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Brief Article

# Endoperoxide Carbonyl Falcipain 2/3 Inhibitor Hybrids: Toward Combination Chemotherapy of Malaria through a Single Chemical Entity

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We extend our approach of combination chemotherapy through a single prodrug entity (O'Neill et al. *Angew. Chem., Int. Ed.* **2004**, *43*, 4193) by using a 1,2,4-trioxolane as a protease inhibitor carbonyl-masking group. These molecules are designed to target the malaria parasite through two independent mechanisms of action: iron(II) decomposition releases the carbonyl protease inhibitor and potentially cytotoxic C-radical species in tandem. Using a proposed target "heme", we also demonstrate heme alkylation/carbonyl inhibitor release and quantitatively measure endoperoxide turnover in parasitized red blood cells.

## Introduction

As a tractable solution to *Plasmodium falciparum* resistance development,[1] we have developed a novel protease inhibitor peroxide hybrid strategy that encapsulates and refines the concept of combination chemotherapy. Our approach is based on the ability of Fe(II) or heme to selectively cleave the peroxide bridge of the endoperoxide class of drug, a process that appears to be restricted to the malaria parasite. We have designed synthetic routes to produce antimalarial drug hybrids that, as a function of heme (or ferrous iron dependent) peroxide cleavage, will liberate not only free radicals (proposed artemisinin type of action)[2] but also a second antimalarial drug with an independent mechanism of action.

Although the specific mechanism of action of the endoperoxide antimalarials remains controversial,[3−5] we have based our hybrid strategy on previous findings using biomimetic Fe(II) chemistry and electron paramagnetic resonance (EPR[a]) spectroscopy. We have provided evidence that the Roche antimalarial arteflene releases a potentially toxic carbon centered radical in tandem with an electrophilic enone.[6] On the basis of a mechanistic understanding of this process, we developed chemistry for the preparation of a new series of endoperoxide cysteine protease inhibitor (ECPI (**1a**)) prodrugs, compounds that have the capacity to selectively deliver two toxic entities to the malaria parasite. Specifically, we demonstrated that under ferrous mediated conditions, our prototype antimalarials (**1a**) degrade by a designed cascade to produce in a concomitant fashion a potentially toxic carbon radical (**2a**) and a chalcone molecule (**2b**), an entity capable of inhibiting the malaria trophozoite cysteine protease falcipain 2.[7]

## Strategy

The selective parasite reductive cleavage of the endoperoxide prodrug (e.g., **1a**) to an enone system and a secondary C-centered radical might suggest that this approach is relatively limited in the type of inhibitor that can be embedded within the prodrug. On the contrary, mechanistic studies by Vennerstrom on the ferrous mediated decompositions of the 1,2,4-trioxolane pharmacophore have revealed that this heterocycle also readily degrades to carbonyl species in tandem with free radical production (Figure 2A).[8] With this knowledge and as a paradigm for a potentially generic approach to combination chemotherapy, we have focused on peroxides incorporating novel peptidic cysteine protease (falcipain 2/3) inhibitors, compounds with antimalarial effects at the level of the parasite digestive vacuole. Falcipain 2 has been selected as a target, since we have a good working knowledge of the biochemistry and structure−activity relationships (SAR) for carbonyl-based inhibitors of this key protease enzyme.[9]

A key consideration for this strategy is the interaction of the two components combined within the single chemical entity. Combining artemisinin with a chalcone derivative independently as an artemisinin combination therapy (ACT) results in synergistic or additive interactions suggesting that endoperoxides and chalcones can be used together effectively.[10] Thus, the prodrug approach previously explored (Figure 1) has significant advantages over the use of either drug in combination or alone, since it allows selective drug delivery (i.e., metabolically stable) to the parasite food vacuole reducing toxicity to

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[a] Abbreviations: ALLN, calpain inhibitor I, *N*-acetyl-Leu-Leu-Norleu-al; ECPI, endoperoxide cysteine protease inhibitor; ACT, artemisinin combination therapy; SAR, structure−activity relationship; CP, cysteine protease; EPR, electron paramagnetic resonance; MRM, multiple reaction monitoring.

