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Computational Study of the Alkylation Reaction of the Nitrogen Mustard Mechlorethamine Using NBO Model and the QTAIM Theory

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ABSTRACT

Substances known as nitrogen mustards turn into aziridinium ion through the intramolecular cyclization SN_1. This ion reacts with the DNA preferably at the N_7 position of the guanine, and because of this, it is an important antineoplastic agent. Based on this, the objective of this study is to quantify the interaction between the nitrogen mustard mechlorethamine and the guanine, using the NBO analysis and the QTAIM theory. The results of the NBO analysis showed that when the triangular cycle C_4-N_1-C_5 is formed, there is some resonance among these atoms. This analysis also showed that the electronic transition at the sigma antibondingorbital σ^* N_1-C_4 presents higher perturbation energy of second order, indicating that this bond is broken at the nucleophilic attack of the N_7 nitrogen of guanine. The analysis that refers to the electron density obtained by the QTAIM theory indicates that the guanine proximity enables an electron density polarization of the BCPs aziridinium ion of mechlorethamine making that the frontal part of the ion becomes electron deficient. Finally, the relative results to the Laplacian of the electron density obtained by the QTAIM theory showed that the guanine approximation increases the "hole" factor at the C_4, proving that the nucleophilic attack based on the "lump-hole" concept causes the region of that atom is the site of alkylation reaction.

Keywords: NBO; QTAIM; Aziridinium; Ion; Nucleophilic Attack; Electron Density; Laplacian of the Electron Density

1. Introduction

Antitumor alkylating agents are classified as Celular-Cycle non specific [1] and form crosslinked bonds with DNA. These crosslinked bonds cause DNA lesions requiring complex repair mechanisms, including replication inhibition. Because of it, in 1942, mechlorethamine was successfully used to induce transient tumor remission in a patient with lymphoma, this event marked the beginning of the modern era cancer chemotherapy [1]. Among the alkylating agents, the mechlorethamine was the first anti-cancer drug effectively used for clinic purpose, and today it is the most common one used against tumor cells [2].

The alkylating agents efficiency, like the mechlorethamine, was studied previously using molecular model [3,4] and also by rational planning [5,6] that highlighted the correlation between the intramolecular distance of the electrophilic centers of these agents and the nucleophilic nitrogen of nucleotide [1]. As the traditional QSAR techniques are laborious and require a long investigation time and high cost [7], the computational analysis of the alkylating agents is becoming more attractive.

The mustard nitrogen compounds are among the most popular agents studied [2,8-11] by the theoretical computational chemistry. These compounds form the aziridinium ion, being this ion highly reactive through the intramolecular cyclization SN_1. The ion reacts with the DNA, preferably at the guanine N_7 position [1], however experiments have been noticing alkylation at the positions N_1, N_3, N_6 e O_6. [1,2]. The physical fundamental understanding of this alkyalting family reaction can be interesting for the development on new drugs.

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With this objective, one of the ways to theoretically quantify the interaction intensity between the nitrogen mustards and the guanine, besides the analysis of the highest occupied molecular orbital (HOMO) [12], lowest unoccupied molecular orbital (LUMO) [13] and its difference [14-16], it is through the NBO model [17]. A distinguishing feature of localized NBO functions is the simultaneous requirement of orthonormality and maximum occupancy, leading to compact expressions for atomic and bond properties [18]. This way, ab initio wavefunctions transformed to NBO form are found to be in good agreement with the Lewis concept and with the classic bond form with hybridization and polarization from Pauling-Slater-Coulson [18-21].

Another way to quantify the nitrogen mustards interaction with the guanines is using the QTAIM theory (Quantum Theory: Atoms in Molecules) [22] from Bader. At this theory, the Laplacian of the electron density $\nabla^2\rho$ is shown at the local form of the virial theorem giving the mechanics of an atom inside of a molecule [23]. This way, the Laplacian can identify the reactivity sites of the molecule [23,24].

So, the objective of the present study is to quantify the interactions of the mechlorethamine molecule with the guanine using the Natural Bond Orbital (NBO) Analysis from Donor-Acceptor Viewpoint, the electron density, and the Laplacian of the electron density obtained by the QTAIM theory.

