Computer-Assisted Design of Pro-drugs for Antimalarial Atovaquone

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Density Functional Theory (DFT) and ab initio calculation results for the proton transfer reaction in Kirby’s enzyme models 1-6 reveal that the reaction rate is largely dependent on the existence of a hydrogen bonding net in the reactants and the corresponding transition states. Further, the distance between the two reacting centers and the angle of the hydrogen bonding formed along the reaction path has profound effects on the rate. Hence, the study on the systems reported herein could provide a good basis for designing antimalarial (atovaquone) pro-drug systems that can be used to release the parent drug in a controlled manner. For example, based on the calculated log EM, the cleavage process for pro-drug 1Pro may be predicted to be about $10^{11}$ times faster than that for a pro-drug 4Pro and about $10^4$ times faster than pro-drug 2Pro: rate $1_{Pro} > rate \ 2_{Pro} > rate \ 4_{Pro}$. Thus, the rate by which the pro-drug releases the antimalarial drug can be determined according to the nature of the linker (Kirby’s enzyme model 1-6).

Key words: ab initio calculations, antimalarial pro-drugs, atovaquone pro-drugs, DFT calculations, Kirby’s enzyme models, proton transfer reaction

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Malaria is, perhaps, the most dangerous protozoan disease. It affects about 300 million people around the world, causing between 1 000 000 and 1 500 000 deaths annually. Children under 5 years old correspond to a large fraction of these fatalities, as malaria kills an African child every 30 seconds. It is estimated that more than 2 billion people (approximately 40% of the world population) are living in regions exposed to malaria infection. Most of the cases of the disease are registered in tropical Africa; though, it is also common in large areas of Latin America, Southern Asia and Oceania. The numerous death cases by malaria in humans are attributed to the most severe type caused by the protozoan parasite Plasmodium falciparum (1).

Chemotherapy is still one of the main control strategies that can be employed in malaria treatment. Generally, all antimalarial medications explore metabolic differences between the parasite and the host, using certain enzymes as targets (1).

The most commonly class of antimalarial agents used in clinical practice are the blood schizonticides like the quinoline-type compounds. Chloroquine is an example of an old affordable medication that is commonly employed in many developing countries. The main challenge facing the world community is the emergence of Plasmodium falciparum strains that are resistant to the chloroquine and other quinoline-type compounds (1).

Another group of antimalarial medications act by inhibiting the enzyme dihydrofolate reductase (DHFR), resulting in protozoan death. However, the development of drug resistance is reducing the efficiency of antifolates as antimalarial drugs. This phenomenon has been linked to the occurrence of mutations in the dihydrofolate reductase of the parasite (1).

In general, the efficacy of old antimalarial drugs has been deteriorated along the last decades, in most cases because of the occurrence of mutations in the primary structure of the target parasite enzyme. For this reason, development of new drugs that are effective against the wild and the mutant strains of the parasite is imperative (1).

The two main advances in drug treatment of malaria have been the development of artemisinin-type compounds and atovaquone as new treatment options for the medical community. Although the new medications helped improve the treatment options, both compounds have many deficiencies that limit their effectiveness (1).

Atovaquone is a highly lipophilic compound with low aqueous solubility, low oral bioavailability (<10% under fasted condition) and high inter-individual variability (2). Improvement in atovaquone pharmacokinetic properties and hence its effectiveness may increase the absorption of the drug via a variety of administration routes. This can be achieved by exploiting a carrier-linked pro-drug strategy (1). The latter can be performed by linking the antimarial drug (atovaquone) to a water-soluble carrier moiety to furnish a drug–host system capable of penetrating the membrane tissues and liberating the antimalarial agent, atovaquone, in a controlled manner (1).

