO; B) artemisinin-acridine adduct **9** (○) and ozonide-acridine adduct **10** (●) with DFO; C) artemisinin-NDB adduct (**12**) in combination with DFO; and D) artemisone (**4**) in combination with DFO (○) or DFP (●). FIC = fractional inhibitory concentration.

clear antagonism between DFO and artemether (**3**), DFO and ozonide ester (**5**), as well as DFO and tetraoxane (**6**).

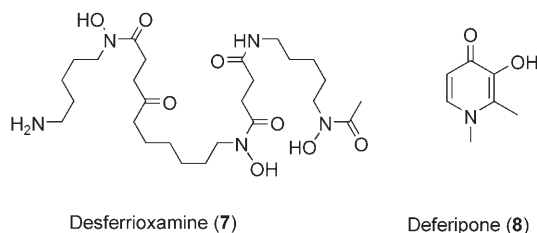
DFO (**7**) is a bulky trishydroxamic acid derivative that chelates ferric iron in a 1:1 molar ratio.^[26] Concerns have been

raised that DFO exerts its effects by chelating extracellular rather than intracellular iron despite data demonstrating that a totally impermeable high-molecular-weight dextran DFO derivative could not mimic the biological effects of DFO iron, thus suggesting an intracellular site for DFO action.^[27] Nonetheless, we have addressed any doubts over the use of DFO by employing the additional, more lipophilic iron chelator deferipone (**8**, DFP).^[28] The results with DFP were similar to those with DFO (not shown). However, the antagonism was more pronounced for some compounds; for example, the combination of artemisone (**4**) and DFO was found to be moderately (but reproducibly) antagonistic, whilst the combination of artemisone and DFP produced a significantly greater degree of antagonism (Figure 1D). In view of the apparent resistance of artemisone to iron activation *in vitro*^[29] and because the iron chelators themselves display antimalarial activity, we needed to rule out any possible nonspecific effects of the iron chelators (it is possible, for example, that the endoperoxides could antagonize the activity of the iron chelators). To address this issue we examined the antagonism of artemisone (and artesunate) by DFP over the much shorter time period of three hours, conditions which had zero effect on parasite viability. By using this protocol we found that the sensitivity of the parasites to artemisone and artesunate was decreased significantly (by tenfold and fivefold, respectively) in the presence of 500 μ M DFP relative to controls (two representative dose-response graphs are shown in Figure S2 of the Supporting Information). These data strongly suggest that non-heme chelatable-free-iron activation is essential for the activity of artemisone (and artesunate).

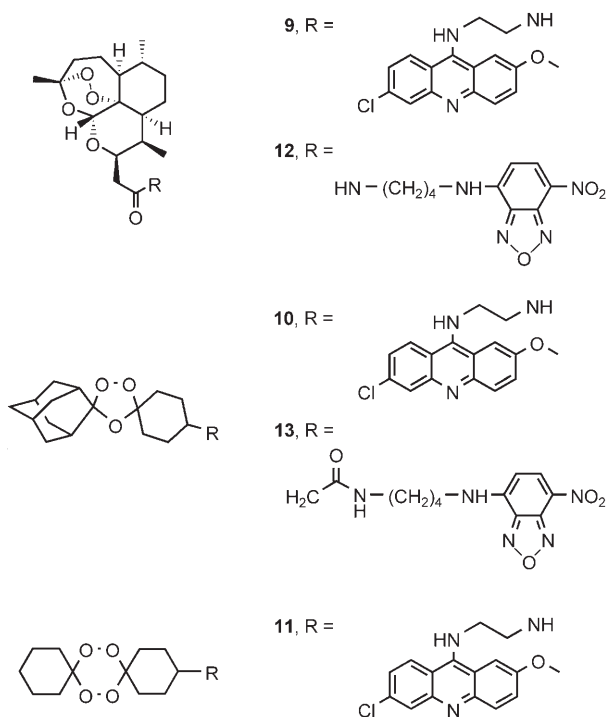
Both chelators employed in our studies are selective for ferric iron, thus suggesting that either bioactivation of the endoperoxide bridge is mediated by a ferric iron source in combination with an endogenous reducing agent or that these chelators alter the equilibrium between ferrous and ferric iron stores within the parasite. (It is well documented that artemisinin derivatives are relatively stable to ferric iron sources in the absence of reducing agents.)^[30]

