The Role of Charge Distribution on the Antimalarial Activity of Artemisinin Analogues

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In this work the calculated nuclear quadrupole coupling constants (NQCC; χ) of ^{17}O in artemisinin and some of its derivatives and the effects of charge density due to the nature of ligands on NQCC of ^{17}O were investigated. All calculations were performed at the HF/3-21G level using the Gaussian 98 program. The results show that the O−O linkage has a characteristic role in the antimalarial activity of artemisinin. In addition, various substitutions on C4 change the charge density on these oxygens and consequently change the pharmaceutical effect of artemisinin. Our results suggest that due to a larger charge density on O1, the heme iron approaches the endoperoxide moiety at the O1 position with preference to the O2 position.

INTRODUCTION

A detailed knowledge of the mechanism of action of any drug is very important in drug development, and one can apply this knowledge to improve the drug-receptor interaction needed for the required activity. Theoretical calculations, in particular calculation of quadrupolar parameters of nuclei, seem to be a proper tool for gaining a better understanding of these interactions.

Malaria as an epidemic disease has been the major cause of death in tropical regions of the world, since the prehistoric age, and new strains of drug-resistant plasmodium falciparum are causing substantial deterioration in clinical situations.[1] Artemisinin, a compound isolated from a Chinese herb,[2] has a unique structure with an endoperoxide linkage (Figure 1). In the early clinical studies, this drug showed fast action, low toxicity and high activity against both drug-resistant and drug-sensitive malaria; however its low solubility in water or oil caused difficulty in the rescue of severe patients. This compound also showed a relatively high recrudescence rate.[3] To overcome these shortcomings, many efforts were made at modifying the chemical structure of artemisinin, which led to the new agents. The biological activity and the challenging structure of artemisinin have prompted extensive synthetic efforts to disclose analogues that have more potency and better pharmacokinetic properties compared to the parent molecule, while retaining its biological crucial endoperoxide functionality.[4]

Among the numerous spectroscopic methods that are used to investigate the electronic structure of compounds, nuclear quadrupole resonance (NQR)[5] spectroscopy is a very sensitive technique for molecular specification and determination of the charge distribution around the nuclei of atoms composing the molecules.

Since NQR parameters are very sensitive to the electronic structure,[6] calculated NQR parameters reveal the details of charge distribution of nuclei, which may not be observed by other methods such as atomic charge determination.

In this work, attention has been focused on the details of the charge distribution on artemisinin and some of its derivatives. For this purpose, we performed a theoretical investigation on the details of charge distribution on endoperoxide linkage of these compounds. Establishing a relation between their charge densities and antimalarial activities may help our understanding of the bioaction phenomena of artemisinin. To achieve this, the electric field gradient (EFG) tensor[7] around the quadrupolar nuclei and subsequently their nuclear quadrupole coupling constants (NQCC; χ) were calculated. The obtained results will give information on how the chemical structure of the drug should be modified to achieve suitable interactions; hence, this could bring about the development of new and more effective drugs.

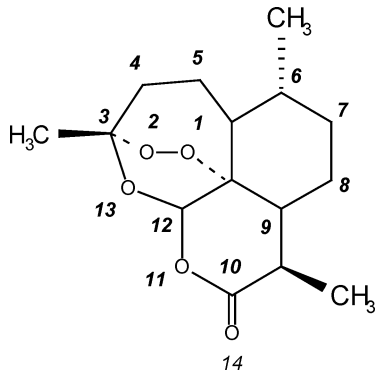

Figure 1. The structure of artemisinin, with atom numbering.

COMPUTATIONAL DETAILS

The molecular structures of the studied derivatives have been fully optimized using the HF method. Previous study[8] in the comparison of various levels of accuracy in artemisinin has shown that the data obtained from HF/3-21G are in good agreement with those of X-ray crystallography.

We examined optimization and ^{17}O−NQCC calculations of artemisinin using 3-21G and some larger basis sets in HF level. The results (summarized in Table 1) showed that NQCC of O1 and O2 are different with all three examined basis sets and in all cases $\chi(O1) > \chi(O2)$.