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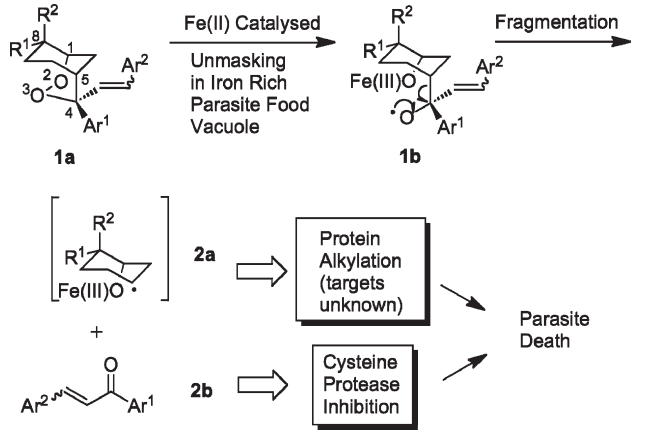



**Figure 1.** Designed ferrous mediated degradation of chalcone prodrug.

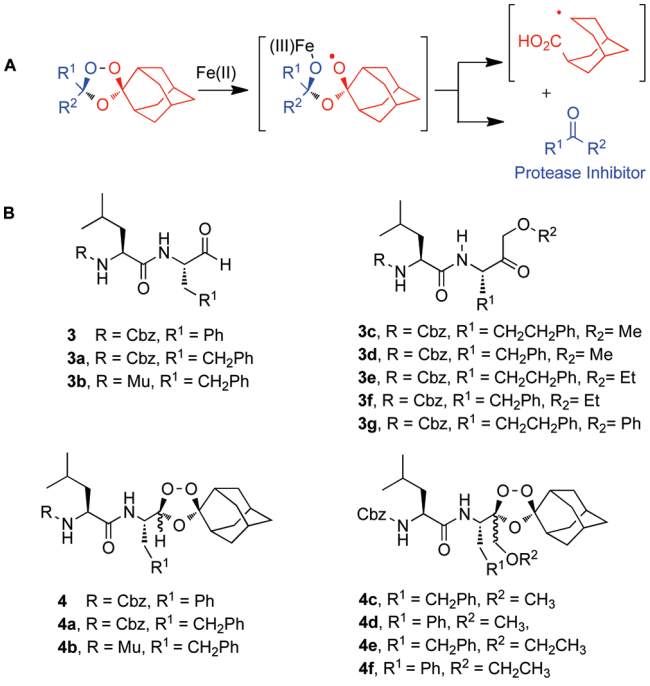



**Figure 2.** (A) Fragmentation of endoperoxide to secondary carbon centred radical species and protease inhibitor. (B) Carbonyl based falcipain 2/3 inhibitors: Cbz = benzyloxycarbonyl; Mu = morpholine urea.

the host cell and its dual mechanism of action should reduce the chance of parasite resistance acquisition.

In line with the above discussion, in the case of peptidic carbonyl based cysteine protease inhibitors (e.g., calpain inhibitor I, *N*-acetyl-Leu-Leu-Norleu-al (ALLN)) we have demonstrated by isobologram analysis that the interaction with artemisinins and synthetic 1,2,4-trioxanes are additive and not antagonistic. With this in mind, for the purposes of comparison, two subsets of carbonyl-based inhibitor were targeted; the carbonyl containing peptides (**3a**−**g**) and the 1,2,4-trioxolane prodrugs (**4a**−**f**) (Figure 2B).

## Chemistry Experimental

The synthesis of target aldehyde inhibitors is presented in Scheme 1A. Briefly, reduction of dipeptide esters **5a** and **5b** followed by Swern oxidation of alcohols of **6a** and **6b** provided aldehydes **3**, **3a**, and **3b**. In studies by Veber on potent low nanomolar peptidic cathepsin K inhibitors it was demonstrated that alkoxymethyl ketones are considerably more stable than their aldehyde counterparts, and for this reason we also included the cysteine protease (CP) inhibitors **3c**−**f** as targets (Figure 2).[11]

