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Computational Study on the Effect of Exocyclic Substituents on the Ionization Potential of Primaquine: Insights into the Design of Primaquine-Based Antimalarial Drugs with Less Methemoglobin Generation

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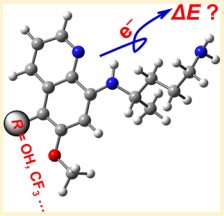
Supporting Information

ABSTRACT: The effect of an exocyclic substituent on the ionization potential of primaquine, an important antimalarial drug, was investigated using density functional theory methods. It was found that an electron-donating group (EDG) makes the ionization potential decrease. In contrast, an electron-withdrawing group (EWG) makes the ionization potential increase. Among all the exocyclic positions, a substituent at the 5- or 7-position has the largest effect. This can be explained by the contribution of the atomic orbitals at those positions to the highest occupied molecular orbital (HOMO). In addition, a substituent at the N8-position has a considerably large effect on the ionization potential because this atom makes the second largest contribution to the HOMO. These findings have potential implications for the design of less hemotoxic antimalarial drugs. We suggest that it is worth considering placement of an EWG at the 5-, 7-, or N8-positions of primaquine in future drug discovery attempts.



1. INTRODUCTION

One of the major infectious diseases that causes significant mortality around the world is malaria. Currently, primaquine (PQ, Figure 1) has been used as the drug to treat the liver stage of this disease.[1] However, a major concern for primaquine is that its metabolites are able to cause hemolytic anemia, particularly in glucose-6-phosphate dehydrogenase (G6PD) deficient patients. The hemotoxicity is related to the conversion of hemoglobin to methemoglobin, which cannot carry molecular oxygen, and the simultaneous generation of reactive oxygen species, such as superoxide radical and hydrogen peroxide.[2−4] From various experimental studies, it is known that the degree of hemotoxicity varies in different primaquine metabolites. For example, 5-hydroxyprimaquine,[5,6] 5,6-dihydroxyprimaquine,[7] and 6-methoxy-8-(*N*-hydroxy)-aminoquinoline[8,9] generate more methemoglobin than the parent molecule.[10,11] In contrast, carboxyprimaquine results in less methemoglobin generation.[2,10,12] Although the attempt to discover less toxic antimalarial drugs has attracted significant attention,[13−25] the chemical mechanism of how methemoglobinemia is caused remained unknown over the past several decades, which, unfortunately, significantly hampered the discovery of new and safer antimalarial drugs.

Since the methemoglobinemia involves a redox reaction, we believe that electron transfer processes are the key to understanding its mechanism. Based on our extensive density functional theory (DFT), molecular dynamics, and quantum mechanics/molecular mechanics (QM/MM) studies, we tested the possibilities that (i) PQ and its derivatives are not involved in the electron transfer process[26] or that (ii) they act as the electron acceptor,[27] and showed proof against both proposed mechanisms. The third possibility is that PQ and its derivatives act as the electron donor in the redox process, which is in fact supported by our computational studies.[28,29] Specifically, we found that upon binding to hemoglobin, 5-hydroxyprimaquine is able to donate an electron to the hemoglobin-bound O_2. In the meantime, the ferrous iron (Fe^{2+}) also donates an electron to O_2. These two electrons help the conversion of O_2 to H_2O_2 and simultaneously generate methemoglobin. Furthermore, we also showed[29] that an electron-donating group (EDG) at the 5-position makes it easier for the corresponding primaquine derivative to lose an electron. In contrast, an electron-withdrawing group (EWG) at the 5-position makes this process more difficult. Hence, a 5-position EWG-substituted primaquine derivative may generate less methemoglobin, which is worth considering in the future development of this class of drugs. In addition to these studies, we also investigated the methemoglobinemia potential and the feasibility of formation of the hydroxylated derivatives of NPC1161, a developmental antimalarial compound with better efficacy and lower toxicity.[30]

In this study, we further considered the effect of a substituent at all other positions of primaquine on its ionization potential. The results obtained from this work may be used to predict the potential of these primaquine derivatives to generate methemoglobin and guide the future design of this class of drugs.