In addition, since there is no experimental data on NQCCs of artemisinin, HF/3-21G is able to lead us to the qualitative results using calculated NQCCs, which seems to be reasonable, because a qualitative prediction may be obtained faster. Furthermore, many ab initio studies on artemisinin and its

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Table 1. Comparison of Calculated $\chi(O1)$ and $\chi(O2)$ Using Various Basis Sets at the HF Level

level of calculation	$\chi(O1)$ (MHz)	$\chi(O2)$ (MHz)
HF/3-21G	16.425	16.535
HF/6-31G*	16.946	17.233
HF/6-311G*	18.406	18.635

analogues (endoperoxides) have been performed at the HF level, and reasonable results have been obtained.[9,10]

Thus, for the sake of saving CPU times, the HF/3-21G method was chosen to predict reliable information for such models. In this work geometries were fully optimized without any restrictions. Calculations of the Hessian matrix of second derivatives of force in each case (to certify that the optimization converged to minimum) and electric field gradients (EFG) have been performed at the HF/3-21G level for the optimized structures at the same theoretical level. The Gaussian 98 program[11] was used in the mentioned calculations. The expression $e^2Qq_{ZZ}/h = \chi$ is termed as nuclear quadrupole coupling constant and has the unit of frequency (*Hz*). q_{ZZ} is the component of EFG in the direction of Z, and Q is the electric quadrupole moment of the nucleus, $Q(^{17}O) = 25.58$ mb.[12]

RESULTS AND DISCUSSION

Comparison of ^{17}O NQCCs in Artemisinin and Deoxyartemisinin. Artemisinin, at the molecular level, was found to generate a carbon-centered free radical intermediate in the parasite on interaction with heme-iron by opening the endoperoxide functionality of the molecule.[13,14] As it has been suggested by others[15−19] and shown in Scheme 1, the reaction between artemisinin and Fe^{2+} of heme starts with a single electron transfer from Fe^{2+} ion to the peroxide bond. It has been proposed that heme iron attacks the endoperoxide linkage of artemisinin either at the O1 or O2 position, and each has its own route to give the final products (Scheme 1).[20,21]

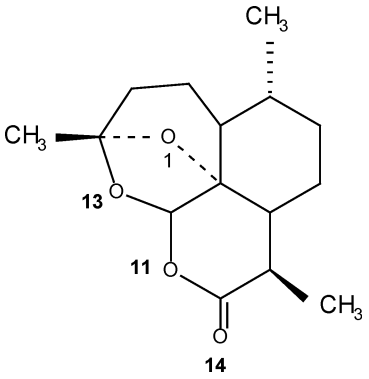

Figure 2. Deoxyartemisinin.

Table 2. Comparison of ^{17}O NQCCs in Artemisinin and Deoxyartemisinin

compound	$\chi(O1)$ (MHz)	$\chi(O2)$ (MHz)	$\chi(O11)$ (MHz)	$\chi(O13)$ (MHz)	$\chi(O14)$ (MHz)
artemisinin	16.425	16.535	11.256	11.703	10.402
deoxyartemisinin	11.537		11.135	11.552	7.084

Since deoxy derivatives are inactive (e.g. deoxyartemisinin; Figure 2) and the only structural difference between these compounds and artemisinin analogues is the endoperoxide bridge, so this moiety is essential for the antimalarial activity of artemisinin. Therefore calculation of NQCC of O1 and O2 may be a useful tool to understand these differences. To further investigate this point, we considered two C4-substituted derivatives of artemisinin that had already been synthesized.[22] The resultant NQCCs from calculated EFG tensors for these compounds are given (Tables 2 and 3).

These results showed that the NQCC of O1 and O2 in artemisinin analogues is greater than that of O1 in deoxyartemisinin by 5 MHz $\chi(O1) = 11.537$ MHz. This important difference could be considered as a criterion in activity of artemisinin analogues.