Docking studies with the falcipain 2 crystal structure (PDB code 2OUL, 2.20 Å)[12] were performed for the aldehyde and ketone based inhibitors, and docking poses are presented in Figure 3. Each ligand was energy-minimized and then subjected to 10 000 docking runs. The top 10 ranking solutions (i.e., those with the highest fitness score[13]) were visually analyzed for (i) the distance between the potentially reactive carbon atoms (i.e., ketone/aldehyde carbonyl and $CH_2OR$) and the sulfur atom of the catalytic Cys42 residue, (ii) hydrogen bond interactions in the proposed oxy anion hole formed by the residues Gln36, His174, and Cys42, and (iii) hydrophobic interactions between the ligand and nonpolar regions of the enzyme surface. Energy minimized structures place the carbonyl warhead of the inhibitor in the vicinity of Cys42 residue (< 4 Å) and the gold scores for **3c**−**f** encouraged ketone inhibitor synthesis.

Peptides **3c**−**g** were prepared in good overall yields using previously reported standard peptide coupling techniques. The synthesis proceeded with hydrolysis of dipeptides **5a** and **5c** to acids **7a** and **7b** followed by Weinreb amide formation and subsequent Grignard addition chemistry to deliver **3c**−**f** (Scheme 1B).[14] Following optimization studies the target prodrugs (**4a**−**f**) were produced by Griesbaum co-ozonolysis using *O*-methyl 2-adamantanone oxime/$O_3$ and the prodrugs were purified by HPLC as an inseparable mixture of 1:1 diastereomers.[15]

## Discussion

Table 1 lists enzyme inhibitory data of all compounds prepared against recombinant falcipains 2 and 3 and the antimalarial activity against 3D7 malaria parasite strain.[16] It is immediately clear that the aldehydes **3a** and **3b** are potent and selective inhibitors of falcipain 2 with low nanomolar activity. In the keto series only α-phenoxy analogue **3g** expresses nanomolar activity against falcipains 2 and 3. As noted previously, **3a** and **3b** also express low nanomolar antimalarial activity versus the 3D7 strain of *Plasmodium falciparum*; apart from **3g**, alkoxy ketone inhibitors had low activity against both enzymes (2−21 uM). This is surprising given the fact that (a) these molecules contain the optimal residues Leu and homophe/Phe within the structure of the inhibitor and (b) the docking studies performed with FP2 suggested a good enzyme fit for this series. The modest activity for **3c**−**g** suggests that the reactivity of the α-alkoxy group, when compared with the reactive aldehyde in **3a** and **3b**, is insufficient to achieve efficient inactivation of Cys 42 through the usual 1,2-addition process.

The most potent endoperoxides from the series are the aldehyde prodrugs **4**, **4a**, and **4b** and ketone prodrugs **4c** and **4e**. Trioxolane **4b** is active in the low nanomolar region (35 nM) with **4a** active at 55 nM versus the 3D7 strain of *Plasmodium falciparum*. The activities of the parent prodrugs **4a** and **4b** were significantly less against the recombinant enzyme (> 400 nM). Since there is more than a 400-fold difference between the inhibitory potency for aldehyde **3a** and endoperoxide **4a** versus falcipain 2, the fact that these molecules express similar activity versus cultured parasites (27 nM versus 55 nM) indicates that activation and inhibitor release are contributing to the observed whole cell parasite growth inhibition for **4a**.

That **4c**−**f** express moderate antimalarial activity demonstrates that molecules containing the privileged 1,2,4-trioxolane heterocycle do not necessarily have potent antimalarial properties. The fact that **4c**−**f** are not as active as some spirocyclohexyl systems may be again a stability issue (or more likely, as suggested later, the alkoxy group could be hindering

