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Full Paper

Interactions between DNA purinic bases and amodiaquine: A theoretical approach

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ABSTRACT: *We study theoretically the amodiaquine-adenine and amodiaquine-guanine adducts formation using Density Functional Theory (B3LYP) and the 6-31G(d) basis set for the geometry optimizations and 6-31+G(d,p) for the analysis of the global indexes: electrophilicity (ω), electronic chemical potential (μ), hardness (η) and softness (S), based in the Frontier Molecular Orbital Theory – FMO. Local softness for nucleophilic reaction (s_k^+) sites over guanine was evaluated using Fukui function (f_k). We also evaluated the guanine Electrostatic Potential (EP) values using the (MSK) charge scheme. The theoretical calculations had demonstrated that the amodiaquine has greater electronic affinity for the guanine, with irreversible formation of the amodiaquine-guanine adduct, as reported before on a previous experimental work.*

Keywords*: amodiaquine, adduct formation, Fukui index, softness, density functional theory, DFT*

Introduction

Electrochemical studies of small molecule/DNA complexes have focused primarily on solution-phase phenomena, in which DNA-induced changes in redox potentials and/or diffusion constants of organic and inorganic species [1, 2]. However, DNA biosensors offer considerable promised for obtaining information necessary for development various

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fields such as biomedical and environmental research. Recent activity in this direction has also centered upon the design of biosensors that exploit the interaction between the surface-confined DNA and target drugs [3-7].

Some studies suggested that interactions of 4-aminoquinolines with DNA might underlie the antimalarial activity of these compounds. In general, 4-aminoquinolines derivatives appear to bind to nucleoproteins and inhibit DNA and RNA polymerase [8, 9].

Amodiaquine (AMD), an 4-aminoquinoline derivative is a potent chemotherapic used in the malaria treatment caused by both resistant and sensitive strains of *Plasmodium falciparum*, used either alone or in combination with sulphadoxine-pyrimethamine, might be a safe and efficacious drug option for malaria treatment in pregnancy and for intermittent preventive treatment [10, 11]. Having in mind the evaluation of the reactivity among the AMD and the purinic bases adenine (ADE) and guanine (GUA) (Figure 1), as well as the possible adducts formation (Scheme 1), we made a theoretical study as follows below.

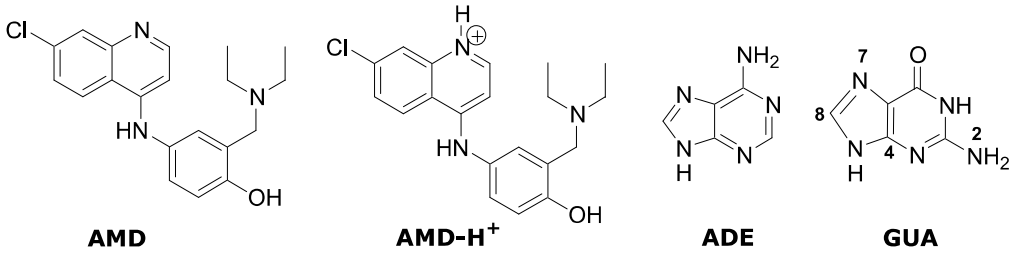


Figure 1. Structures used in the theoretical calculations.

Since the classical definition of Mulliken with regards to the electronegativity (χ) in 1934 [12], and the appearance of the hardness (η) and softness (S) [13, 14], in the 60's, to explain the acid-base reactions, see HSAB principle [13, 15], several works were set out [16] and new theories developed. Among them we point out the application of the density functional theory to best describe the molecular electronic structure and chemical reactions [17, 18]. To foreseen the chemical reactivity of a molecule and more than that, the precise attack sites is, without doubt, a very hard and primordial task to the Chemistry [19-21]. In this sense, the indexes of Fukui, proposed in 1952 [22], give good results in many cases. Works were developed from the Molecular Orbital Theory, including applications in QSAR [23-26].

In this article we analyze local and global properties, aiming to distinguish the difference between the formation of the N7- and N2-adducts, which come from the reaction between the amodiaquine (AMD) and guanine (GUA) (Scheme 1).