Scheme 1. Proposed Mechanisms of the Action of Artemisinin

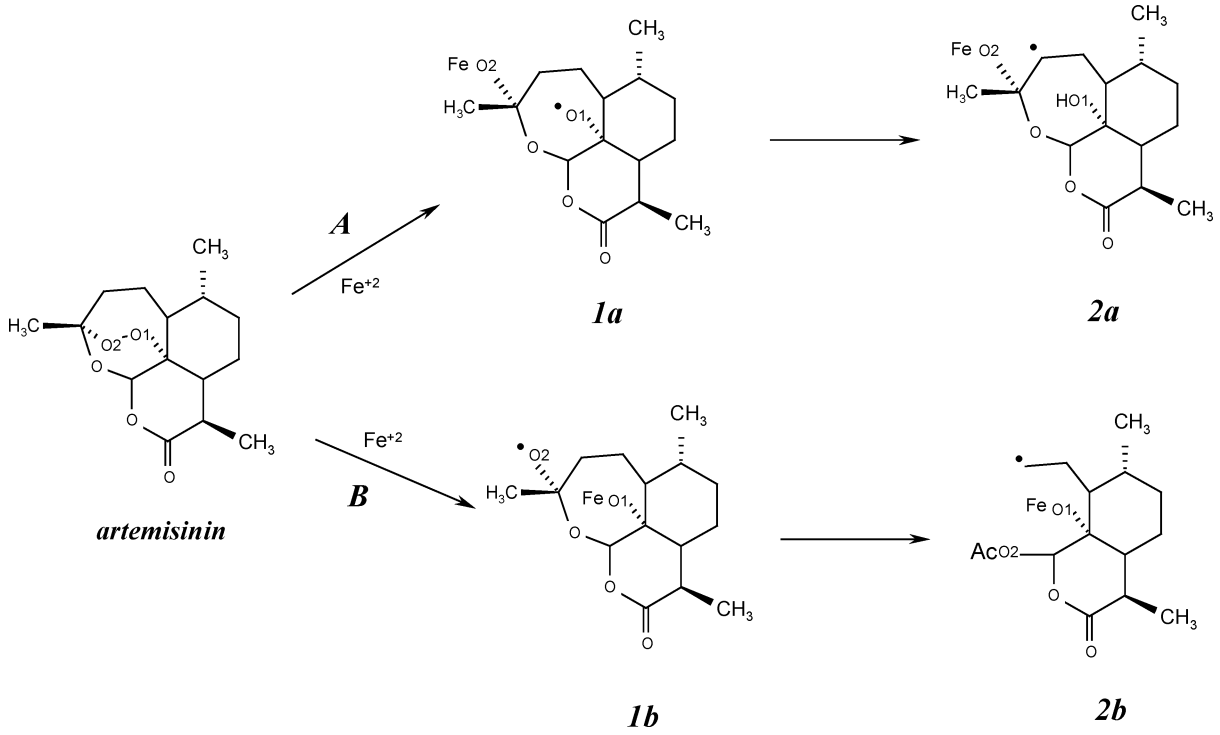

Table 3. Comparison of NQCCs of O1 and O2 in Some Artemisinin Analogues

substituent on C4(R)	$\chi(O1)$ (MHz)	$\chi(O2)$ (MHz)	$\chi(O11)$ (MHz)	$\chi(O13)$ (MHz)	$\chi(O14)$ (MHz)	IC_{50} (ng/mL)[a] D6	W2
H	16.425	16.535	11.256	11.703	10.402	0.5	0.2
OAc	17.071	16.393	10.846	11.724	10.548	11.1	2.07
OH	17.235	16.419	10.795	11.684	10.528		

[a] IC_{50}: inhibitory concentration 50%. *Plasmodium falciparum* D6 (chloroquine sensitive, mefloquine resistant) and W2 (chloroquine resistant, mefloquine sensitive) are as standard controls for IC_{50} values.

From expression e^2Qq_{ZZ}/h, it is obvious that NQCC of nuclei is directly proportional to q_{zz}. There are two factors controlling the value of q_{zz} in a nucleus, charge density on the nucleus and symmetry of EFG around the quadrupolar nucleus. It is evident that increase of charge density causes the q_{zz} and consequently χ to be increased. If charge distribution were such that the symmetry of EFG increased, then q_{zz} and consequently χ will be decreased.

Since the contribution of nonbonding electrons (lone pairs p and d) in the nonspherical charge distribution is greater than the bonding electrons and charges on neighboring ions, therefore in atoms with nonbonding electron pairs (such as oxygen), the EFG is more asymmetric than that of the others and increasing the charge density in these atoms caused the effect of the nonbonding electron pairs to become modest and the symmetry of EFG around the nucleus to be increased. Therefore, in these cases, the values of q_{zz} and χ for oxygen atoms decrease with the increase of charge density.