To achieve this goal, the antimalarial pro-drug must possess the following characteristics: (i) to be readily soluble in a physiological environment (ii) to have a moderate hydrophilic lipophylic balance.
Scheme 1: Proton transfer reactions in 1Pro-4-Pro.
(HLB) value (iii) to release upon chemical cleavage the active drug in a controlled manner, and (iv) to provide upon cleavage a safe and non-toxic by-products. By fulfilling the four requirements listed previously, the following goals may be accomplished: (i) a maximum absorption of the pro-drug into the body tissues. (ii) The feasibility to use the drug in different dosage forms. (iii) A chemically driven sustained release system that liberates the antimalarial drug in a controlled manner once the pro-drug reaches the human blood

Scheme 2: Proton transfer reactions in 1-6, where GM and P are the reactants and products, respectively.
circulation system; and (iv) a drug with physical–chemical properties that leading to a high bioavailability and efficient pharmacokineti- 
c properties.

Recently, we have been engaged in investigating the driving force(s) 
responsible for significant accelerations in rate of some intramolecu-
lar processes that have been used as enzyme models and pro-drug 
hosts (3–15). Using different molecular orbital calculation methods, 
we have explored: (i) the acid-catalyzed lactonization of hydroxy 
acids as studied by Cohen (16–21) and Menger (22–27); (ii) the 
intramolecular proton transfer in rigid systems as investigated by 
Menger (22–27); and (iii) the $S_{N}2$-based cyclization as researched 
by Brown, Bruce and Mandolini (28–31) arriving at the following 
conclusions: the driving force for rate enhancements in intramolecu-
lar processes can be attributable to proximity and/or steric effects, 
depending on the nature of the system, and the accelerations in 
rate for intramolecular reactions are a result of both entropy and 
enthalpy effects.

Further, we have concluded from our previous studies that under-
standing the mechanisms by which systems execute their reactions 
is a stepping stone for the design of chemical devices (pro-drugs). 
These pro-drugs have the capability to undergo cleavage reactions 
in physiological environments in rates that are completely depen-
don the structural features of the host (inactive linker) (6–15).

Continuing our research in this area, we sought to study the proton 
transfer reactions in some of Kirby’s enzyme models that are capa-
ble of being good hosts (linkers, carriers) to the antimalarials ato-
vaquone (32–41). Our rational for Kirby’s enzyme model-atovaquone 
pro-drug system is depicted in Scheme 1.

Based on the calculations made, three pro-drugs of atovaquone are 
proposed. As shown in the scheme, the atovaquone pro-drugs, 
1Pro-4Pro, have a carboxylic acid group (hydrophilic moiety) and a 
lipophilic moiety (atovaquone), where the combination of both 
groups ensures a reasonable HLB. Furthermore, in a physiologic envi-
ronment, 1Pro-4Pro will undergo ionization to the carboxylate 
ion form which has a better water solubility than the parent drug 
(atovaquone). Hence, it is expected that pro-drugs 1Pro-4Pro may 
have a better bioavailability than the parent drug because of 
improved absorption. In addition, those pro-drugs may be used in dif-
ferent dosage forms (tablets, suppositories, and etc.) because of 
their potential solubility in organic and aqueous media owing to the 
ability of the carboxyl group to be converted to the carboxylate salt.

It should be emphasized that our proposal is to exploit pro-drugs 
1Pro-4Pro for per os use in the form of enteric coated tablets. 
The latter are stable at the highly acidic pH found in the stomach 
but break down rapidly at a less acidic (relatively more basic) pH. 
For example, they will not dissolve in the acidic juices of the stom-
ach (pH ~3), but they will in the higher pH (above pH 5.5) environ-
ment present in the small intestine. At this physiologic environment, 
those pro-drugs will exist in the acidic and ionic forms where the equilibration constant for the exchange between both 
forms is dependent on the pKa of the given pro-drug. The experi-
mentally determined pKas for 1Pro-4Pro linkers are in the range 
of 3.5–4.0. Therefore, it is expected that the pKas of the corre-
sponding pro-drugs will be in the same range. Because the pH for 
the small intestine lies in the range of 5.5–7.5, and the calculated 
unionized (acidic/ionized ratio will be in the range of 20%–35%. 
Although the percentage of the acidic form is not significantly high, 
we expect that pro-drugs undergoing an efficient proton transfer 
(rate-limiting step) to yield the antimalarial drug (atovaquone) and 
Kirby’s enzyme model by-products (Scheme 1) will have the poten-
tial to be effective pro-drugs. In the blood circulation (pH 7.4), the 
calculated acidic form for those pro-drugs is around 20%, and it is 
expected that the efficiency for delivering the parental drug (ato-
vaquone) will be relatively reduced.