We will also use the Electrostatic Potential (EP) values to help our decision with regards to the most probable adduct to be formed. Once and for all we will compare ours

results with the experimental data.

Model Equations

The stabilization in energy when the system acquires an additional electronic charge, ΔN, from the environment has been defined [27, 28], as the global electrophilicity index, ω:

$$\omega = \mu^2/2\eta \qquad (1)$$

in terms of the electronic chemical potential μ, and the chemical hardness η, μ and η may be approached in terms of the one electron energies of the frontier molecular orbital HOMO (ε_H) and LUMO (ε_L), as $\mu \approx \varepsilon_H + \varepsilon_L /2$ and $\eta \approx \varepsilon_L - \varepsilon_H$, respectively [18, 29]. The electrophilicity index encompasses both the propensity of the electrophile to acquire an additional electronic charge driven by μ^2 (the square of the electronegativity) and the resistance of the system to exchange electronic charge with the environment described by η, simultaneously. A good electrophile is in this sense characterized by a high value of μ and a low value of η. Associated with the definition of global electrophilicity, there is an additional and useful relationship that accounts for the maximum electronic charge, ΔN_{max}, that the electrophile may accept from the environment. Note that in the present approach, the environment may be represented by either external effects coming for instance from the interaction with the solvent or more simply as field effects coming from the presence of substituent groups in the molecule. It has been defined as [27]:

$$\Delta N_{max} = - \mu/\eta \qquad (2)$$

Also, the following partition for ΔN_{max} is possible in terms of the electrophilic Fukui functions:

$$\Delta N_{max}(k) = \Delta N_{max} f_k^+ \qquad (3)$$

Note that within the present model, the maximum electrophilicity power within a molecule will be located at the softest site of the system. If we further use the exact relationship between local softness and electrophilic Fukui function, namely $s_k^+ = S^* f_k^+$ [18, 29], the local softness for nucleophilic reaction (s_k^+), can be generalize as:

$$s_k^\alpha = S^* f_k^\alpha \qquad (4)$$

where α = +, - , 0 refer to nucleophilic, electrophilic, and radical reactions respectively [18, 30]. A local version of the HSAB principle has been proposed in terms of theses quantities [31, 32].

The Fukui function is by far the most important local reactivity index. It is defined as [33]:

$$f(\mathbf{r}) = \partial\rho(\mathbf{r})/\partial N)_{v(\mathbf{r})} = (\delta\mu/\delta v(\mathbf{r}))_N \quad (5)$$

Because of the discontinuities in slope of the $\rho(r)$ versus N curve. Three types of Fukui functions can be written as follows:

for nucleophilic attack:

$$f^{+}(\mathbf{r}) = (\partial\rho(\mathbf{r})/\partial N)^{+}_{v(\mathbf{r})} \approx \rho_{N+1}(\mathbf{r}) - \rho_N(\mathbf{r}) \approx \rho_{LUMO}(\mathbf{r}) \quad (6)$$

for electrophilic attack:

$$f^{-}(\mathbf{r}) = (\partial\rho(\mathbf{r})/\partial N)^{-}_{v(\mathbf{r})} \approx \rho_N(\mathbf{r}) - \rho_{N-1}(\mathbf{r}) \approx \rho_{HOMO}(\mathbf{r}) \quad (7)$$

for radical attack:

$$f^{0}(\mathbf{r}) = 1/2\,[f^{+}(\mathbf{r}) + f^{-}(\mathbf{r})] \quad (8)$$

which lead us back to what exists in the essence of the Fukui frontier orbital theory.

Use these local $f(r)$ functions sometimes become difficult, so to deal with this problem, the related condensed-to-atom variants are written for the atomic site k of the molecule. The corresponding Fukui functions can be written [30] by replacing the associated electron densities by the respective electron populations q_k, as:

for nucleophilic attack:

$$f_k^{+} = q_k(N+1) - q_k(N) \quad (9)$$

for electrophilic attack:

$$f_k^{-} = q_k(N) - q_k(N-1) \quad (10)$$

for radical attack:

$$f_k^{0} = 1/2\,[(f_k^{+}) + (f_k^{-})] \quad (11)$$

Global softness is defined us [18]:

$$S = 1/\eta \quad (12)$$

We will use eqs. 4, 9 and 12 to calculate the nucleophilic attack sites at guanine atoms (s_k^{+}).