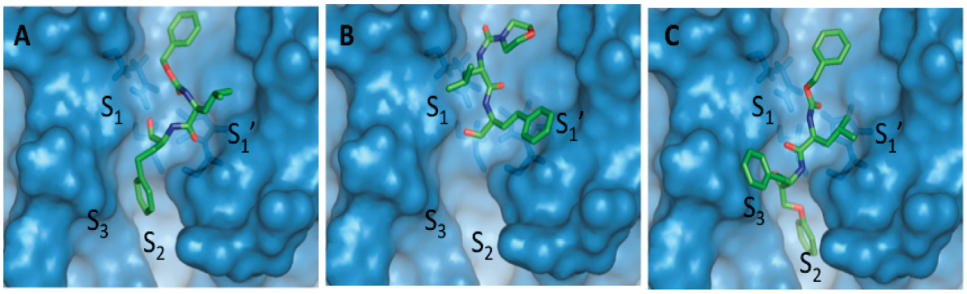
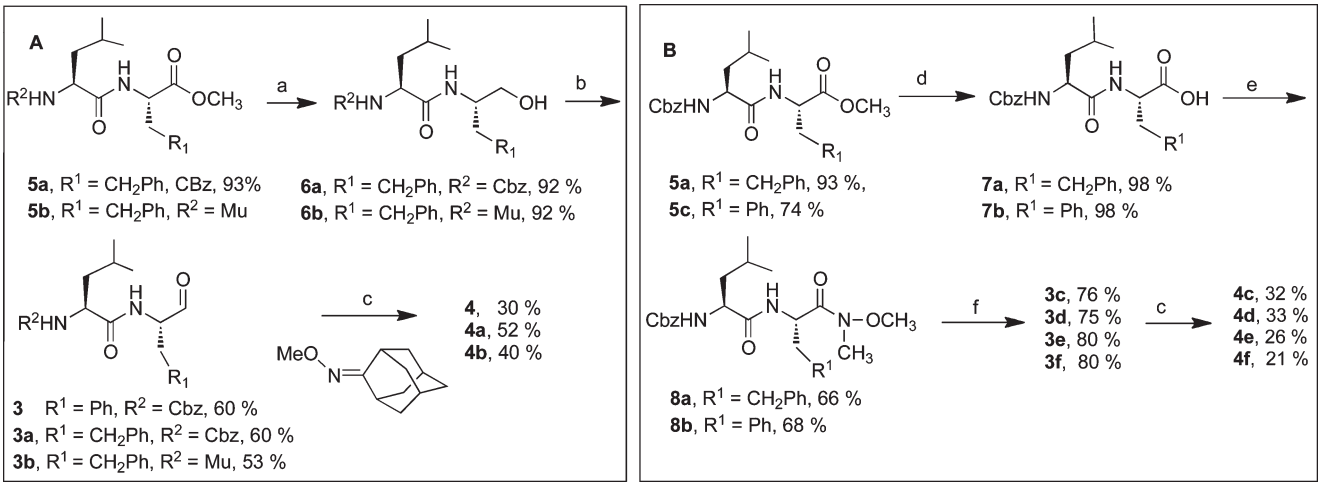



**Figure 3.** Docking poses of aldehydes **3a** (A), **3b** (B), and **3g** (C) in the active site of falcipain 2, obtained from PDB 2OUL. Figures were prepared using Pymol.

**Scheme 1.** Synthesis of 1,2,4-Trioxolane Prodrugs[a]




[a] Part A: (a) $CaCl_2$, $NaBH_4$, MeOH, −5 °C, 2 h; (b) $(COCl)_2$, DMSO, $Et_3N$, $CH_2Cl_2$, 0 °C to room temp, 3 h; (c) $O_3$, pentane/$CH_2Cl_2$ (4:1), carbonyl protease inhibitor. Part B: (d) 2 N NaOH, MeOH, room temp, 16 h; (e) MeONHMe·HCl, HOBt, EDAC, DMAP, DIEA, $CH_2Cl_2$, 0°C, 3 h; (f) Mg, $HgCl_2$, $ROCH_2Cl$, THF, −25 °C, 1.5 h.