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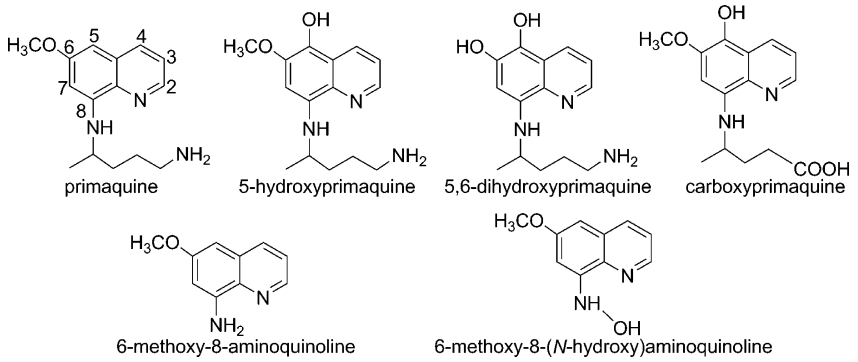

Figure 1. Structure of primaquine and some of its metabolites.

2. COMPUTATIONAL METHODS

The Gaussian 09 program[31] was used for all calculations. Geometries were optimized in the gas phase using the B3LYP[32−34] method with the 6-31G(d,p) basis set. Frequency calculations were also performed at this level to confirm that all the optimized structures correspond to minima on the potential energy surface and to obtain zero-point vibrational energies (ZPVE). Relative energies in the gas phase were obtained by performing single point calculations at the B3LYP/6-311+G(2df,p) level based on the above optimized geometries, and these were corrected with the ZPVE from the smaller basis set, that is, B3LYP/6-311+G(2df,p)//B3LYP/6-31G(d,p) + ZPVE[B3LYP/6-31G(d,p)]. All the gas phase energies reported in this work were obtained at this level. To investigate the effects of the polarity of the environment, the optimized gas-phase structures were used for single point calculations employing the integral equation formalism polarizable continuum model (IEF-PCM)[35] at the B3LYP/6-311+G(2df,p) level. A dielectric constant (ε) of 78.36 was used to model aqueous solution. The obtained relative energies were further corrected with the gas-phase ZPVE. The ionization potentials (IPs) were calculated according to the following definition:

$$\mathrm{IP} = E(\text{radical cation}) - E(\text{neutral})$$

The atomic orbital contributions to the highest occupied molecular orbital (HOMO) were obtained by performing population analysis using Gaussian 09. The B3LYP method has been used previously for calculation of IPs that agree well with experiment.[36−39]

3. RESULTS AND DISCUSSION

3.1. Effect of Electron-Donating Groups at All Exocyclic Positions. Previously, we studied the interaction between 5-hydroxyprimaquine and hemoglobin, which led to our proposed methemoglobinemia mechanism.[28] In this study, we first considered the effect of an -OH group at all other exocyclic positions on the ionization potential (IP) of primaquine. An extended side chain conformation was used for all the PQ derivatives considered in this work, as was done in our previous study.[27] The IPs of all the derivatives are listed in Table 1. When any of the exocyclic positions is substituted with an -OH group, the IP of the resulting derivatives is smaller than that of PQ itself. In the gas phase, 5-OH-PQ has the smallest IP of 585.3 kJ mol^{-1}, while 3-OH-PQ has the largest IP of 620.1 kJ mol^{-1}, which is 34.8 kJ mol^{-1} higher in energy than that of 5-OH-PQ and is still 3.2 kJ mol^{-1} less than that of PQ.

In solution, the IPs of all the derivatives significantly decrease by 128.0−135.1 kJ mol^{-1}, most likely due to the stabilization effect of the solution. However, compared with the gas phase, the same trend was observed in solution. For example, 5-OH-PQ has the smallest IP, while 3-OH-PQ has the largest IP, 1.4 kJ mol^{-1} less than that of PQ.