Material and Methods

Computational Methods

All calculations were performed with Gaussian 03 package of programs [34]. A pre-optimization was performed with semi-empirical PM3 method. Full geometry optimization for amodiaquine AMD, protonated-amodiaquine AMD-H^{+}, adenine ADE and

guanine GUA were performed applying both Hartree-Fock method (3-21G basis set) and B3LYP hybrid functional [35, 36] with the standard 6-31G(d) basis set. All stationary points were characterized as minima or transition structures by calculating the harmonic vibrational frequencies and ZPE corrections were applied in energy calculations using the same theoretical level used in optimization. The global indexes (μ, η and ω) were calculated at B3LYP/6-31+G(d,p)// B3LYP/6-31G(d) level and the EP values at B3LYP/6-31+G(d,p) level with full geometry optimization. The charges were derived from the EP using the MKS (Merz-Singh-Kollman) scheme [37, 38].

Results and Discussion

To decide if the AMD reacts with GUA or ADE we analyze the global indexes (μ, η and ω), obtained from the energy of the FMO (HOMO-LUMO), as described before.

Through the analysis of the global indexes results pointed out in the Table 1, we show recently [39] that the protonated-amodiaquine, AMD-H^+, is the best electrophile (ω = 8.75). On the other hand, guanine, GUA, is the worse electrophile and best nucleophile (ω = 1.10).

Table 1. Global indexes* (μ, η and ω) [39].

Compounds	HOMO	LUMO	μ	η	ω
AMD-H^+	-0.3208	-0.2109	-0.2659	0.1099	8.75
AMD	-0.2083	-0.0627	-0.1355	0.1456	1.72
ADE	-0.2310	-0.0337	-0.1324	0.1973	1.21
GUA	-0.2241	-0.0279	-0.1260	0.1962	1.10

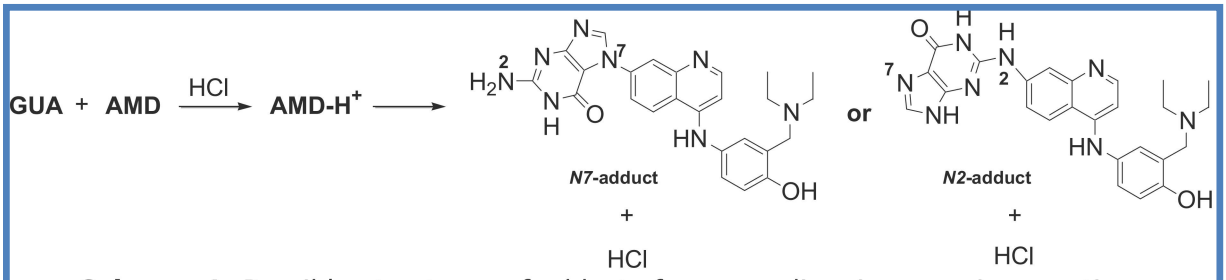
*HOMO, LUMO, electronic chemical potential, μ, and chemical hardness, η, values are in au, electrophilicity power values, ω, are in eV. We used eq. 1, and references [19, 28] as explained in the text, to calculate ω, μ and η, respectively.

With this information we were able to propose two different possible structures for the adduct formed between AMD and GUA, which differ only for the N7 or N2 GUA attack sites (Scheme 1). Schemes 2 and 3 shows the proposed mechanisms for N7- and N2-adducts formation, respectively. Obtained data in our laboratory suggests the formation of the guanine adduct.