What is clear is that since the chelators employed are accepted to be selective for non-heme free iron it must be this species of iron that is involved in the activation process. To our knowledge, this is the first direct evidence in malaria parasites that support the role of non-heme iron in the intraparasitic activation process of all endoperoxides, including the artemisone-like semisynthetic compounds which appear to be resistant to degradation mediated by biomimetic ferrous sulfate.^[29] Having demonstrated clear antagonism for both fluorescently tagged and untagged probe derivatives, we then set about investigating the intraparasitic sites of accumulation of representative examples as well as the role of iron chelators in modulating cellular accumulation.

In a recent study in collaboration with the Krishna research group a fluorescently labeled artemisinin was employed in combination with confocal laser scanning microscopy (CLSM), and the results demonstrated that the labeled drug accumulates in the parasite cytoplasm with exclusion from the digestive vacuole.^[31] We also demonstrated that the activation of labeled artemisinin can be prevented by DFO. In

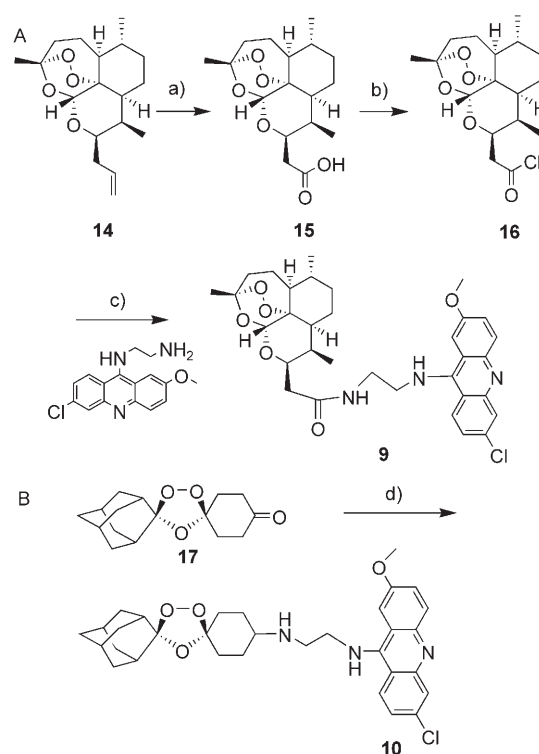


this fluorescent probe an acridine moiety was conjugated to the artemisinin framework through a C-10 ether linkage. To allay concerns that this linkage may be prone to intraparasitic hydrolysis (as is the case for several C-10 ether-linked derivatives in acidic media^[32,33]) we have repeated these studies using a more stable C-10 carba-linked acridine probe. We have extended our initial observations to include appropriately labeled ozonides **10** and **13**, and tetraoxane derivatives **11**.



The synthesis of acridine artemisinin and OZ probe molecules is depicted in Scheme 1. The C-10 allyldeoxyartemisinin **14** was converted into the carboxylic acid with NaIO₄/KMnO₄. The carboxylic acid **15** was quantitatively converted into the acid chloride **16** with two equivalents of oxalyl chloride. Coupling of *N*-1-(6-chloro-2-methoxyacridin-9-yl)ethane-1,2-diamine to **16**, in the presence of triethylamine provided the acridine conjugate **9** in 43% yield. The OZ conjugate **10** was prepared by reductive amination of **17** with the appropriate amino acridine side chain as shown in Scheme 1B.