Table 1. Calculated Ionization Potentials (IP, kJ mol^{-1}) of PQ and of PQ Hydroxylated at All Possible Exocyclic Positions in the Gas Phase and in Solution

	gas phase		solution	
	IP	ΔIP	IP	ΔIP
PQ	623.3	0.0	488.2	0.0
2-OH-PQ	613.7	−9.6	479.0	−9.2
3-OH-PQ	620.1	−3.2	486.8	−1.4
4-OH-PQ	611.2	−12.1	483.2	−5.0
5-OH-PQ	585.3	−38.0	452.3	−35.9
7-OH-PQ	608.8	−14.5	478.5	−9.7

According to our previously proposed mechanism[28] that the methemoglobinemia caused by the PQ derivatives may be determined by their ionization potentials, hydroxylation at any of the exocyclic positions should make the corresponding derivative able to generate more methemoglobin. In addition, the 5-OH substituted derivative should be able to generate the most significant methemoglobinemia, because it has the smallest IP.

In addition to the hydroxylated derivatives, we also considered all the possible methylated derivatives. The calculated IPs are shown in Table 2. Similar to the effect of the hydroxyl group, the -CH_3 substituent at the 5-position results in the smallest IP in both the gas phase and solution, compared with the same substituent at all other positions. However, the difference between the IP of 5-CH_3-PQ and of those PQ derivatives with the same substituent at other positions is much smaller than the differences found for the hydroxylated derivatives. Specifically, the IP of 5-OH-PQ is smaller than those of the other -OH substituted derivatives by

Table 2. Calculated Ionization Potentials (IP, kJ mol^{-1}) of Methylated PQ at All Possible Exocyclic Positions in the Gas Phase and in Solution[a]

	gas phase		solution	
	IP	ΔIP	IP	ΔIP
PQ	623.3	0.0	488.2	0.0
2-CH_3-PQ	611.4	−11.9	481.0	−7.2
3-CH_3-PQ	614.8	−8.5	484.7	−3.5
4-CH_3-PQ	615.2	−8.1	484.4	−3.8
5-CH_3-PQ	604.8	−18.5	472.3	−15.9
7-CH_3-PQ	610.3	−13.0	478.3	−9.9

[a]For comparison, the IPs of PQ in the gas phase and in solution are also listed.

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26.2−34.5 kJ mol^{-1} in solution. In contrast, the IP of 5-CH_3-PQ is smaller than those of the other -CH_3 substituted derivatives by only 6.0−12.4 kJ mol^{-1} in solution. This is likely due to the weaker electron-donating ability of the -CH_3 group compared with the -OH group.

3.2. Effect of Electron-Withdrawing Groups at All Exocyclic Positions. We then considered EWG substituents at the exocyclic positions. The nitro and trifluoromethyl groups were chosen in this work as typical EWGs. The calculated IPs of all possible nitro and trifluoromethyl derivatives are listed in Tables 3 and 4, respectively. It can be seen that all of them have larger IP than the parent PQ molecule both in the gas phase and in solution, which is the opposite of the effect of having an EDG substituent at the exocyclic position. For both the nitro and trifluoromethyl compounds, the 4-position substituted derivatives have the lowest IP in the gas phase, while the 3-position substituted derivatives have the lowest IP in solution. This is different from the EDG-substituted derivatives, in which substitution at the 5-position results in the smallest IP. However, it should also be noted that the IPs of the 2-, 3-, and 4-position substituted derivatives are quite close, with the largest difference being only 7.7 kJ mol^{-1}. The largest IP was found with substitution at the 5-position in the gas phase and at the 7-position in solution. However, the difference between the IPs of the 5-position and 7-position substituted derivatives is very small and is within ∼2 kJ mol^{-1}. Hence, the 5-position seems to be particularly sensitive to the substituent with respect to change in the IP. Specifically, an EDG at this position results in the lowest IP both in the gas phase and in solution. In contrast, an EWG at this position results in the largest IP in the gas phase and the second largest IP in solution, which is only marginally smaller than the largest IP.

Table 3. Calculated Ionization Potentials (IP, kJ mol^{-1}) of the Nitro-Substituted PQ at All Possible Exocyclic Positions in the Gas Phase and in Solution[a]

	gas phase		solution	
	IP	ΔIP	IP	ΔIP
PQ	623.3	0.0	488.2	−24.0
2-NO_2-PQ	670.1	46.8	519.0	30.8
3-NO_2-PQ	667.0	43.7	512.2	24.0
4-NO_2-PQ	662.4	39.1	515.7	27.5
5-NO_2-PQ	672.3	49.0	536.4	48.2
7-NO_2-PQ	672.2	48.9	538.1	49.9

[a]For comparison, the IPs of PQ in the gas phase and in solution are also listed.