The smaller value of $\chi(O1)$ compared to $\chi(O2)$ in artemisinin (about 110 kHz) indicates the larger charge density on O1 compared with O2 (it is worth noting that the resolution of modern NQR spectrometers are about a few kHz).[23] Therefore, between the two proposed mechanisms of action for artemisinin (Scheme 1), based on calculated NQCC parameters the formation of radical with unpaired electron on O2 is preferred (route B). This point corresponds to the results of the automated molecular docking of artemisinin to heme[24] that iron in heme interacts with O1 more preferably than O2.

Therefore, it is expected that for a better interaction of Fe^{2+}, the charge density on O1 should be more than that of O2, and thus, any status that increases the charge distribution on O1 (i.e. better condition for Fe^{2+} interaction) and decreases the charge distribution on O2 (i.e. a better situation for the locating of bare electron and radical formation) may cause the antimalarial activity of artemisinin to be increased.

The Effect of C4 Substituents on Artemisinin Activity. Soo-Un et al. indicated that the activity of artemisinin is very sensitive to bulkiness of the side chain, for example by substitution of OH and OAc in the C4(R) position (Figure 3), its antimalarial activity becomes less than that of artemisinin.[22] In comparison to artemisinin, NQCCs calculations show that these derivatives have a larger value of $\chi(O1)$ and a smaller value of $\chi(O2)$ (Table 3). In other words, these substituents cause the charge density on O1 to be decreased and on O2 to be increased. Due to the smaller charge density on O1, the interaction of Fe^{2+} with O1 is less likely. On the other hand, due to the larger charge density on O2, the formation of a radical with an unpaired electron on O2 (Scheme 1, route B) in these derivatives is harder than with artemisinin.

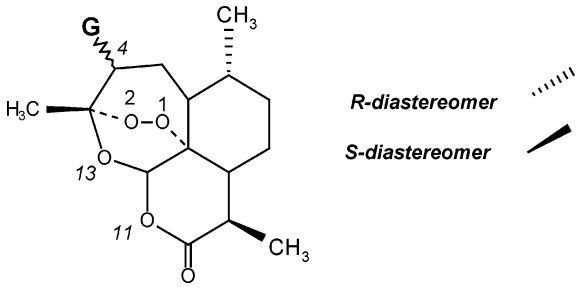

Figure 3. R and S diastereomers of C4-substituted artemisinin derivatives. (The compound could be R or S depending on the position of the G group.)

Table 4. Calculated NQCCs of O1 and O2 for Some Proposed Artemisinin Analogue

substituent on C4	R or S	$\chi(O1)$ (MHz)	$\chi(O2)$ (MHz)	$\chi(O11)$ (MHz)	$\chi(O13)$ (MHz)	$\chi(O14)$ (MHz)
F	R	16.732	16.315	11.290	11.694	10.436
	S	16.288	16.467	11.294	11.711	10.437
Cl	R	16.772	16.247	11.306	11.738	10.448
	S	16.336	16.785	11.297	11.749	10.457
Br	R	16.704	16.262	11.294	11.743	10.442
	S	16.380	16.457	11.277	11.744	10.454
NO_2	R	16.542	16.266	11.330	11.729	10.446
	S	16.332	16.513	11.325	11.764	10.473
CH_3	R	16.839	16.494	10.777	11.723	10.523
	S	16.777	16.477	10.781	11.690	10.519
CH_3CH_2	R	16.884	16.507	10.764	11.712	10.542
	S	16.775	16.475	10.769	11.695	10.519

From Table 3, for artemisinin $\Delta\chi = \chi(O1) - \chi(O2) = 16.425 - 16.535 \langle 0$ and IC_{50} $(W2) = 0.2$ ng/mL, while for OAc(R) substituted artemisinin $\Delta\chi = \chi(O1) - \chi(O2) = 17.071 - 16.393 \rangle 0$ and IC_{50} $(W2) = 2.07$ ng/mL. This implies that for artemisinin there is a larger charge density on O1 and hence better heme-drug interaction so greater artemisinin activity than there is for OAc(R) substituted artemisinin. This is borne out by experimental IC_{50} values; the smaller IC_{50} value means greater activity.