2. Computationaldetails

The tridimensional molecule structures were built using the software *GaussView* 3.0 [25]. The structures of the mechlorethamine molecule were drawn in several steps of thealkylation reaction with the guanine. These structures were built in four states: isolated mechlorethamine, isolated aziridinium ion of mechlorethamine, aziridinium ion in the presence of guanine and the transition state of the aziridinium ion. All these structures were optimized to the lower energy state with the program *Gaussian03* [26] at the level B3LYP with the basis set 6-31G (d, p). All the post calculations were made with the same wavefunction, B3LYP/6-31G (d, p). The transition state of the aziridinium ion was obtained with the QST3 algorithm. With the optimized geometry, using the same software [26], the Natural Bond Orbitals (NBO) were obtained and also the electronic transitions determined by this theory. The electronic transitions chosen for the discussion were the ones with the second order perturbation energy $\Delta E^2 \geq 5$ kcal·mol^{-1}. All these calculations were made in a machine SGI Altix 1350/Altix 450 with 174 CPU's Intel Itanium2, 866GB of RAM memory, technology NUMAFlexGeração 4, interconnection Infiniband and storage system SGI TP9300 with 43 TB. This system is installed at CENAPAD-SP [27].

Using personal computers, continuing from the optimized geometries obtained, the critical points position (3,−1) and (3,−3) of the gradient of the electron density, were determined. Later, the electron density of these critical points were calculated and also the Laplacian of these densities, using the QTAIM theory. These quantities were calculated using the software *AIM*2000 [28]. This software was also used to generate the relief maps of Laplacian of the electron density.

3. Results and Discussion

3.1. Construction of the Mechlorethamine Molecule in Different States of the Alkylation Reaction

The different steps of the mechlorethamine molecule alkylation with the guanine have been widely discussed at the previous art [1,2,8-11,29,30]. It is know that this molecule reacts with aintramolecular cyclization SN_1 releasing a chloride ion, forming the aziridinium ion. Right after the aziridinium ion gets close to the guanine, it goes to a transition state and lately suffers a nucleophilic attack from the N_7 of these nitrogenous base.

This way, the molecules used in this study, according to the reaction steps, were built using the software *Gaussview*03 and they were optimized to the lowest energy state using the program *Gaussian*03. The molecules were built in the following order:

State (1)—isolated mechlorethamine molecule

State (2)—isolated aziridinium ion of mechlorethamine molecule

State (3)—molecular cluster formed by the aziridinium ion of mechlorethamine + guanine

State (4)—mechlorethamine molecule at the transition state + guanine (obtained with the algorithm QST3 from *Gaussian*03)

These geometries obtained for the four states of the reaction, shown at **Figure 1**, were used at the NBO analysis and also to obtain the electron density of the QTAIM theory.

3.2. NBO Analysis of the Intermolecular Interaction between Donor and Acceptor at the Four Reactions States

Thedata obtained with the NBO analysis represent the electronic transition within only one reaction state. The NBO analysis of the mechlorethamine molecule (state 1) did not show any electronic transition that satisfies the minimum stabilization energy condition $\Delta E^2 \geq 5$ kcal·mol^{-1}. However, **Table 1** shows that when the mechlorethamine becomes the aziridinium ion, forming the triangle C_4-N_1-C_5 (state 2), there is a kind of resonance between the electrons of the sigma bond N_1-C_4 and N_1-C_5. However, a slight asymmetry can be noticed at