Tetraoxane conjugate **11** was prepared in a similar manner by reductive amination of **6** with *N*-1-(6-chloro-2-methoxyacridin-9-yl)ethane-1,2-diamine in 68% yield (not shown, but included in the Supporting Information). Figure 2 shows data obtained with fluorescent acridine adducts of artemisinin, tetraoxane, and ozonide ester, and it can be seen that accumulation is selective only for infected erythrocytes. In each case, there is a marked accumulation of fluorescent probe in the parasite cytoplasm, both in the presence and absence of DFO. These data suggest that there may be a parasite-specific accumulation mechanism for all of these lipophilic endoperoxides, perhaps the vacuolar membrane



Scheme 1. Synthesis of: A) C-10 carba-artemisinin conjugate **9** and B) OZ conjugate **10**. a) NaIO₄ (0.004 equiv), acetone/water 1:1, 25 °C, 60%; b) oxalyl chloride (2 equiv), CH₂Cl₂, 0 °C, 100%; c) Et₃N (0.3 equiv), 6-aminoethylamino-9-chloro-2-methoxyacridine (2 equiv), CH₂Cl₂, 0 °C, 25 °C, 75%; d) ClCH₂CH₂Cl, NaBH(OAc)₃, 25 °C, 43%.

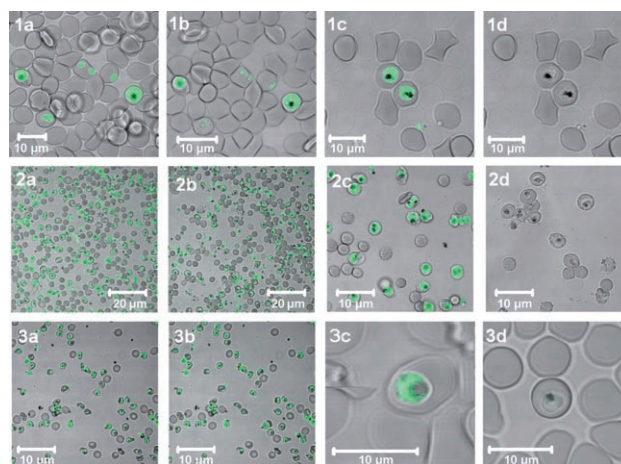


Figure 2. Confocal microscope images of infected red blood cells with acridine conjugates (1 μm) in the presence or absence of DFO (100 μm). All fluorescent-labeled compounds were added 10 min before imaging. For imaging experiments using DFO and DFP (at 100 μm in media), the parasite cultures were preincubated at 37 °C for 30 minutes with the appropriate iron chelator prior to use. After uptake of the drug, the perfusion chamber was washed with 250 × its volume of media to examine the retention of fluorescence. Artemisinin–acridine adduct **9** without DFO before (1a) and after wash (1b), and with DFO (100 μm) before (1c) and after wash (1d). Ozonide–acridine adduct **10** without DFO before (2a) and after wash (2b), and with DFO (100 μm) before (2c) and after wash (2d). Tetraoxane–acridine adduct **11** without DFO before (3a) and after wash (3b), and with DFO (100 μm) before (3c) and after wash (3d).

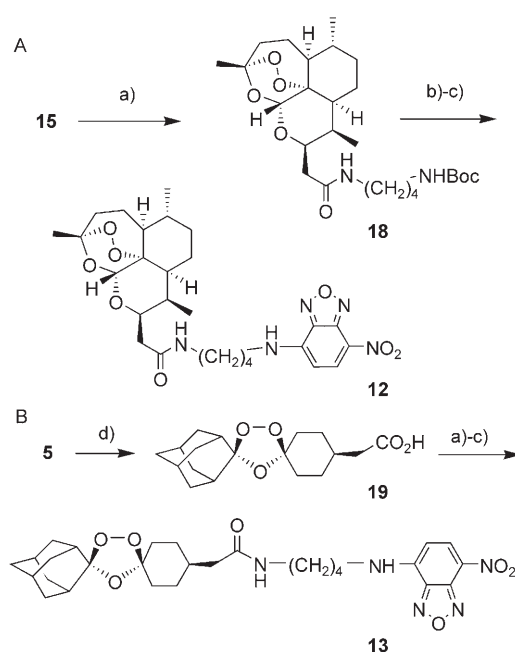
network, as has been suggested for artemisinin.^[31,34] Also of significance is the fact that the drugs can be completely washed out in the presence of DFO (Figure 2, 1c versus 1d, 2c versus 2d and 3c versus 3d), but remain irreversibly bound when the iron chelator is absent (Figure 2, 1a versus 1b, 2a versus 2b, and 3a versus 3b). The simplest interpretation of these data is that the labeled compounds are being accumulated by the parasite and then activated by iron to form stable covalent adducts with parasite macromolecules.

