Artemisinin Derivatives

# Reply to Comments on "Highly Antimalaria-Active Artemisinin Derivatives: Biological Activity Does Not Correlate with Chemical Reactivity"

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**Stichwörter:**
alkylation · antiviral agents · heme proteins · iron · porphyrinoids

Laurent, Robert, and Meunier describe the efficient alkylation of the artemisinin derivatives **18** and **23** (our original numbering;[1a] see Figure 1) by iron(II)

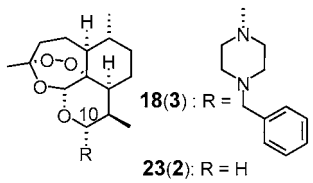


***Figure 1.*** Artemisinin derivatives **18** and **23**, as numbered in our original communication.[1a] In parentheses are the compound numbers (**3** and **2**, respectively) according to Laurent, Robert, and Meunier.[2]

protoporphyrin-IX ($Fe^{II}$-heme) generated under strong reducing conditions (sodium dithionite in dimethylsulfoxide (DMSO) containing 4% water).[2] Glutathione (GSH) is also used as reductant, although no further details are given. According to an earlier publication,[3] this is likely to be a large excess (10 equiv) in DMSO, hardly conditions which can be said to mimic the biological system. We found previously that compound **18** reacts substantially (82%; see Table 2 in Ref. [1a]) with $Fe^{II}$-heme, generated from heme by treatment with cysteine (1 equiv) under aqueous conditions (1:1 MeCN/$H_2O$, pH 7.4 with phosphate buffer), although compound **23** did not.[1a] For compound **18**, besides the organic-soluble product dihydroartemisinin (6%) and non-heme decomposition products (10%), the major organic-insoluble products were heme–artemisinin adducts. However, no attempt was made to isolate or characterize these adducts, and we give credit to Laurent, Robert, and Meunier for their work in this regard.

The authors go on to ascribe the difference in reactivities observed to differences in the solubility of heme in our solvent system and their own. Whether the heme used by the authors contains iron as $Fe^{II}$ or $Fe^{III}$ is not specified, but one may assume that as the "heme" descriptor is used, it is in the $Fe^{III}$ state. Thus, the authors' assumption is tenuous, particularly as compound **18** does react with $Fe^{II}$-heme under our conditions. They also state that derivatives **18** and **23** are "not very soluble" in aqueous solution. However, **18** reacts with $Fe^{II}$-heme in a 1:1 mixture of MeCN and $H_2O$, while **23** is completely decomposed by $Fe^{II}$ sulfate (0.3 or 1.0 equiv) in 1:1 MeCN/$H_2O$. In contrast, compound **18** is much more resistant to the effect of $Fe^{II}$ sulfate, with only a maximum of 27% undergoing decomposition.[1a] Thus, the contrasting reactivities of compounds **18** and **23** towards $Fe^{II}$ and $Fe^{II}$-heme is not a result of solubility factors of the compounds.[1b] Furthermore, the comments made by the authors about the stereochemistry at C10 are not alluded to in our paper and are taken out of context. Nevertheless, if one does examine 3D structures of compound **18**, the piperazine ring is disposed such that access of $Fe^{II}$ to the peroxide bridge may be blocked. However, this is speculative—it is not a statement of fact. Our preparative method,[1a] which could not be reproduced in Meunier's laboratory, works on multigram scales for the preparation of both **18** and all other 10-amino derivatives.

For a drug to cross a membrane, in particular from the blood stream as for an antimalarial drug, it must be dissolved in the medium outside the membrane. The use of DMSO as a vehicle for administering drugs, usually in vitro, cannot be taken to justify the authors' use of DMSO as the medium for their reactions. However, it does raise the question of the detailed environment under which heme is generated in the breakdown of hemoglobin in the food vacuole of the parasite. Whatever the environment it cannot be lipophilic, as apparently assumed by the authors. After all, the pH of the food vacuole can be measured, the food vacuole contains free $Ca^{2+}$ ions, and fluorescence indicators,[4] which are hardly lipophilic molecules, can readily enter. Therefore, we submit that our conditions used for the heme reactions are a rather better mimic of the food vacuole than those of Laurent, Robert, and Meunier.

Laurent, Robert, and Meunier subscribe to the idea that generation of "C-centered radicals" by heme is responsible for antimalarial activity, and substantial effort has been vested thereby in the development of their "trioxaquines". As the heme–artemisinin ad-

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ducts generated from the carbon-centered radicals are inactive as antimalarials, the ability of artemisinins to generate such adducts is irrelevant. If indeed the artemisinins function as antimalarials by generating radicals, according to the heme-activation idea, this requires 1) encounter of artemisinin with $Fe^{II}$-heme in the food vacuole, 2) binding of the peroxide to the iron center in $Fe^{II}$-heme for inner-sphere electron transfer, 3) generation of the C4-centered radical and conversion of $Fe^{II}$-heme to $Fe^{III}$-heme, 4) dissociation of the oxygen ligand (arising from cleavage of the peroxide bridge) of the C4-centered radical from the iron center in $Fe^{III}$-heme, and 5) escape of the resulting "free" C4-centered radical from the environment of the heme, migration, and encounter with a vital parasite protein. The inner-sphere electron transfer requires that Fe is coordinated to the oxygen atom of the peroxide during the decomposition step in which it transforms to an alkoxyl. Therefore, ligand exchange with a putative external ligand (water?) is required to release the C-centered radical from the heme more rapidly than the very fast intramolecular reaction between the radical and the heme.[5] Not surprisingly, it is not possible to trap the C4 radical with cysteine, or indeed glutathione (GSH), when this reagent is used to reduce $Fe^{III}$-heme. The radical, however, does react with cysteine, and much less effectively with glutathione, when free $Fe^{II}$ is used for reductive cleavage of the peroxide. Not all antimalarial-active peroxides alkylate heme-$Fe^{II}$,[6] although there is no doubt that some artemisinins may be sequestered by heme in a biologically irrelevant process.[7] During P-450-mediated Phase I metabolism of artemisinins, inhibition of the enzyme is not observed and hydroxylation of the artemisinins takes place without affecting the peroxide function by heme within P-450.[8] Furthermore, the food vacuole does not contain the bioactive target of the artemisinins.[9]

The thrust of our original paper (see Reference [1a]) is to show that there is no correlation between the ability of artemisinins to react with either heme or non-heme $Fe^{II}$ and their antimalarial activity. The work described by Laurent, Robert, and Meunier does not provide sufficient evidence to the contrary to force a change of view. The extremely high activities of artemisinins described in Reference [1a] are also consistent with binding to a specific target and not to a heme-mediated process that requires conversion of artemisinins into reactive intermediates—C-centered radicals—whose application as parasiticidal agents has so often been presumed but never demonstrated.


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