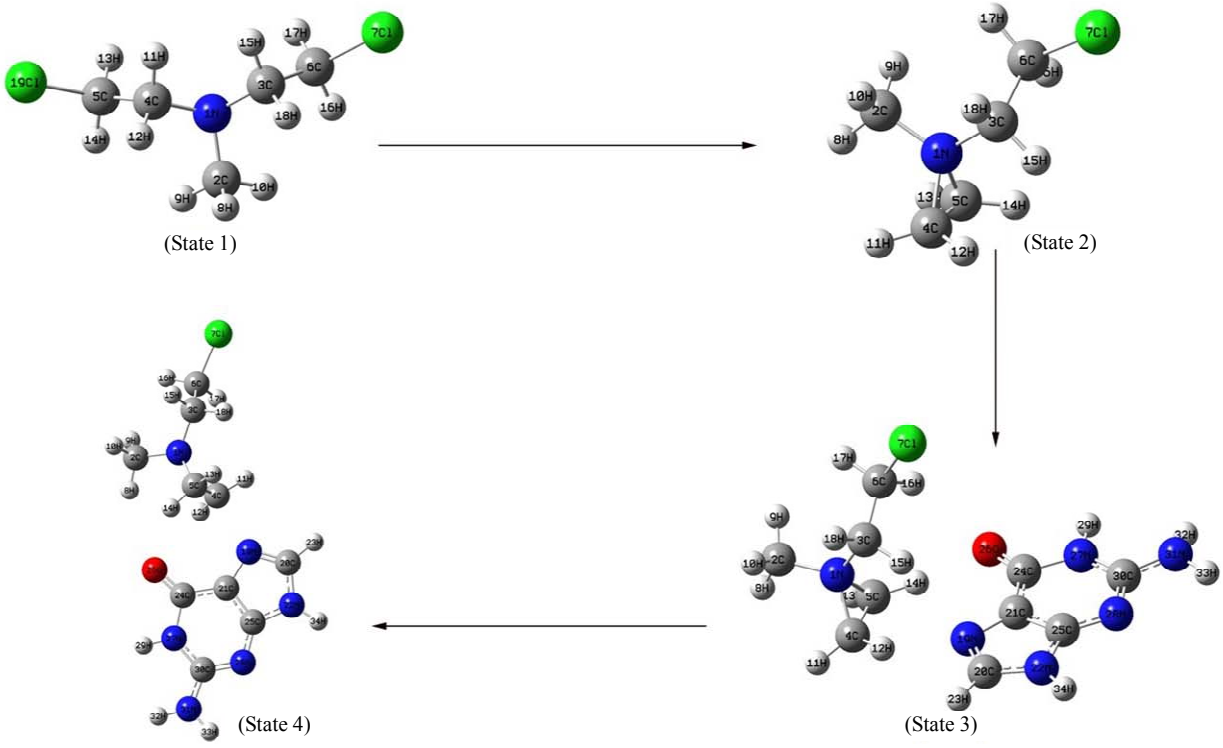

Figure 1. Alkylation reaction mechanism of the mechlorethamine with the DNA guanine, separated in 4 states.

Table 1. Electronic transitions obtained by NBO Analysis of states (2) and (3) with the wavefunction B3LYP/6-31G (d, p).

Donor NBO	Acceptor NBO	ΔE^2 (kcal/mol)	Donor NBO	Acceptor NBO	ΔE^2 (kcal/mol)
Intramolecular Interactions State (2)			**Intramolecular Interation State (3)**		
BD (σ) N_1-C_4	BD*(σ*)N_1-C_5	6.81	BD (σ) N_1-C_4	BD*(σ*)N_1-C_5	8.10
BD (σ) N_1-C_5	BD*(σ*)N_1-C_4	6.89	BD (σ) N_1-C_5	BD*(σ*)N_1-C_4	8.39
			BD (σ) N_1-C_4	BD*(σ*)N_1-C_3	5.28
			BD (σ) N_1-C_4	BD*(σ*)C_4-C_5	5.63
Intramolecular Interactions of Guanine (Cluster)					
BD (σ) N_{19}-C_{20}	BD*(σ*)C_{21}-C_{24}	5.42	LP N_{22}	BD*(π*)C_{21}-C_{25}	39.47
BD (π) N_{19}-C_{20}	BD*(π*)C_{21}-C_{25}	13.17	LP N_{22}	BD*(σ*)N_{22}-H_{34}	9.83
BD (σ) C_{20}-N_{22}	BD*(σ*)C_{25}-N_{28}	6.07	LP O_{26}	$RY^*_{C_{24}}$	14.09
BD (π) C_{21}-C_{25}	BD*(π*)N_{19}-C_{20}	17.18	LP O_{26}	BD*(σ*)C_{21}-C_{24}	17.91
BD (π) C_{21}-C_{25}	BD*(π*)C_{24}-O_{26}	31.18	LP O_{26}	BD*(σ*)C_{24}-N_{27}	28.82
BD (π) C_{21}-C_{25}	BD*(π*)N_{28}-C_{30}	7.11	LP N_{27}	BD*(π*)C_{24}-O_{26}	50.76
BD (π) C_{24}-O_{26}	BD*(π*)C_{21}-C_{25}	5.08	LP N_{27}	BD*(π*)N_{28}-C_{30}	60.57
BD (σ) C_{25}-N_{28}	BD*(σ*)C_{30}-N_{31}	5.88	LP N_{28}	BD*(σ*)C_{21}-C_{25}	10.30
BD (σ) N_{28}-C_{30}	BD*(σ*)N_{22}-C_{25}	6.07	LP N_{28}	BD*(σ*)N_{27}-C_{30}	14.22
BD (π) N_{28}-C_{30}	BD*(π*)C_{21}-C_{25}	28.46	LP N_{31}	BD*(π*)N_{28}-C_{30}	64.29
BD (σ) N_{31}-H_{33}	BD*(σ*)N_{27}-C_{30}	6.28	BD* (π) N_{19}-C_{20}	BD*(π*)C_{21}-C_{25}	29.24
CR O_{26}	RY* C_{24}	5.11	BD* (π) N_{28}-C_{30}	BD*(π*)C_{21}-C_{25}	72.65
LP N_{22}	BD*(π*)N_{19}-C_{20}	48.98			
Intermolecular Interactions					
LP N_{19}	BD*(σ*)N_1-C_4	35.26			