In this manuscript, we describe our DFT and ab initio quantum 
molecular orbital investigations into ground state and transition 
state structures, vibrational frequencies and reaction trajectories for 
the intramolecular proton transfer in six of Kirby’s enzyme model 
systems 1-6 (Scheme 2). It is expected that the calculations study 
on the proton transfers in systems 1-6 will provide a good basis 
for the prediction of the pharmacokinetic behavior of the pro-drugs 
of the type 1Pro-4Pro.

Calculations Methods

The ab initio and DFT calculations were carried out using the quant-
um chemical package Gaussian-98. The starting geometries of all 
the molecules presented in this study were obtained using the 
Argus Lab program (42) and were initially optimized at the Austin 
Model 1 (AM1) level of theory (43). The calculations were carried 
out based on the restricted Hartree-Fock (RHF) method with full 
optimization of all geometrical variables. To avoid results with local 
minima optimization, frequency calculations were carried out for 
these systems. An energy minimum (a stable compound or a reac-
tive intermediate) has no negative vibrational force constant. A 
transition state is a saddle point which has only one negative 
vibrational force constant (44). The “reaction coordinate method” 
(45) was used to calculate the activation energy in systems 1-6. In 
this method, one bond length is constrained for the appropriate 
degree of freedom, while all other variables are freely optimized. 
The activation energy values for the proton transfer reactions were 
calculated from the difference in energies of the global minimum 
structures and the derived transition state (TS). Verification of the 
desired reactants and products was accomplished using the “intrinsic 
coordinate method” (45). The transition state structures were veri-
fied by their only one negative frequency. Full optimization of the 
transition states was accomplished after removing any constrains 
imposed while executing the energy profile. The activation energies 
obtained from DFT and HF levels of theory for 1-6 were calculated 
with and without the inclusion of solvent (water). The calculations 
with the incorporation of a solvent were performed using the inte-
gral equation formalism model of the polarizable continuum model 
(PCM) (46–49).

Results and Discussion

To prolong the pharmacological activity of atovaquone, enhance 
absorption, decrease pharmacokinetic variability and improving the
antimalarial therapeutic strategy, the pro-drug approach of linking atovaquone to an entity, which upon exposure to a physiologic environment, releases the parental drug seems to be promising. Kirby’s kinetic study on some enzyme models has inspired us to utilize these models as appropriate linkers to atovaquone. Our choice is based on Kirby’s experimental kinetic studies that indicate that the rate-limiting step in these processes (processes 1-6, Scheme 2) is a transfer of a proton from the carboxylic group into the neighboring ether oxygen. In addition, the proton transfer rate is largely affected by the formation of strong hydrogen bonding in the products and consequently in the transition state leading to them (32–41). Hence, it is feasible to assume that the rate of the proton transfer will be dependent on the structural features of the enzyme model as evident from the different experimental rate values determined for processes 1-6.

Replacing the methoxy group in 1-6 (Scheme 2) with atovaquone (for example, 1Pro-4Pro, Scheme 1) is not expected to have any effect on the relative rates of these processes. Thus, theoretical investigation into the kinetic and thermodynamic properties for these models will shed some light on the rates for the chemical cleavage of pro-drugs 1Pro-4Pro to atovaquone.

**General consideration**

Because the energy of aliphatic and aromatic carboxylic acids is strongly dependent on its conformation and its ability to be engaged intramolecularly in hydrogen bonding, we were concerned with the identification of the most stable conformation (global minimum) for each of Kirby’s enzyme models (1–6) calculated in this study. This was accomplished by 360° rotation of the carboxylic acid hydroxy group (OH) about the bond O3-C4 in increments of 10° (i.e. variation of the dihedral angle H2O3C4C5, see Chart 1) and calculation of the conformational energies.