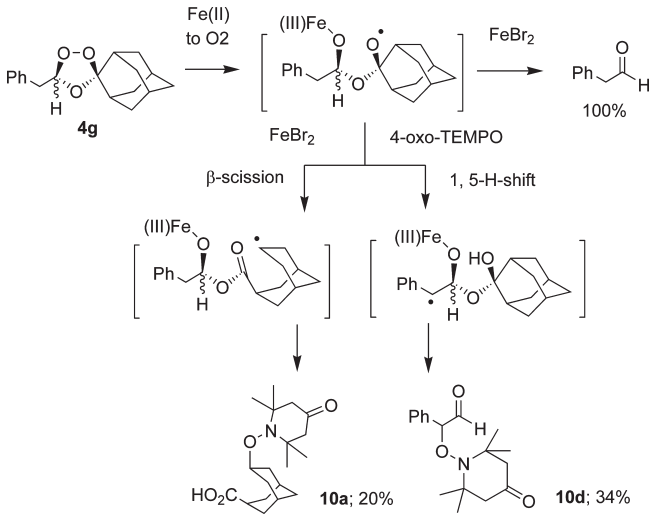
**Table 1.** Enzyme Inhibition (Falcipain 2/3) and Antimalarial Activity of Inhibitors and ECPI Prodrugs

| | $IC_{50}$ (nM ± SD) | | $IC_{50}$ (nM) |
|---|---|---|---|
| analogue | recombinant falcipain 2 | recombinant falcipain 3 | *Plasmodium falciparum* 3D7 |
| **3a** | 1.4 ± 0.2 | 198.9 ± 1.06 | 27.1 |
| **3b** | 16.3 ± 0.9 | 214.6 ± 11.3 | 9.3 |
| **3c** | 7149.5 ± 245 | 5597 ± 1563.7 | > 10000 |
| **3d** | 1966 ± 254 | 8613 ± 913.5 | > 10000 |
| **3e** | 2048.5 ± 14.8 | 853 ± 149 | > 10000 |
| **3f** | 1961 ± 247 | 21600 ± 198 | > 10000 |
| **3g** | 437.7 ± 47.7 | 222 ± 77.8 | 310 |
| **4a** | 425.3 ± 34.5 | 947 ± 133 | 55.5 |
| **4b** | 510.23 ± 25.2 | 332 ± 18.1 | 35.3 |
| **4c** | < 11500 ± 452 | < 19780 ± 3903 | 582 |
| **4d** | 1200 ± 1230 | 44155 ± 7332 | 2564 |
| **4e** | < 8668 ± 501 | < 24215 ± 5239 | 572 |
| **4f** | < 8583 ± 794 | > 25000 | ND |
| artemisinin | NA | NA | 12.5 |

the approach of reducing iron to the endoperoxide-bridge in this trioxolane).

Initial mechanistic studies conducted with trioxolane model compound **4g** ($IC_{50}$ = 100 nM vs 3D7) indicated the feasibility of our approach, since this aldehyde prodrug was efficiently turned over to the requisite aldehyde as shown in Scheme 2 in addition to two distinct carbon centered radical species. The expected adduct **10a** was obtained in 20% yield in combination with **10d**. We propose that **10d** is derived from

**Scheme 2.** Degradation of 1,2,4-Trioxolane Aldehyde Model




the benzylic C-radical intermediate produced itself by a 1,5-H-shift of the initially formed oxyl centered radical as shown in Scheme 2.

With this result in hand we turned to peptide aldehyde prodrug analogue **3**. Scheme 3 provides data on the conversion of selected trioxolane **4** in the presence of ferrous bromide and with heme. For **4**, using $FeBr_2$, we were able to characterize a secondary carbon centered radical spin-trapped adduct