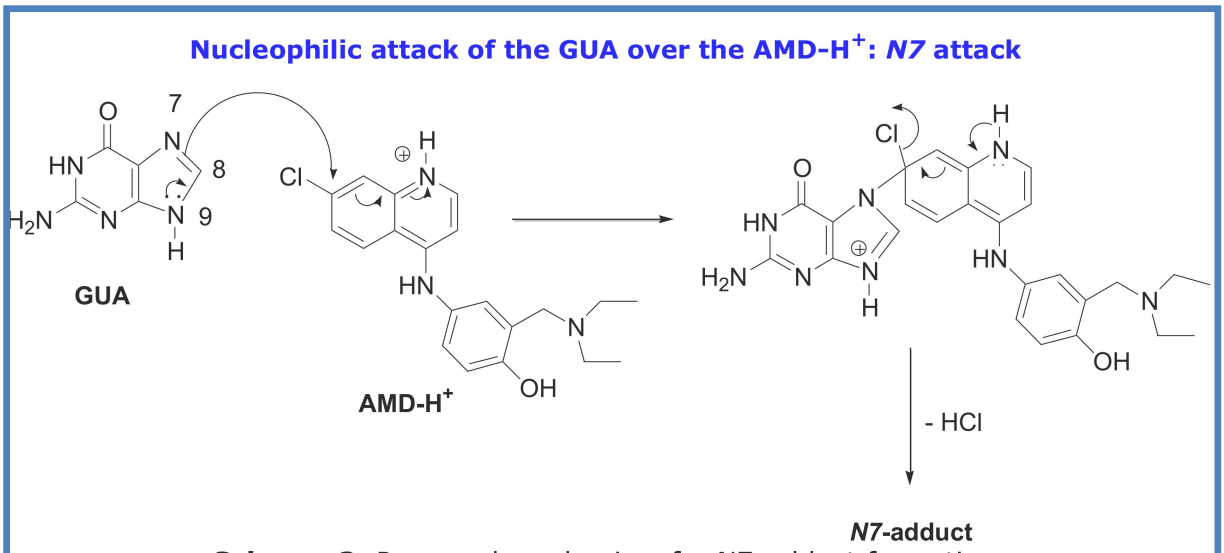
To obtain some information about the favored position for the nucleophilic attack of the GUA over the AMD-H^+, our first approach was to evaluate the local Fukui indexes which were calculated according to eq. 9. It is worthwhile reminding that the results of enthalpy (ΔH) reported by Munk, B. H et al., with calculations at B3LYP/6-31G(d) level [40] indicated that C8 oxidation is thermodynamically preferred to the C4.

They obtained the following values of ΔH: C8 -33.06 Kcal/mol, C4 -18.23 Kcal/mol – for hydroxyl radical and ΔH: C8 -18.98 Kcal/mol, C4 -2.86 Kcal/mol for methoxy.

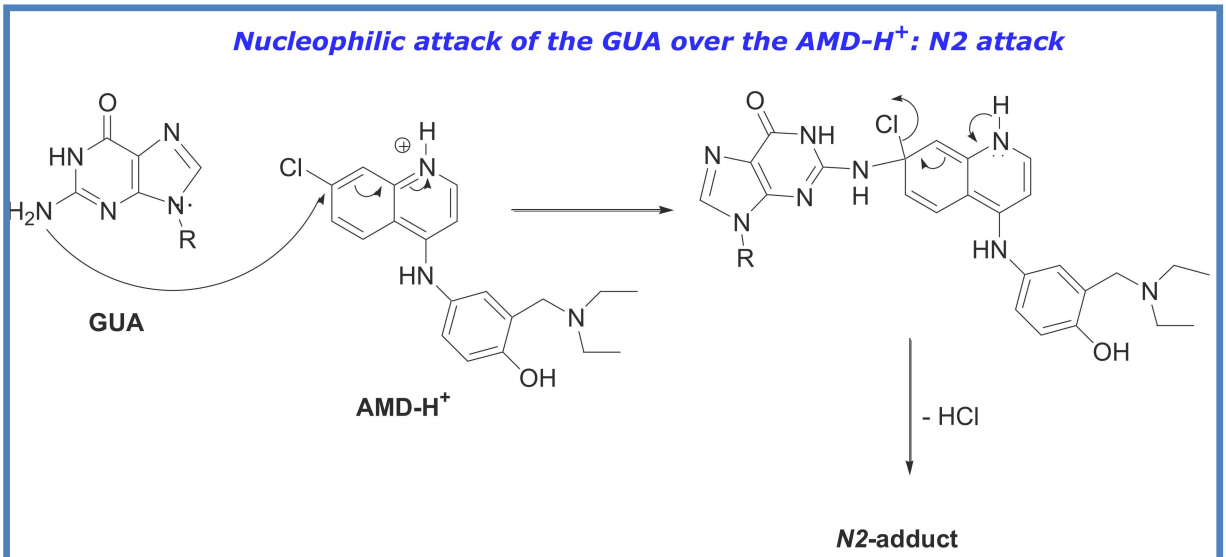
radical (See reference 38). Our calculations shown in Table 2 are in accordance with these values, once a higher C8 oxidation value, implies in a greater softness.