In the DFT and *ab initio* calculations for 1-6, two types of conformations in particular were considered: one in which the carboxylic proton is syn to the alkoxy group in β position of the carboxylic acid and another in which it is anti to the alkoxy group. It was found that for 4, the global minimum structure is with the conformation where the carboxylic proton is far away from the alkoxy oxygen (no hydrogen bonding exists between the carboxylic proton and the alkoxy oxygen) and instead it forms a hydrogen bond with a water molecule. For Kirby’s enzyme models 1-3 and 5-6, the conformations with global minimum energies were those having hydrogen bonds between the carboxylic proton and the alkoxy oxygen (syn orientation) (see Figure 1).

**Conformational analysis for the entities involved in the proton transfer processes of Kirby’s enzyme models 1-6 (see Data S1)**

### Starting geometries (GM)

Because the proton transfer reactions for Kirby’s enzyme models 1-6 were carried out in aqueous medium, we have calculated the geometries of the entities involved in these processes in the presence of one molecule of water. The DFT-calculated properties for the starting geometries of 1-6 (1GM-6GM) are shown in Figure 1A and Table 1. Inspections of the calculated geometries of 1GM-6GM indicate that all of them except 4GM exhibit conformation by which the carboxylic group is engaged in a hydrogen bonding net with the neighboring alkoxy oxygen. This engagement results in the formation of seven-membered ring for 1GM and 5GM and six-membered ring for 2GM-4GM and 6GM (see Figure 1A). The DFT-calculated hydrogen bonding length for 1GM-3GM and 5GM-6GM was found in the range of 1.67 Å–1.77 Å and that for the attack angle α (the hydrogen bond angle, O1H2O3) in the range of 147°–171°. Furthermore, the hydrogen bonding strength, rGM (O1-H2), varies according to the structural features of the starting geometry. On the other hand, no intramolecular hydrogen bond was found in the global minimum structure of 4 (4GM). The interatomic distance, rGM (O1-H2), between the carboxylic proton and the ether oxygen in 4GM is 3.62 Å (Figure 1A). This is because the carboxyl group in 4GM prefers to be engaged in hydrogen bonding with a molecule of water rather than intramolecularly, because the latter is energetically expensive owing to a high energy barrier for the rotation of the carboxyl group around the cyclohexyl moiety (50). It should be indicated that Fife reported that the acetal 4 shows no intramolecular general acid catalysis by the neighboring carboxyl group (51).

### Transition state geometries (TS)

The DFT-calculated properties for the transition state geometries of 1-6 (1TS-6TS) are illustrated in Figure 1B and Table 1. Examination of the calculated structures for 1TS-6TS reveals that all the geometries involve a strong hydrogen bonding between the carboxylic proton and the ether oxygen and the hydrogen bonding

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**Chart 1**: Schematic representation of the proton transfer in Kirby’s enzyme models 1-6. GM, TS and P are global minimum, transition state and product structures, respectively. rGM and rP are the O-H distance in the GM and P, respectively. α, β and γ are the angle O-H-O in the GM, TS and P, respectively.
Figure 1: (A) DFT-optimized structures for the global minimum (GM) structures in the intramolecular proton transfer reaction of 1-6. (B): DFT-optimized structures for the transition state (TS) structures in the intramolecular proton transfer reaction of 1-6. (C) DFT-optimized structures for the product (P) structures in the intramolecular proton transfer reaction of 1-6.
**Table 1:** HF and DFT (B3L)-calculated properties for the proton transfer in 1-6

<table>
<thead>
<tr>
<th>System</th>
<th>( r_{GM} ) (Å)</th>
<th>( x ) (°)</th>
<th>( \beta ) (°)</th>
<th>( r_{TS} ) (Å)</th>
<th>( x ) (°)</th>
<th>( \beta ) (°)</th>
<th>( \gamma ) (°)</th>
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<td>144</td>
<td>1.69</td>
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<td>131</td>
<td>3.66</td>
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<td>131</td>
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<td>1.72</td>
<td>170</td>
<td>162</td>
<td>1.72</td>
<td>1.45</td>
<td>171</td>
<td>162</td>
</tr>
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<td>146</td>
<td>161</td>
<td>1.76</td>
<td>1.66</td>
<td>147</td>
<td>161</td>
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</table>

HF and B3L refer to values calculated using HF/6-31G and B3LYP/6-31G (d, p) methods, respectively. GM and TS refer to global minimum and transition state structures, respectively. \( r_{GM} \) and \( r_{TS} \) are the O-H distance in GM and P, respectively (Chart 1). \( x, \beta, \gamma \) are the OHO angle in GM, TS and P, respectively (Chart 1).