Ponser et al. showed that alkyl groups substitution in C4-(R) in trioxanes, which encourages formation of the C4 radical, increased antimalarial activity as compared to C4-unsubstituted trioxanes, whereas C4(S) substitutions which prevents formation of the C4-radical decrease the efficacy relative to the parent systems.[25] It is evident that in both pathways of suggested mechanisms of action of artemisinin (Scheme 1) C4 has a characteristic role and the C4-centered free radical is an important intermediate in antimalarial activity.[22]

We examine the effect of some electron withdrawing (Cl, F, Br and NO_2) and alkyl group substitutions ($-CH_3$ and $-CH_2-CH_3$) on the activity of C4-substitutions of artemisinin based on the value of NQCC of O1. For this purpose, several substituted derivatives of artemisinin were considered. The EFG tensors and their corresponding ^{17}O NQCCs were calculated (Table 4). By inspection of these results, it has been suggested that in all the mentioned derivatives, the C4-(S) diastereomer has a smaller $\chi(O1)$ relative to the C4(R) diastereomer.

Based on the results of Table 3, the smaller algebraic $\Delta\chi$ $(= \chi(O1) - \chi(O2)$ values may be correlated with more activity. As OH(R) substituted artemisinin ($\Delta\chi = 0.816$,

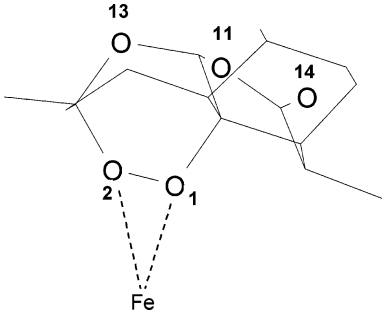

Figure 4. Docking configuration between artemisinin and Fe^{2+} in heme-pdb.

Table 3) is less active than artemisinin ($\Delta\chi = -0.110$), therefore using $\Delta\chi$ of these derivatives, it may be possible to compare the antimalarial activity of those with artemisinin. For example Cl(S)-substituted artemisinin ($\Delta\chi = -0.449$) would be more active than artemisinin ($\Delta\chi = -0.110$).

With further analysis, we explain these results in three groups based on the nature of the ligands:

•**Halogens (F, Cl, Br), Substitutions with High Electronegativity.** Because of the high electronegativity of halogens, the difference of $\chi(O1)$ in two C4 diastereomers (R and S) is significant (around 400 kHz). In other words, the position of these substitutions on C4 may play a major role in determining the ability of these derivatives to interact with heme iron.

•**NO_2, an Electron-Withdrawing Group.** NO_2 is also an electron-withdrawing group, but its effect is less than halogens. This different effect is inferred from smaller $\Delta\chi(O1)$ for various diastereomers of this derivative (about 200 kHz) than that of the halogen derivatives.

•**$-CH_3$ and $-CH_2CH_3$, Alkyl Groups.** Alkyl groups are electron-donating groups. As the NQCC results show, the effect of these substitutions on the charge density of O1 is less than the other above-mentioned groups. The value of $\Delta\chi(O1)$ for these groups is about 80 kHz.

In short, it is concluded that the charge density on O1 in the C4(S) diastereomer is more than that of the C4(R) diastereomer and the binding of the iron ion to the C4(S) diastereomer may be stronger and more complete than the other. Therefore it is expected that the antimalarial activity of C4(S) of these derivatives be more than C4(R) diastereomers.

How Does NQCCs of Oxygens Change Due to Binding of Fe^{II} with Artemisinin? Previous studies[24] showed that heme structures with no or little steric hindrance at the Fe position (such as in heme-pdb; the structure of heme which is stored in the Protein Data Bank) facilitate binding of heme with endoperoxide oxygens. Based on the docking results, artemisinin points its endoperoxide moiety toward the heme iron for the most occurring configuration. Its O1−Fe and O2−Fe distances were measured and found to be 2.49 *A*° and 3.12 *A*°, respectively (Figure 4). For this artemisinin-heme binding, NQCCs of oxygens in the endoperoxide moiety and other oxygens in artemisinin were calculated at the above-mentioned level of theory. These results were compared with those of artemisinin (Table 5). As Table 5 clearly shows, the values of $\Delta\chi(O1)$ and $\Delta\chi(O2)$ are significant, indicating that O1 and O2 atoms are more affected by heme iron than others and the variation of the charge density of these atoms is the most. It further supports our previous suggestion that Fe^{2+} interacts with O−O linkage.