It is also apparent from the images in Figure 2 that acridine-labeled compounds are accumulated in the cytoplasm and excluded from the digestive vacuole. At first glance this seems to rule out drug activation in the digestive vacuole. However, the fluorescence of acridine-based fluorophores is quenched in lysosomes because of their acidic pH value^[35] (the digestive vacuole is a secondary lysosome that maintains an acidic pH at around 4.7).^[36] In addition, we have previously demonstrated that hematin readily quenches the fluorescence of the acridine conjugates **9** (see Figure S3 in the Supporting Information, MJ51) while acridine-based probes demonstrate a propensity to bind to DNA.^[37] These problems raise a number of concerns based on the quenching of the probe in the food vacuole as a result of the low pH value and the presence of hematin as well as the potential for an exaggerated nonvacuolar signal arising from acridine/DNA binding in the relatively large and poorly defined parasite nucleus. To completely eliminate these concerns from the data set we synthesized an additional set of fluorescent probes based on the nitrobenzylidiazole (NBD) fluorochrome. These compounds provide an important control since NBD does not bind to DNA, is insensitive to pH changes in the physiological range,^[38] and is very poorly quenched by hematin.

The syntheses of NBD-artemisinin conjugate **12** and OZ probe **13** are depicted in Scheme 2. The carboxylic acid **15** was activated in situ with dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt), and *N*-methylmorpholine (4-NMM). Subsequent reaction with *tert*-butyl 4-aminobutylcarbamate provided the Boc-protected derivative **18**. Amine deprotection with 4 M HCl and coupling of the hydrochloride with 4-chloro-7-nitrobenzo[*c*][1,2,5] oxadiazole (NBD-Cl) provided the target molecule **12** in 70% yield. The corresponding OZ conjugate **13** was prepared in a similar manner from ozonide ester **5**. Base-catalyzed hydrolysis provided carboxylic acid **19**; this was then allowed to couple with *tert*-butyl 4-aminobutylcarbamate. Cleavage of the Boc group on this amide-coupled derivative provided the free amine which was then coupled with NBD-Cl to give the conjugate **13** (see the Supporting Information).

Table 1 lists the in vitro antimalarial activity^[39] of all the analogues used in this study. All of the compounds express antimalarial activity at less than 13 nM, and it is clear that the presence of the fluorescent group (for example, NBD) has little effect on the inherent drug potency, thereby validating the use of this probe in the experiments that follow. Data were also recorded for artemisinin and artemether versus human peripheral blood mononuclear type B cells, where the IC₅₀ values are greater than 250 μM.

Results of confocal experiments using artemisinin-NBD conjugate **12** and ozonide ester-NBD **13** are presented in



Scheme 2. Synthesis of: A) C-10 carba-NBD conjugate **12** and B) OZ-NBD conjugate **13**. a) 1. DCC (1.25 equiv), HOBt (1.2 equiv), 4-NMM (2.5 equiv), CH₂Cl₂, RT, 24 h; 2. H₂N(CH₂)₄NHBoc (2 equiv), 25 °C, 16 h, 58% for **18**; b) HCl (4 M), 1,4-dioxane, 0 °C, 24 h; c) NBD-Cl (1.1 equiv), NaHCO₃ (0.1 M), MeOH, 60 °C, 2 h, 70% for **13**; d) 1. NaOH (15%), EtOH (95%), 25 °C, 24 h; 2. HCl (3 M) (70%). Boc = *tert*-butoxycarbonyl.