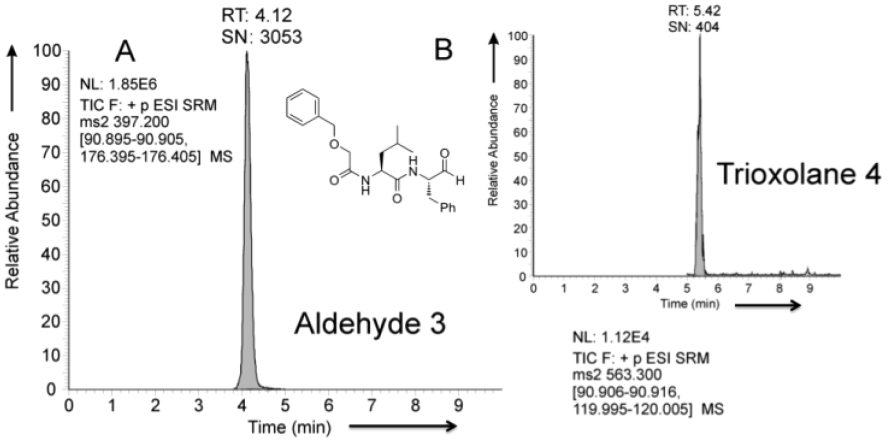
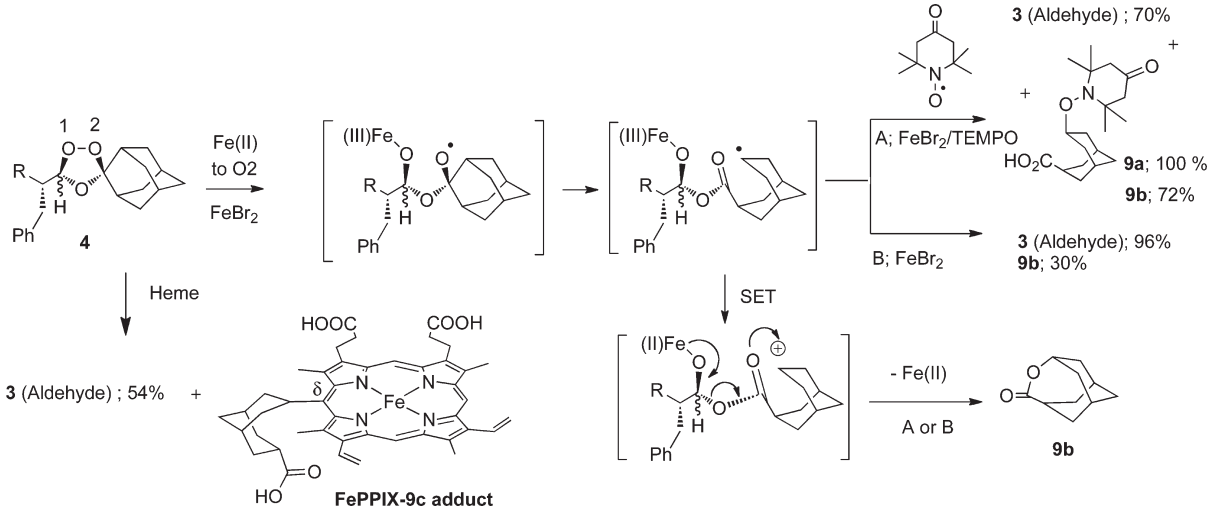



**Figure 4.** Representative chromatograms of **3** and **4** obtained after the analysis of 20 $\mu$L aliquot of 100 nM incubation of **4** with 3D7 malaria parasites. Ion current intensities and multiple reaction monitoring (MRM) transitions are shown in each chromatogram.

**Scheme 3.** Ferrous Mediated Fragmentation of Prodrug **4** (R = Cbz-Leu) and Spin Trapping with 4-Oxo TEMPO[a]




[a] Reactions were performed at 35 °C under nitrogen. Solvent for $FeBr_2$ is ACN/DCM (1:1). Solvent for heme is ACN/$H_2O$ (3:1).

**9a** (quantitative yield) along with lactone **9b** (see mechanism involving a single electron transfer (SET)) from ferric iron and oxidation of the radical to a carbocation with subsequent ring closure. Treatment of trioxolane **4** with Fe(II) in the absence of 4-oxo TEMPO provided a 96% yield of the aldehyde along with 30% of the lactone **9b**. The corresponding reaction with heme (generated from hemin chloride and sodium dithionite) also led to cleavage of the prodrug to the aldehyde in 54% yield in tandem with heme alkylated adduct **9c** which was characterized by LC/MS.

In terms of carbonyl release the reactivity of the corresponding α-alkoxy ketone inhibitor **4e** was much slower with 62% turnover depending on the iron source used. It was considered that the alkoxy group could be hindering the approach of reducing iron to the endoperoxide bridge in this prodrug.