the stabilization energy of the electronic transition at the acceptor antibonding orbitals σ^* of the carbons of the triangular cycle C_4-N_1-C_5. The data indicate that the electronic transition at the antibonding orbital σ^* N_1-C_4 stabilizes the system more, making that this carbon (C_4) be more susceptible to a nucleophilic attack.

Looking at the data regarding state 3, at **Table 1**, an increase of around 20% can be noticed at the stabilization energy ΔE^2 of the acceptor antibonding σ^* of the traingular cycle carbons C_4-N_1-C_5. It is also observed the appearance of two new intramolecular transitions of the aziridinium ion in which the electronic donor is the bond orbital σ N_1-C_4, another indication that this bond is the most probable to be cleaved with the guanine nucleophilic attack. The electronic transitions obtained for the guanine are the typical resonance transitions of the aromatic rings and of the carbonyl formed by the atoms C_{24} and O_{26}. The most important transitions that occur at the guanine were $\pi_{N28\text{-}C30} \rightarrow \pi^*_{C21\text{-}C25}$ with 72.65 kcal·mol^{-1} and $LP_{N31} \rightarrow \pi^*_{N28\text{-}C30}$ with 64.29 kcal·mol^{-1}.

Another relevant electronic transition is the intermolecular transition $LP_{N19} \rightarrow \sigma^*_{N1\text{-}C4}$ of ΔE^2 = 35.26 kcal·mol^{-1} indicating that a nucleophilic attack of the N_{19} (the guanine N_7 mentioned at the references [1,2,9]) possibly contributes to the σN_1-C_4 bond break.

The algorithm QST3 that determined the geometry of theaziridinium ion of mechlorethamine transition state (state 4) shows that before the alkylation, there is a break at the triangular cycle C_4-N_1-C_5. This new geometry is obtained with the bond break of σ N_1-C_4, what was already predicted by the NBO analysis of **Table 1** and by the new intramolecular transition $\sigma_{N1\text{-}C4} \rightarrow \sigma^*_{N1\text{-}C5}$ with 10.75 kcal·mol^{-1} from **Table 2**. Among the guanine intramolecular transitions at this reaction state, two Lone Pair transitions of the C_{21}, $LP_{C21} \rightarrow \pi^*_{N22\text{-}C25}$ e $LP_{C21} \rightarrow \pi^*_{C24\text{-}O26}$ can be highlighted, with stabilization energies of 244.46 and 97.92 kcal·mol^{-1} respectively.

It is interesting to notice that at this reaction step, where there is the transition state, the intermolecular transition $LP_{N19} \rightarrow \sigma^*_{N1\text{-}C4}$ presents a second order energy of ΔE^2 = 63.59 kcal·mol^{-1}. This value corresponds to an increase of 80.35% in the ΔE^2 energy of this transition when compared to the value obtained at the previous state (3). This result demonstrates that there is a transition state (4) of the aziridinium ion of mechlorethamine that favors the nucleophilic attack of the guanine N_7 at the ion C_4.

It is thought that this fact happens due to the proximity of the C_4 to N_7 at the transition state (4), because the distance between these two atoms at the state (4) is 2.11 Å, while at state (3) this same distance is 3.34 Å (**Figure 2**).

3.3. Application of the QTAIM Theory to Obtain the Electron Density and the Laplacian of the Electron Density of Molecules at the Four Reaction Sites

The QTAIM theory (Quantum Theory: Atoms in Molecules) was used to calculate the electron density and the Laplacian of the electron density for the critical points (3,−1) and (3,−3) and for all the structures at each alkylation reaction state of the mechlorethamine with the guanine (**Figure 3**).