Table 1: In vitro antimalarial activities of selected endoperoxides versus the 3D7 strain of *Plasmodium falciparum* and insensitivity of human PBMC cells to **1** and **3**.

Endoperoxide drug	3D7 <i>Plasmodium falciparum</i> mean IC ₅₀ [nM] ^[a]
artemisinin 1	7.5 ± 0.2
artemether 3	0.9 ± 0.2
OZ ester 5	1.7 ± 1.1
tetraoxane 6	6.7 ± 3.2
artemisinin-acridine conjugate 9	10.7 ± 5.2
OZ-acridine conjugate 10	9.1 ± 5.6
tetraoxane-acridine conjugate 11	12.3 ± 0.9
artemisinin-NBD conjugate 12	13.2 ± 1.9
OZ-NBD conjugate 13	5.2 ± 2.3
	human PBMC (μM) ^[b]
artemisinin 1	> 250
artemether 3	> 250

[a] Drug susceptibilities were assessed by the measurement of fluorescence after the addition of SYBR Green I as previously described by Smilkstein et al.^[39a] Drug IC₅₀ values were calculated from the log of the dose/response relationship, as fitted with Grafit software (Erithacus Software, Kent, United Kingdom). Results are given as the means of at least three separate experiments. [b] IC₅₀ values were calculated using the MTT assay following 72 h incubation with the endoperoxides according to the method of Holl et al.^[39b]

Figure 3. In most respects, the images obtained using the NBD probes are similar to those obtained using acridine probes; both sets of probes were accumulated in the parasite

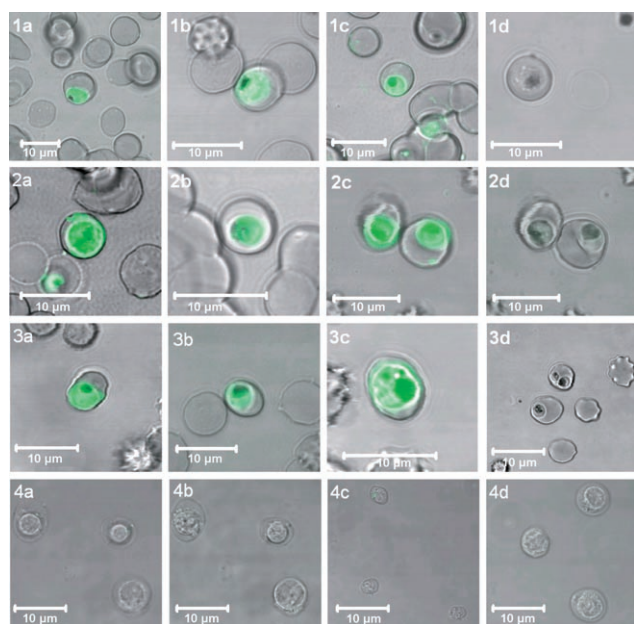


Figure 3. Confocal microscope images of infected red blood cells with fluorescent NBD conjugates (1 μM) in the presence or absence of iron chelators DFO and DFP (100 μM). The same protocols as described in Figure 2 were used. Artemisinin–NBD adduct **12** without DFO before (1 a) and after wash (1 b), and with DFO (100 μM) before (1 c) and after wash (1 d). Artemisinin–NBD adduct **12** without DFP before (2 a) and after wash (2 b), and with DFP (100 μM) before (2 c) and after wash (2 d). Ozonide–NBD adduct **13** without DFP before (3 a) and after wash (3 b), and with DFP (100 μM) before (3 c) and after wash (3 d). Peripheral mononuclear type B cells (PBMC) cells with adduct **12** before (4 a) and after wash (4 b), and with adduct **13** before (4 c) and after wash (4 d).