In order to obtain definitive proof of inhibitor release within the living malaria parasite two experiments were performed in late ring/early trophozoites. Parasites were exposed to 1 $\mu$M or 100 nM prodrug **4**. After this time, the media were removed via centrifugation (2000*g* × 5 min), and red blood cells were lysed by the addition of 5 mL of acetonitrile/methanol/water (40:40:20). The lysed material was triturated through a pipet and the lysis solution separated by centrifugation (3000*g* × 5 min). The lysis solution was then analyzed via LC/MS/MS (Supporting Information). As a control, nonparasitised red blood cells were treated in exactly the same manner. Figure 4 shows the representative chromatograms of **3** (Figure 4A) and **4** (Figure 4B) obtained after the analysis of 20 $\mu$L aliquot of 100 nM incubation of **4** (incubation of 20 $\mu$L aliquot of 100 nM) with chloroquine-sensitive *Plasmodium falciparum* (3D7) parasitized red blood cells for 4 h. It was found that after 4 h of incubation **4** was converted to 54 nM **3** (54% yield) with only minor amounts of the trioxolane remaining (1.5 nM detected by LC/MS/MS). Notably, in uninfected erythrocytes, turnover to the aldehyde **3** was considerably lower.

## Conclusion

We have completed the synthesis and assessment of carbonyl based protease inhibitors and their endoperoxide hybrids. Studies on the ferrous mediated decomposition of these molecules have definitively proven that these systems degrade by carbonyl inhibitor formation in tandem with C-radical formation. In studies with a viable parasitic target we have demonstrated efficient heme alkylation with concomitant carbonyl inhibitor release. Furthermore, using LC/MS/MS, we have described for the first time a quantitative intraparasitic estimation of endoperoxide turnover in 3D7 parasites following a 4 h exposure to trophozoites. One potential caveat to this

approach with the 1,2,4-trioxolane heterocycle is the capacity of this system to undergo Hock cleavage under mildly acidic conditions (see Supporting Information Figure S4). While our in vitro studies with iron(II) species definitively prove C-radical formation in tandem with carbonyl release, we cannot rule out that this process is occurring within the acidic digestive vacuole of *Plasmodium falciparum*. A Hock mediated release of the inhibitor, while ensuring efficient delivery, would not provide the desired dual targeting effect. This competing degradation pathway could explain, in part, the lower than expected antimalarial activity for **4a** and **4b**. Further work will focus on the optimization of the endoperoxide carbonyl masking group in terms of tuning the reactivity to achieve selective and controlled drug release within the parasite target while maintaining appropriate pharmacokinetic properties.

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**Supporting Information Available:** Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

**Note Added after ASAP Publication.** The version of this paper that was published ASAP October 27, 2010, was missing author Nuna C. Araujo from the author list. The revised version was published October 29, 2010.

## References

(1) Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; An, S. S.; Yeung, S.; Singhasivanon, P.; Day, N. P. J.; Lindegardh, N.; Socheat, D.; White, N. J. Artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* **2009**, *361* (17), 1714–1714.

(2) O'Neill, P. M.; Posner, G. H. A medicinal chemistry perspective on artemisinin and related endoperoxides. *J. Med. Chem.* **2004**, *47* (12), 2945–2964.

(3) Jefford, C. W. Why artemisinin and certain synthetic peroxides are potent antimalarials. Implications for the mode of action. *Curr. Med. Chem.* **2001**, *8* (15), 1803–1826.

(4) Olliaro, P. L.; Haynes, R. K.; Meunier, B.; Yuthavong, Y. Possible modes of action of the artemisinin-type compounds. *Trends Parasitol.* **2001**, *17* (3), 122–126.

(5) Meshnick, S. R. Artemisinin: mechanisms of action, resistance and toxicity. *Int. J. Parasitol.* **2002**, *32* (13), 1655–1660.

(6) O'Neill, P. M.; Bishop, L. P. D.; Searle, N. L.; Maggs, J. L.; Storr, R. C.; Ward, S. A.; Park, B. K.; Mabbs, F. Biomimetic Fe(II)-mediated degradation of arteflene (Ro-42-1611). The first EPR spin-trapping evidence for the previously postulated secondary carbon-centered cyclohexyl radical. *J. Org. Chem.* **2000**, *65* (5), 1578–1582.