Table 5. Effect of Binding of Fe^{2+} Ion on ^{17}O NQCCs in Artemisinin

compound	$\chi(O1)$ (MHz)	$\chi(O2)$ (MHz)	$\chi(O11)$ (MHz)	$\chi(O13)$ (MHz)	$\chi(O14)$ (MHz)
artemisinin	16.425	16.535	11.256	11.703	10.402
heme-artemisinin	20.209	10.167	11.526	12.192	10.866
$\Delta\chi$	3.784	−6.368	0.270	0.489	0.464

CONCLUSION

From the calculated NQCC results in this work we conclude that

•NQCCs of nuclei can be used as a useful tool to understand the antimalarial activity of drugs.

•In artemisinin, the smaller value of $\chi(O1)$ compared with $\chi(O2)$ suggests that the heme iron approaches the endoperoxide moiety at the O1 position in preference to the O2 position.

•Substitutions of an electronegative group on C4(S) suggest an increase of the antimalarial activity of artemisinin.

•Calculated ^{17}O NQCCs in artemisinin-heme model supports the idea that the O1−O2 moiety is more affected with Fe^{2+} rather than other oxygen atoms.

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REFERENCES AND NOTES

(1) Marshall, E. Reinventing an ancient cure for malaria. *Science* **2000**, *290*, 438−439.
(2) Qinghaosu Antimalaria Coordination Research Group. *Chin. Med. J.* **1979**, *92*, 811.
(3) China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials, Clinical Studies on the treatment of malaria with qinghaosu and its derivatives. *J. Trad. Chin. Med.* **1982**, *2*, 45−50.
(4) Dong, Y.; Matile, H.; Chollet, J.; Kamisky, R.; Wood, J. K.; Vennerstrom, J. L. Synthesis and Antimalarial Activity of 11 Dispiro-1, 2, 4, 5-tetraoxane Analogues of WR 148999. 7,8,15,16-Tetraoxa-dispiro [5.2.5.2] hexadecanes Substituted at the 1 and 10 Positions with Unsaturated and Polar Functional Groups. *J. Med. Chem.* **1999**, *42*, 1477−1480.
(5) Lucken, E. A. C. *Nuclear Quadrupole Coupling Constant*; Academic Press: London, 1969.
(6) Hadipour; N. L.; Rafiee, M. A.; Javaheri, M.; Mousavie, M. K. Ab initio calculations of NQR ^{35}Cl frequency in organo-germanium chlorides and its dependency on Ge−O distance. *Chem. Phys. Lett.* **2002**, *356*, 445−450.
(7) Slichter, C. P. *Principle of Magnetic Resonance*; Harper and Row: London, 1992.
(8) Tonmunphean, S.; Kokpol, S.; Parasuk, V.; Wolschann, P.; Winger, R. H.; Liedl, K. R.; Rode, B. M. Comparative molecular field analysis of artemisinin derivatives: Ab initio versus semiempirical optimized structures. *J. Comput.-Aided Mol. Des.* **1998**, *12*, 397.
(9) Bhattacharjee, A. K.; Skanchy, D. J.; Hicks, R. P.; Carvalho, K. A.; Chmurny, G. N.; Klose, J. R.; Scovill, J. P. Structure of β-Artelinic acid clarified using NMR analysis, molecular modeling and cyclic voltammetry, and comparison with α-Artelinic Acid and β-Arteether, Internet Electron. *J. Mol. Des.* **2004**, *3*, 55−72.
(10) Pinheiro, J. C.; Kiralj, R.; Ferreira, M. M. C.; Romero, O. A. S. Artemisinin derivatives with antimalarial activity against Plasmodium falciparum designed with the aid of quantum chemical and partial least squares methods. *QSAR Comb. Sci.* **2003**, *22*, 830−842.
(11) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, Jr., R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.;

Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A.; Gaussian, 1998.