The results indicate that the presence of a water as a solvent has a profound effect on the relative rate values. This is in accordance with previously reported studies by Kirby and Fife that indicate the importance of water in the mechanistic pathway for the proton transfer in such systems (32–41,51).

**Product geometries (P)**
The DFT-calculated geometries for the products of 1-6 (1P-6P) shown in Figure 1C and listed in Table 1 indicate the presence of a strong hydrogen bonding net between O1-H2-O3 where the bond length ranges from 1.45 Å to 1.66 Å and the O1-H2-O3 angle is in the range of 154°–170°. This is similar to that found for the corresponding transition state structures, 1TS-6TS.

**Calculations of the kinetic parameters (activation energy, \( \Delta AG^\ddagger \)) for the proton transfer in Kirby’s enzyme models 2-6**
Using the quantum chemical package Gaussian-98 (9), we have calculated the \textit{ab initio} HF/6-31G and the DFT B3LYP/6-31G (d,p) kinetic and thermodynamic parameters for the intramolecular proton transfer in processes 1-6 (Scheme 2).

The HF/6-31G and the B3LYP/6-31G (d,p) activation energy values were calculated with and without the inclusion of solvent (water).

**Table 2:** HF and DFT (B3LYP)-calculated properties for the proton transfer reactions of 1-6

<table>
<thead>
<tr>
<th>Structure</th>
<th>HF Enthalpy, ( H ) (gas phase)</th>
<th>HF (gas phase)</th>
<th>HF Frequency/Cm</th>
<th>B3LYP Enthalpy, ( H ) (gas phase)</th>
<th>B3LYP (gas phase)</th>
<th>B3LYP Frequency/Cm</th>
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<tbody>
<tr>
<td>1GM</td>
<td>–986.66834</td>
<td>144.16</td>
<td>–</td>
<td>–993.05253</td>
<td>153.17</td>
<td>–</td>
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<tr>
<td>1TS</td>
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<td>142.59</td>
<td>99.35i</td>
<td>–993.00826</td>
<td>144.17</td>
<td>181.03i</td>
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<tr>
<td>2GM</td>
<td>–1069.46182</td>
<td>168.96</td>
<td>–</td>
<td>–1076.50693</td>
<td>172.52</td>
<td>–</td>
</tr>
<tr>
<td>2TS</td>
<td>–1069.41300</td>
<td>162.59</td>
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<td>–1076.45691</td>
<td>160.07</td>
<td>611.61i</td>
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<td>3GM</td>
<td>–951.27252</td>
<td>157.29</td>
<td>–</td>
<td>–957.37381</td>
<td>159.64</td>
<td>–</td>
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<tr>
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<td>184.58i</td>
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<td>4GM</td>
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<td>–</td>
<td>–961.00056</td>
<td>152.14</td>
<td>–</td>
</tr>
<tr>
<td>4TS</td>
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<td>144.84</td>
<td>289.93i</td>
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<td>578.23i</td>
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<td>–893.73201</td>
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<td>–</td>
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<tr>
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<td>165.49i</td>
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<tr>
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HF and B3LYP refer to values calculated by HF/6-31G and B3LYP/6-31G (d, p), respectively. GM and TS are global minimum and transition state structures, respectively.
The rGM distance values were achieved when the values of the attack angle (α) in the GM conformations were high and close to 180°, whereas small values of α resulted in longer rGM distances (Figure 1A and Table 1). Figure 2A illustrates the linear correlation between the distance rGM and the attack angle α. Similarly, the corresponding transition state structures, 1TS-6TS, shown in Figures 1B indicate that the angle β value formed at the transition state is largely determined by the conformational structural features of the corresponding starting geometry. In fact, linear correlation was found between the three parameters ΔH‡, β and α as shown in Figure 2B.