Scheme 1. Possible structures of adducts from amodiaquine-guanine reaction.



Scheme 2. Proposed mechanism for N7-adduct formation.



Scheme 3. Proposed mechanism for N2-adduct formation.

The EP values analyzes (Table 2) indicates N7 us the best nucleophilic attack site when compared with N2.

We believe that adduct reaction formation of aromatic amines and phenols with guanine, occurs preferentially with the nitrogen N2 or N7 of the guanine structure. Our hypotheses are supported by biological studies revealing the formation of these types of aniline´s adducts [41, 42] and quinoline derivatives [43, 44].

Others studies indicated that electrophilic quinines react with DNA by 1,4-Michael addition and nitrogen N7 of the guanine is the principal nucleophilic region involved in the adduct formation . In all the cases there are structural likenesses with amodiaquine region [45, 46].

Table 2. Calculated local indexes q, f_k^-, f_k^+, S_k^-, S_k^+, $|S_k^+|$ and EP for amodiaquine.

Atoms	q(0) [a]	f_k^- =q (-)-q(0)	f_k^+=q(0)-q(+)	$s_k^+ = S*f_k^+$ [b c]	$s_k^- = S*f_k^-$	$\|s_k^+\| = S*f_k^+$ [d]	EP [b c]
N2	-0.857598	-0.062799	-0.056385	-0.320076	-0.287385	0.320076	-18.289193
N7	-0.589348	-0.030391	-0.070529	-0.154898	-0.359475	0.154898	-18.356188
C8	0.297498	-0.099224	-0.172429	-0.505729	-0.878843	0.505729	-14.665376
C4	0.478210	-0.030722	-0.144345	-0.156585	-0.735703	0.1565851	-14.652578

[a]The charges were calculated using the MSK scheme.
[b]Global softness (S) was calculated using equation 12 and is equal to 5.096840.
[c] s_k^+ and f^+ were calculated using equations 4 and 9, respectively.
[d] $|s_k^+|$ module of the s_k^+.

On the other hand the softness for nucleophilic attack (s_k^+), its module, is much higher over N2. In fact to decide which reactive site (N7 or N2) will be attached by AMD is not trivial, and both contribution: electron-transfer and electrostatically controlled reactions probable are competing [21].

We hope this study became a good indication for laboratory work synthesis, in order to propose new antimalarial drugs.

Conclusion

The redox behavior of amodiaquine within the DNA environment is important in the studies of the toxicity effects in malarial therapy. The intercalations of the amodiaquine in the DNA structure with the subsequent adduct formation between the amodiaquine and guanine can contribute to bigger vulnerability of the DNA to oxidative processes.

Theoretical calculations had demonstrated that the amodiaquine has greater electronic affinity for the guanine. The preferential way for the formation of the adduct betweeen the antimalaric and the purinic base GUA (N2-adduct or N7-adduct) was determined according to the local softness. This analysis predicted the preference for the N2-adduct formation.

The local softness for C8 and C4 atoms of GUA, indicated correctly the most

favorable oxidation site, the C8 atom, in agreement with experimental data, indicating that the basis we used are precise enough for these calculations.

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