Table 4. Calculated Ionization Potentials (IP, kJ mol^{-1}) of the Trifluoromethyl Substituted PQ at All Possible Exocyclic Positions in the Gas Phase and in Solution[a]

	gas phase		solution	
	IP	ΔIP	IP	ΔIP
PQ	623.3	0.0	488.2	0.0
2-CF_3-PQ	651.8	28.5	505.5	17.3
3-CF_3-PQ	651.8	28.5	504.4	16.2
4-CF_3-PQ	648.1	24.8	506.4	18.2
5-CF_3-PQ	663.0	39.7	523.3	35.1
7-CF_3-PQ	661.2	37.9	523.9	35.7

[a]For comparison, the IPs of PQ in the gas phase and in solution are also listed.

3.3. Analysis of the Highest Occupied Orbital of Primaquine. It has been previously shown[40] that the IP of a molecule is determined in part by its highest occupied molecular orbital (HOMO). In order to understand the significant effect of a substituent at the 5-position, we plotted the HOMO of primaquine, which is shown in Figure 2. It can be seen that the HOMO is primarily localized on the aminoquinoline ring as well as the exocyclic −NH moiety at the 8-position. The contribution of the individual atomic orbitals to the HOMO was also analyzed and is provided in Table 5. The top contributors were all p-orbitals. It can be seen that the 2p orbital of C5 contributed the most (0.28) to the HOMO. Hence, any substituent at this position should have the largest effect on the IP. This thus explains the above findings that either an EDG or an EWG at this position results in the largest effect on the IP. In contrast, the contributions of the atomic orbitals of C2, C3, and C4 to the HOMO are all much smaller than that of C5.

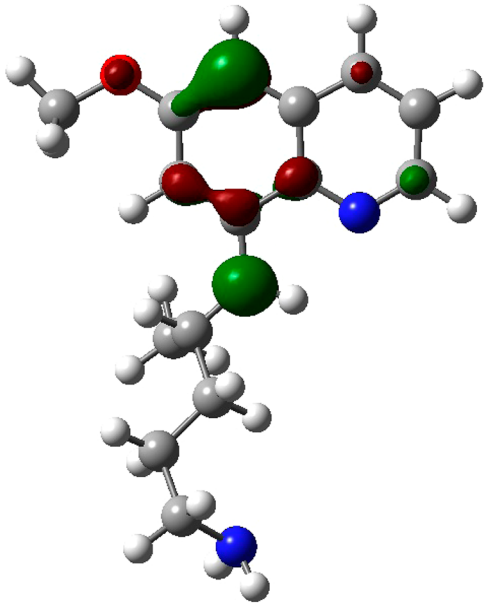
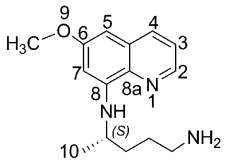


Figure 2. Highest occupied molecular orbital of primaquine.

Table 5. Contribution of Atomic Orbitals to the Highest Occupied Molecular Orbital of Primaquine

	Atomic orbital	contribution
	C5-*p*	0.28
	N8-*p*	0.22
	C7-*p*	0.09
	C8a-*p*	0.09
	C8-*p*	0.07
	C2-*p*	0.06
	C4-*p*	0.04
	C6-*p*	0.04
	O9-*p*	0.03
	N1-*p*	0.02
	C10-*p*	0.02

The atomic orbital that makes the second largest contribution to the HOMO is N8 (Table 5). As a result, any substituent at this position should also result in a considerable change to the IP compared with the parent compound. Therefore, the IPs of the molecules having an -OH, -CH_3, or

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-CF_3 substituent on the 8-amino group were calculated and are listed in Table 6. It was again found that an EDG (-OH, -CH_3) at the N8-position decreases the IP while an EWG (-CF_3) at this position increases the IP. For both the -OH and -CF_3 substituents, the change of IP is more significant for substitution at N8 than for the same substituent at the 5-position. It should be noted that in the optimized structure of the radical cation of the 8-N-OH derivative, the proton of the N-OH moiety is transferred to N1, probably due to the better delocalization of the positive charge on the aminoquinoline ring.