compartment and in both cases, the formation of stable adducts was inhibited by co-incubation with iron chelators (Figure 3, 1c, 2c, and 3c versus 1d, 2d, and 3d). The only major difference is that the NBD probes were not excluded from the digestive vacuole (Figure 3, 1b–3b and 1c–3c). This is a critical observation as it confirms that the peroxidic drugs do indeed accumulate within the acidic hematin-containing food vacuole, a view discounted by the data presented in our earlier study. In stark contrast to the malaria parasites, nonsusceptible mammalian cell lines (PBMC) apparently take up very little labeled artemisinin conjugate **12** or OZ conjugate **13**. The small amount that is taken up by the PBMCs is freely washed out (Figure 3, 4a–4d), irrespective of whether or not DFO is present.

Based on our data, we propose that the antimalarial selectivity of endoperoxide compounds comes in a large part from common mechanisms of accumulation and bioactivation within the intracellular malarial parasite. Our studies on the intracellular localization of tagged molecules confirm that non-heme chelatable-iron activation in the parasite produces stable adducts that cannot be washed out by cell perfusion with buffer. This does not happen in two peroxide-insensitive mammalian cell lines, which suggests that the activation step is crucial to the selectivity of these drugs. It is important for us to state clearly that the interaction of artemisinins with specific protein targets such as PfATPase6 is not ruled out by

these data;^[31] indeed it is difficult to explain the potency of artemisone without proposing an interaction with this target. Nevertheless, we believe that parasite-specific mechanisms of activation are indispensable and they may be all that is required to explain the antimalarial effects of distant endoperoxide structures such as the ozonide and tetraoxane derivatives. The fact that these compounds are not excluded from the digestive vacuole leaves the door open for a role for chelatable non-heme iron mediated activation in this parasite compartment. As noted, several studies have demonstrated that artemisinin and simplified 1,2,4-trioxanes readily alkylate heme but the impact of this event in the mechanism of action remains unclear, particularly given the fact that other endoperoxides such as arteflene, which fail to produce covalent adducts with heme, express antimalarial activity in the nanomolar range.^[1,40] Chelatable sources of parasite iron play an important role in the mechanism of action of all the important classes of endoperoxide antimalarial drugs. This includes totally synthetic compounds that may lack the structural motifs for binding to PfATPase6, namely the tetraoxanes,^[41] the tetraoxane dimers,^[42] and ozonide derivatives.^[43,44]

Received: November 17, 2006

Revised: May 31, 2007

Published online: July 19, 2007

Keywords: antimalarial agents · chelates · confocal imaging · fluorescence · iron