<table>
<thead>
<tr>
<th>System</th>
<th>Medium</th>
<th>ΔH‡</th>
<th>TΔS‡</th>
<th>ΔG‡</th>
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<th>B3LYP calculated log EM</th>
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HF and B3LYP refer to values calculated by HF/6-31G and B3LYP/6-31G (d, p) methods, respectively. ΔH‡ is the activation enthalpic energy (kcal/mol). TΔS‡ is the activation entropic energy in kcal/mol. ΔG‡ is the activation free energy (kcal/mol). EM = e^((ΔH‡−ΔS‡)/R)T.

$y = 1.3965x + 26.082$
$R^2 = 0.8379$

$y = 1.7373x + 23.555$
$R^2 = 0.9277$

$y = 0.1962x + 24.225$
$R^2 = 0.9315$

$y = 5.0055x – 129.13$
$R^2 = 0.7404$

**Figure 2:** (A) Plot of the DFT-calculated rGM vs. angle α in 1-6, where α is the attack (hydrogen bond) angle in the GM structure. (B) Plot of the DFT-calculated ΔH‡ vs. angle β – angle α in 1-6, where α is the attack (hydrogen bond) angle in the GM structure and β is the hydrogen bond angle at TS. (C) Plot of the DFT-calculated ΔH‡ and ΔG‡ vs. rGM^2 × sin(180–α) in 1-6, where α is the attack angle and r is the distance between the two reactive centers in the GM structure. (D) Plot of the DFT-calculated ΔG‡ vs. angle β – angle α in 1-6, where α is the attack (hydrogen bond) angle in the GM structure and β is the hydrogen bond angle at TS.
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Inspection of Tables 1 and 3 reveals that the free activation energy ($\Delta G^0$) in the gas phase and water needed to execute a proton transfer in systems 1-6 is largely affected by both the distance $r_{GM}$ (O1-H2) and the attack angle $\alpha$ (O1H2O3) (see Chart 1). Systems with low $r_{GM}$ and high $\alpha$ values in their global minimum structures, such as 1 and 5, exhibit much higher rates (lower $\Delta G^0$) than those having high $r_{GM}$ and low $\alpha$ values, such as 4. Linear correlation of the calculated DFT free activation ($\Delta G^0$) and enthalpic activation energies ($\Delta H^0$) with $r_{GM}$ and $\alpha$ values gave good correlations with relatively high correlation coefficients, $R = 0.92$ and 0.99, respectively (Figure 2C).

To shed light on the mode and action of the proton transfer in systems 1-6, the different values of ($\beta$-$\alpha$) were examined for linear correlation with both the free ($\Delta G^0$) and the enthalpic activation energies ($\Delta H^0$). The correlation results shown in Figure 2D indicate a relatively good correlation with correlation coefficients of $R = 0.87$–0.99. Proton transfer in systems with low ($\beta$-$\alpha$) values is faster than that in systems having higher ($\beta$-$\alpha$) values. For example, the ($\beta$-$\alpha$) value for process 1 is 0, and its activation energy is 24.15 kcal/mol, whereas for process 4 the ($\beta$-$\alpha$) value is 49.8 and its $\Delta G^0$ is 39.11 kcal/mol (Table 3). This is intuitively reasonable as one would expect that an extra energy is needed when the structures of the starting geometry and the transition state are so different.

The effective molarity parameter (EM) is defined as the rate ratio ($k_{intra}/k_{inter}$) for corresponding intramolecular and intermolecular processes driven by identical mechanisms. The main factors affecting the effective molarity are ring size, solvent and reaction type. The EM is considered the best tool to evaluate the efficiency of a certain intramolecular process (52). Because absolute EM values for processes 1-6 are not available, we sought to introduce our computational rational for calculating these values based on the DFT-calculated activation energies ($\Delta G^0$) for 1-6 and the corresponding intermolecular process 10 (Scheme 3).