Table 6. Calculated Ionization Potentials (IP, kJ mol^{-1}) of the Derivatives with a -CH_3, -OH, or -CF_3 Substituent at the N8 Position of Primaquine[a]

	IP (gas phase)	IP (solution)
PQ	623.3	488.2
8-N-CH_3-PQ	615.3	482.9
8-N-OH-PQ	573.2	431.1
8-N-CF_3-PQ	699.3	558.1

[a]For comparison, the IPs of PQ in the gas phase and in solution are also listed.

3.3. Implication for Future Design of Less Hemotoxic Antimalarial Drugs. The results in this work provide insights into the future design of less hemotoxic antimalarial drugs. We recently proposed that the methemoglobinemia caused by the primaquine derivatives may be determined by their electron-donating ability.[28] If one wants to lower the hemotoxicity of primaquine by adding an exocyclic substituent, the findings in this work suggest that an EWG should be considered. In addition, among all the possible positions, the 5- and 7-positions deserve particular attention, because a substituent at these positions should have a larger effect than those at other positions. We note that tafenoquine (Figure 3), a promising antimalarial drug candidate that has entered clinical trials, has a -CF_3 exocyclic substituent, although it is not directly connected to the C5 position of the aminoquinoline ring. It is worth testing other EWG-substituted derivatives. However, the efficacy of the resulting derivative against the malaria parasite will also need to be taken into account.

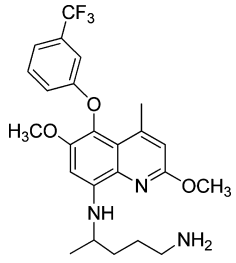

Figure 3. Structure of tafenoquine.

Another position that is worth considering in future research is N8. This nitrogen has the second largest contribution to the HOMO of primaquine. It has been known that when this position is substituted by a hydroxyl group in 6-methoxy-8-aminoquinoline (Figure 1), the resulting 6-methoxy-8-(*N*-hydroxy)aminoquinoline derivative generates much more significant amounts of methemoglobin than 6-methoxy-8-aminoquinoline does.[8,9] This is in agreement with our finding that an EDG substituent at N8 decreases the IP. Further experimental studies should also be performed to determine the advantages of placing an EWG at this position.

4. CONCLUSIONS

In this work, the ionization potentials of the exocyclic EDG- and EWG-substituted primaquine derivatives were considered. It was found that for all the positions, an EDG causes the IP to decrease. In contrast, an EWG increases the IP. However, among all the possible exocyclic positions, the 5- and 7-positions each play a special role. In solution, an EDG at the 5-position results in the smallest IP, while an EWG at the 7-position results in the largest IP. This can be explained by the contribution of each atomic orbital to the HOMO of primaquine. It was found that p-orbitals from C5 and C7 are among the first three atomic orbitals with the largest contribution to the HOMO of primaquine. The atomic orbital with the second largest contribution to the HOMO is that of N8. Hence, any substituent at the N8 position should also have a considerable effect on the IP. This was indeed confirmed by our calculations. When the 8-amino group is substituted by a -CF_3 group, the resulting derivative has a larger IP compared with placing the same substituent at any other position.

The mechanism of the methemoglobinemia caused by primaquine-based antimalarial drug has been unknown for decades. As a result, a rational approach to the development of PQ-based antimalarial drugs with less methemoglobin generation was lacking. Our previous studies[26−29] suggested that the potential for methemoglobin generation by primaquine derivatives is determined in part by their ability to lose an electron. The results reported in this study further suggest that the future design of less hemotoxic primaquine-based antimalarial drugs should consider use of an EWG as an exocyclic substituent, in particular at the C5, C7, or N8 positions, because substituents at these positions have the most significant effect on their ionization potentials.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Optimized *xyz* coordinates of all the structures discussed in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

PQ, primaquine; G6PD, glucose-6-phosphate dehydrogenase; DFT, density functional theory; QM/MM, quantum mechanics/molecular mechanics; EDG, electron-donating group; EWG, electron-withdrawing group; ZPVE, zero-point vibrational energy; IEF-PCM, integral equation formalism polarizable continuum model; IP, ionization potential; HOMO, highest occupied molecular orbital

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