- [1] P. M. O'Neill, G. H. Posner, *J. Med. Chem.* **2004**, *47*, 2945–2964.
- [2] R. K. Haynes, *Curr. Top. Med. Chem.* **2006**, *6*, 509–537.
- [3] J. A. Vroman, M. Alvim-Gaston, M. A. Avery, *Curr. Pharm. Des.* **1999**, *5*, 101–138.
- [4] G. H. Posner, P. M. O'Neill, *Acc. Chem. Res.* **2004**, *37*, 397–404.
- [5] Y. Q. Tang, Y. X. Dong, J. L. Vennerstrom, *Med. Res. Rev.* **2004**, *24*, 425–448.
- [6] D. L. Klayman, *Science* **1985**, *228*, 1049–1055.
- [7] C. W. Jefford, M. G. H. Vicente, Y. Jacquier, F. Favarger, J. Mareda, P. Millasson-Schmidt, G. Brunner, U. Burger, *Helv. Chim. Acta* **1996**, *79*, 1475–1487.
- [8] G. H. Posner, C. H. Oh, *J. Am. Chem. Soc.* **1992**, *114*, 8328–8329.
- [9] G. H. Posner, C. H. Oh, D. Wang, L. Gerena, W. K. Milhous, S. R. Meshnick, W. Asawamahsakda, *J. Med. Chem.* **1994**, *37*, 1256–1258.
- [10] G. H. Posner, D. Wang, J. N. Cumming, C. H. Oh, A. N. French, A. L. Bodley, T. A. Shapiro, *J. Med. Chem.* **1995**, *38*, 2273–2275.
- [11] S. R. Meshnick, *Int. J. Parasitol.* **2002**, *32*, 1655–1660.
- [12] S. Krishna, A. C. Uhlemann, R. K. Haynes, *Drug Resist. Updates* **2004**, *7*, 233–244.
- [13] W. Asawamahsakda, I. Ittarat, Y. M. Pu, H. Ziffer, S. R. Meshnick, *Antimicrob. Agents Chemother.* **1994**, *38*, 1854–1858.
- [14] Y. Yang-Zi, B. Little, S. R. Meshnick, *Biochem. Pharmacol.* **1994**, *48*, 569–573.
- [15] S. R. Meshnick, Y. Z. Yang, V. Lima, F. Kuypers, S. Kamchonwongpaisan, Y. Yuthavong, *Antimicrob. Agents Chemother.* **1993**, *37*, 1108–1114.
- [16] Y. L. Hong, Y. Z. Yang, S. R. Meshnick, *Mol. Biochem. Parasitol.* **1994**, *63*, 121–128.
- [17] A. Robert, B. Meunier, *J. Am. Chem. Soc.* **1997**, *119*, 5968–5969.
- [18] a) A. Robert, J. Cazelles, B. Meunier, *Angew. Chem.* **2001**, *113*, 2008–2011; *Angew. Chem. Int. Ed.* **2001**, *40*, 1954–1957.