(7) Wang, S. X.; Pandey, K. C.; Scharfstein, J.; Whisstock, J.; Huang, R. K.; Jacobelli, J.; Fletterick, R. J.; Rosenthal, P. J.; Abrahamson, M.; Brinen, L. S.; Rossi, A.; Sali, A.; McKerrow, J. H. The structure of chagasin in complex with a cysteine protease clarifies the binding mode and evolution of an inhibitor family. *Structure* **2007**, *15* (5), 535–543.

(8) Creek, D. J.; Charman, W. N.; Chiu, F. C. K.; Prankerd, R. J.; McCullough, K. J.; Dong, Y. X.; Vennerstrom, J. L.; Charman, S. A. Iron-mediated degradation kinetics of substituted dispiro-1,2,4-trioxolane antimalarials. *J. Pharm. Sci.* **2007**, *96* (11), 2945–2956.

(9) Verissimo, E.; Berry, N.; Gibbons, P.; Cristiano, M. L. S.; Rosenthal, P. J.; Gut, J.; Ward, S. A.; O'Neill, P. M. Design and synthesis of novel 2-pyridone peptidomimetic falcipain 2/3 inhibitors. *Bioorg. Med. Chem. Lett.* **2008**, *18* (14), 4210–4214.

(10) Bhattacharya, A.; Mishra, L. C.; Sharma, M.; Awasthi, S. K.; Bhasin, V. K. Antimalarial pharmacodynamics of chalcone derivatives in combination with artemisinin against *Plasmodium falciparum* in vitro. *Eur. J. Med. Chem.* **2009**, *44* (9), 3388–3393.

(11) Votta, B. J.; Levy, M. A.; Badger, A.; Bradbeer, J.; Dodds, R. A.; James, I. E.; Thompson, S.; Bossard, M. J.; Carr, T.; Connor, J. R.; Tomaszek, T. A.; Szewczuk, L.; Drake, F. H.; Veber, D. F.; Gowen, M. Peptide aldehyde inhibitors of cathepsin K inhibit bone resorption both in vitro and in vivo. *J. Bone Miner. Res.* **1997**, *12* (9), 1396–1406.

(12) Kerr, L. D.; Lee, J. H.; Pandey, K. C.; Harrison, A.; Sajid, M.; Rosenthal, P. J.; Brinen, L. S. Structures of falcipain-2 and falcipain-3 bound to small molecule inhibitors: implications for substrate specificity. *J. Med. Chem.* **2009**, *52* (3), 852–857.

(13) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **1997**, *267* (3), 727–748.

(14) Martinez, J.; Bali, J. P.; Rodriguez, M.; Castro, B.; Magous, R.; Laur, J.; Lignon, M. F. Synthesis and biological-activities of some pseudo-peptide analogs of tetragastrin: the importance of the peptide backbone. *J. Med. Chem.* **1985**, *28* (12), 1874–1879.

(15) Tang, Y. Q.; Dong, Y. X.; Karle, J. M.; DiTusa, C. A.; Vennerstrom, J. L. Synthesis of tetrasubstituted ozonides by the griesbaum coozonolysis reaction: diastereoselectivity and functional group transformations by post-ozonolysis reactions. *J. Org. Chem.* **2004**, *69* (19), 6470–6473.

(16) (a) Smilkstein, M.; Sriwilaijaroen, N.; Kelly, J. X.; Wilairat, P.; Riscoe, M. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. *Antimicrob. Agents Chemother.* **2004**, *48* (5), 1803–1806. (b) Bennett, T. N.; Paguio, M.; Gligorijevic, B.; Seudieu, C.; Kosar, A. D.; Davidson, E.; Roepe, P. D. *Antimicrob. Agents Chemother.* **2004**, *48*, 18. (c) Sijwali, P. S.; Shenai, B. R.; Gut, J.; Singh, A.; Rosenthal, P. J. *Biochem. J.* **2001**, *360*, 481. (d) Shenai, B. R.; Lee, B. J.; Alvarez-Hernandez, A.; Chong, P. Y.; Emal, C. D.; Neitz, R. J.; Roush, W. R.; Rosenthal, P. J. *Antimicrob. Agents Chemother.* **2003**, *47*, 154.