(12) Pyykko, P. Spectroscopic nuclear quadrupole moments. *Mol. Phys.* **2001**, *99*, 1617.

(13) Meshik, S. R. Artemisinin antimalarials: mechanisms of action and resistance. *Med. Trop.* **1998**, *58*, 13−17.

(14) Posner, G. H.; Wang, D.; Cumming, J. N.; Ho Oh, C.; French, A. N.; Bodley, A. L.; Shapiro, T. A. Further Evidence Supporting the Importance of and the Restrictions on a Carbon-Centered Radical for High Antimalarial Activity of 1,2,4-Trioxanes Like Artemisinin. *J. Med. Chem.* **1995**, *38*(13), 2273−2275.

(15) Wu, W.; Wu, Y.; Wu, Y. L.; Yao, Z.; Zhou, C.; Li, Y.; Shan, F. Unified Mechanistic Framework for the Fe (II)-Induced Cleavage of Qinghaosu and Derivatives/Analogues. The First Spin-Trapping Evidence for the Previously Postulated Secondary C-4 Radical. *J. Am. Chem. Soc.* **1998**, *120*, 3316−3325.

(16) Posner, G. H.; Oh, C. H.; Wang, D. S.; Gerena, L.; Milhous, W. K.; Meshnick, S. R. Mechanism-Based Design, Synthesis, and in vitro Antimalarial Testing of New 4-Methylated Trioxanes Structurally Related to Artemisinin: The Importance of a Carbon-Centered Radical for Antimalarial Activity. *J. Med. Chem.* **1994**, *37*, 1256−1258.

(17) Bloodworth, A. J.; Shah, A. Iron (II)-mediated rearrangement of 1,2,4-trioxanes into 1,2-diol monoesters via 1,5-hydrogen transfer. *Tetrahedron Lett.* **1995**, *36*, 7551−7554.

(18) Posner, G. H.; McGarvey, D. J.; Oh, C. H.; Kumar, N.; Meshnick, S. R.; Asawamahasadka, W. Structure−activity relationships of lactone ring-opened analogues of the antimalarial 1,2,4-trioxane artemisinin. *J. Med. Chem.* **1995**, *38*, 607−612.

(19) Robert, A.; Meunier, B. Characterization of the First Covalent Adduct between Artemisinin and a Heme Model. *J. Am. Chem. Soc.* **1997**, *119*, 5968−5969.

(20) Jefford, C. W.; Vicente, M. G. H.; Jacquier, Y.; Favarger, F.; Mareda, J.; Millasson Schmidt, P.; Brunner, G.; Burger, U. The Deoxygenation and isomerization of artemisinin and artemether and their relevance to antimalarial action. *Helv. Chim. Acta* **1996**, *79*, 1475.

(21) Posner, G. H.; Cummings, J. N.; Ploypradith, P.; Oh. C. H. Evidence for Fe (IV):O in the Molecular Mechanism of Action of the Trioxane Antimalarial Artemisinin. *J. Am. Chem. Soc.* **1995**, *117*, 5885−5886.

(22) Han, J. H.; Lee J. G.; Min, S. S.; Park, S. H.; Angerhofer, C. K.; Cordell, G. A.; Kim, S. U. Synthesis of new artemisinin analogues from artemisinic acid modified at C-3 and C-13 and their antimalarial activity. *J. Nat. Prod.* **2001**, *64*, 1201−1205.

(23) Day, R. O.; Hadipour, N. L.; Ragle, J. L. A deuterium NQR study of imidazolidone, imidazolidone hemihydrate, phthalimide and benzamide. *J. Magn. Reson.* **1986**, *67*, 466−475.

(24) Tonmunphean, S.; Parasuk, V.; Kokpol, S. Automated calculation of docking of artemisinin to heme. *J. Mol. Model.* **2001**, *7*, 26−33.

(25) Cumming, J. N.; Wang, D.; Park, S. B.; Shapiro, T. A. A.; Ponser, G. H. Design, Synthesis, Derivatization, and Structure−Activity Relationships of Simplified, Tricyclic, 1,2,4-Trioxane Alcohol Analogues of the Antimalarial Artemisinin. *J. Med. Chem.* **1998**, *41*, 952−964.

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