- [19] J. Cazelles, A. Robert, B. Meunier, *J. Org. Chem.* **2002**, *67*, 609–619.
- [20] A. Robert, B. Meunier, *Chem. Soc. Rev.* **1998**, *27*, 273–279.
- [21] A. Robert, O. Dechy-Cabaret, J. Cazelles, B. Meunier, *Acc. Chem. Res.* **2002**, *35*, 167–174.
- [22] A. Robert, F. Benoit-Vical, C. Claparols, B. Meunier, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 13676–13680.
- [23] a) R. K. Haynes, W. Y. Ho, H. W. Chan, B. Fugmann, J. Stetter, S. L. Croft, L. Vivas, W. Peters, B. L. Robinson, *Angew. Chem.* **2004**, *116*, 1405–1409; *Angew. Chem. Int. Ed.* **2004**, *43*, 1381–1385.
- [24] R. J. Tallarida, *J. Pharmacol. Exp. Ther.* **2006**, *319*, 1–7.
- [25] S. R. Meshnick, Y. Z. Yang, V. Lima, F. Kuypers, S. Kamchonwongpaisan, Y. Yuthavong, *Antimicrob. Agents Chemother.* **1993**, *37*, 1108–1114.
- [26] H. Glickstein, W. Breuer, M. Loyevsky, A. M. Konijn, J. Libman, A. Shanzer, Z. I. Cabantchik, *Blood* **1996**, *87*, 4871–4878.
- [27] M. Loyevsky, C. John, B. Dickens, V. Hu, J. H. Miller, V. R. Gordeuk, *Mol. Biochem. Parasitol.* **1999**, *101*, 43–59.
- [28] Z. D. Liu, R. C. Hider, *Coord. Chem. Rev.* **2002**, *232*, 151–171.
- [29] a) R. K. Haynes, B. Fugmann, J. Stetter, K. Rieckmann, H. D. Heilmann, H. W. Chan, M. K. Cheung, W. L. Lam, H. N. Wong, S. L. Croft, L. Vivas, L. Rattray, L. Stewart, W. Peters, B. L. Robinson, M. D. Edstein, B. Kotecka, D. E. Kyle, B. Beckermann, M. Gerisch, M. Radtke, G. Schmuck, W. Steinke, U. Wollborn, K. Schmeer, A. Romer, *Angew. Chem.* **2006**, *118*, 2136–2142; *Angew. Chem. Int. Ed.* **2006**, *45*, 2082–2088.
- [30] a) D. J. Creek, F. C. K. Chiu, R. J. Pranker, S. A. Charman, W. N. Charman, *J. Pharm. Sci.* **2005**, *94*, 1820–1829; b) R. K. Haynes, S. C. Vonwiller, *Tetrahedron Lett.* **1996**, *37*, 257–260; c) R. K. Haynes, S. C. Vonwiller, *Tetrahedron Lett.* **1996**, *37*, 253–256.
- [31] 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* **2003**, *424*, 957–961.
- [32] T. T. T. Nga, C. Menage, J. P. Begue, D. Bonnet-Delpon, J. C. Gantier, *J. Med. Chem.* **1998**, *41*, 4101–4108.
- [33] P. M. O'Neill, N. L. Searle, K. W. Kan, R. C. Storr, J. L. Maggs, S. A. Ward, K. Raynes, B. K. Park, *J. Med. Chem.* **1999**, *42*, 5487–5493.
- [34] T. Akompong, J. VanWye, N. Ghori, K. Haldar, *Mol. Biochem. Parasitol.* **1999**, *101*, 71–79.
- [35] Y. Moriyama, T. Takano, S. Ohkuma, *Biochemistry* **1982**, *92*, 1333–1336.
- [36] R. Hayward, K. J. Saliba, K. Kirk, *J. Cell Sci.* **2006**, *119*, 1016–1025.
- [37] R. Shukla, M. Mishra, S. N. Tiwari, *Prog. Cryst. Growth Charact. Mater.* **2006**, *52*, 107–113.
- [38] H. W. Meyers, R. Jurss, H. R. Brenner, G. Fels, H. Prinz, H. Watzke, A. Maelicke, *Eur. J. Biochem.* **1983**, *137*, 399–404.
- [39] a) M. Smilkstein, N. Sriwilaijaroen, J. X. Kelly, P. Wilairat, M. Riscoe, *Antimicrob. Agents Chemother.* **2004**, *48*, 1803–1806; b) V. Holl, P. Jung, D. Weltin, J. Dauvergne, A. Burger, D. Coelho, P. Dufour, A. M. Aubertin, P. L. Bischoff, J. F. Biellmann, *Anticancer Res.* **2000**, *20*, 1739–1742.
- [40] For model studies on reactions of artemisinin with non-heme iron in the presence of cysteine, see Y. K. Wu, Z. Y. Yue, Y. L. Wu, *Angew. Chem.* **1999**, *111*, 2730–2733; *Angew. Chem. Int. Ed.* **1999**, *38*, 2580–2582.
- [41] a) J. L. Vennerstrom, Y. X. Dong, S. L. Andersen, A. L. Ager, H. N. Fu, R. E. Miller, D. L. Wesche, D. E. Kyle, L. Gerena, S. M. Walters, J. K. Wood, G. Edwards, A. D. Holme, W. G. McLean, W. K. Milhous, *J. Med. Chem.* **2000**, *43*, 2753–2758; b) K. J. McCullough, J. K. Wood, A. K. Bhattacharjee, Y. Dong, D. E. Kyle, W. Milhous, J. L. Vennerstrom, *J. Med. Chem.* **2000**, *43*, 1246–1249.
- [42] D. Opsenica, G. Pocsfalvi, Z. Juranic, B. Tinant, J. P. Declercq, D. E. Kyle, W. K. Milhous, B. A. Solaja, *J. Med. Chem.* **2000**, *43*, 3274–3282.
- [43] 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* **2004**, *430*, 900–904.
- [44] A. C. Uhlemann, S. Wittlin, H. Matile, L. Y. Bustamante, S. Krishna, *Antimicrob. Agents Chemother.* **2006**, *51*, 667–672.