Using Eqns 1–4, we have derived Eqn 5 that describes the EM term as a function of the difference in the activation energies of the intramolecular and corresponding intermolecular processes. Using Eqn 5, we have calculated the EM values for processes 1-6. The calculated EM values are listed in Table 3.

\[
EM = \frac{k_{intra}}{k_{inter}} \quad (1)
\]

\[
\Delta G^0_{inter} = -RT \ln k_{inter} \quad (2)
\]

\[
\Delta G^0_{intra} = -RT \ln k_{intra} \quad (3)
\]

\[
\Delta G^0_{inter} - \Delta G^0_{intra} = -RT \ln k_{intra}/k_{inter} \quad (4)
\]

\[
EM = e^{-(\Delta G^0_{intra} - \Delta G^0_{inter})/RT} \quad (5)
\]

where $T$ is 298°K and $R$ is the gas constant.

Inspection of the EM values listed in Table 3 reveals that 5 is the most efficient process among 1-6 (log EM >12), and the least efficient is process 4 with log EM <1. Although the EM values for 1-6 were not experimentally determined, Kirby and coworkers estimated the experimental EM value for process 1 in the order of $10^{10}$. The DFT-calculated value for 1 is $3.8 \times 10^{10}$, which is in agreement with the experimental estimated value (32–41).

Conclusions and Future Directions

The ab initio and DFT calculation results revealed that the proton transfer rate in systems 1-6 is quite responsive to geometric disposition, especially to distance between the two reactive centers, $r_{GM}$, and the angle of attack, $\alpha$. Requirements for a system to achieve a high intramolecular proton transfer rate are: (i) a short distance between the two reactive centers ($r_{GM}$) in the ground state (GM) which subsequently results in strong intramolecular hydrogen bonding and (ii) the value difference between the attack angle $\alpha$ in the ground state and the angle $\beta$ formed at the transition state should be minimal. This is to maximize the orbital overlap of the two reactive centers when they are engaged along the reaction pathway.

In summary, we conclude that the study on systems 1-6 reported herein could provide a good basis for designing pro-drug systems that can be used to release the parent drug in a controlled manner. For example, based on the calculated log EM, the cleavage process for pro-drug 1Pro may be predicted to be about $10^{11}$ times faster than that for a pro-drug 4Pro and about $10^{9}$ times faster than pro-drug 2Pro: rate 1Pro > rate 2Pro > rate 4Pro (Scheme 1). Hence, the rate by which the pro-drug releases the antimalarial drug can be determined according to the nature of the linker (Kirby’s enzyme model).

Our future directions include the synthesis of pro-drug 1Pro-4Pro according to known procedures followed by in vitro kinetic studies at different pH values. The kinetic data results will provide us with the data basis for the pharmacokinetic studies, in vivo.

Based on the in vitro results, one or more of the pro-drug systems will be tested in vivo in addition to atovaquone as a control. The pro-drug will be administered to animals by i.v. injection and per

\[
\Delta G^0_{intra} - \Delta G^0_{inter} = -RT \ln k_{intra}/k_{inter} \quad (4)
\]

\[
EM = e^{-(\Delta G^0_{intra} - \Delta G^0_{inter})/RT} \quad (5)
\]

\[
\Delta G^0_{inter} - \Delta G^0_{intra} = -RT \ln k_{intra}/k_{inter} \quad (4)
\]

\[
EM = e^{-(\Delta G^0_{intra} - \Delta G^0_{inter})/RT} \quad (5)
\]

Scheme 3: Intermolecular proton transfer in 7, where GM and P are the reactant and product, respectively.
os, blood and urine samples will be collected at different times. The concentration of the antimalarial drug, atovaquone, will be determined using a reliable bioanalytical method. Further, pharmacokinetic parameter values will be calculated including oral bioavailability, terminal elimination half-life and other pharmacokinetic parameters as deemed necessary.

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References


50. Enthalpic energy of 5 kcal/mol was needed to rotate the carboxyl group such that the attack angle increases from 48° to 88°.

Notes
5http://w/w/gaussian.com.

Supporting Information
Additional Supporting Information may be found in the online version of this article:
Data S1. Xyz Cartesian coordinates for the calculated GM and TS optimized structures in processes 1–6.

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