It can be noticed by **Table 3**, that in all changes of state, the critical points of electron density variation

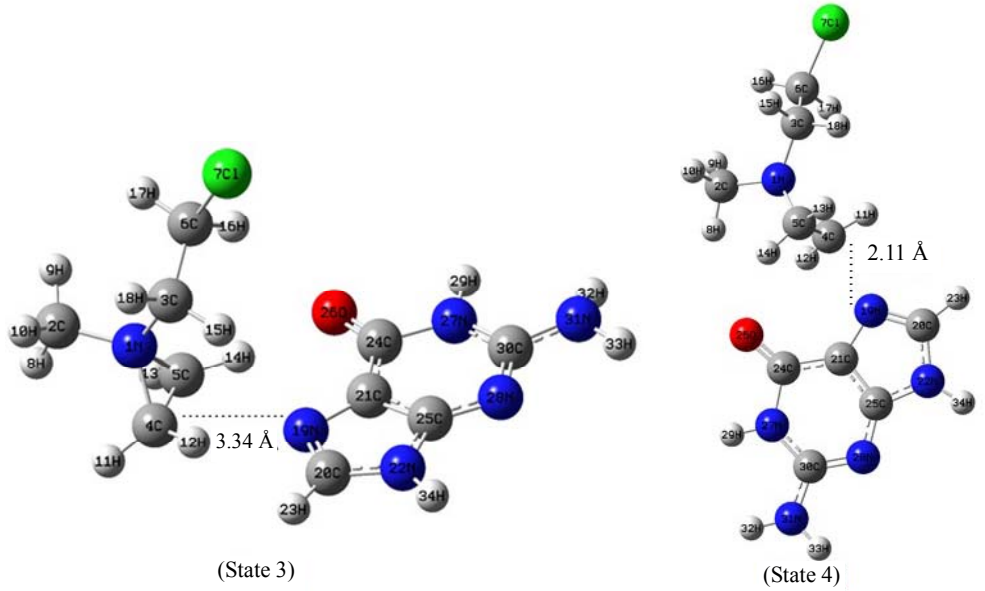

Figure 2. Distance between the N_{19} (guanine N_7) and themechlorethamine C_4 at the state (3) and (4) of the alkylation reaction.

Table 2. Electronic Transitions obtained by NBO Analysis of state (4) with the wavefunction B3LYP/6-31G (d, p).

Donor NBO	Acceptor NBO	ΔE^2 (kcal/mol)	Donor NBO	Acceptor NBO	ΔE^2 (kcal/mol)
Intramolecular Interactions of Aziridinium Ion (TS)					
BD (σ) N_1-C_4	BD*(σ*)N_1-C_5	10.75			
Intramolecular Interactions of Guanine (Cluster)					
BD (σ) N_{19}-C_{20}	LP C_{21}	26.91	LP N_{19}	BD*(σ*)C_{20}-N_{22}	6.81
BD (σ) C_{20}-N_{22}	BD*(σ*)C_{25}-N_{28}	5.40	LP C_{21}	BD*(π*)N_{19}-C_{20}	60.94
BD (σ) N_{19}-C_{21}	BD*(σ*) C_{21}-C_{24}	5.28	LP C_{21}	BD*(π*)N_{22}-C_{25}	244.46
BD (π) N_{22}-C_{25}	LP C_{21}	12.34	LP C_{21}	BD*(π*)C_{24}-O_{26}	97.92
BD (π) N_{22}-C_{25}	BD*(π*) N_{19}-C_{20}	29.35	LP O_{26}	RY* C_{24}	14.07
BD (π) C_{24}-O_{26}	LP C_{21}	6.89	LP O_{26}	BD*(σ*)C_{21}-C_{24}	16.87
BD (σ) C_{25}-N_{28}	BD*(σ*)C_{30}-N_{31}	5.33	LP O_{26}	BD*(σ*)C_{24}-N_{27}	29.32
BD (σ) N_{28}-C_{30}	BD*(σ*)N_{22}-C_{25}	6.84	LP N_{27}	BD*(π*)C_{24}-O_{26}	49.44
BD (π) N_{28}-C_{30}	BD*(π*)N_{22}-C_{25}	40.59	LP N_{27}	BD*(π*)N_{28}-C_{30}	58.95
BD (σ) N_{31}-H_{33}	BD*(σ*)N_{27}-C_{30}	6.19	LP N_{28}	BD*(σ*)C_{21}-C_{25}	10.84
CR O_{26}	$RY^*_{C_{24}}$	5.40	LP N_{28}	BD*(σ*)N_{27}-C_{30}	14.64
Intermolecular Interactions					
LP N_{19}	BD*(σ*)N_1-C_4	35.26			

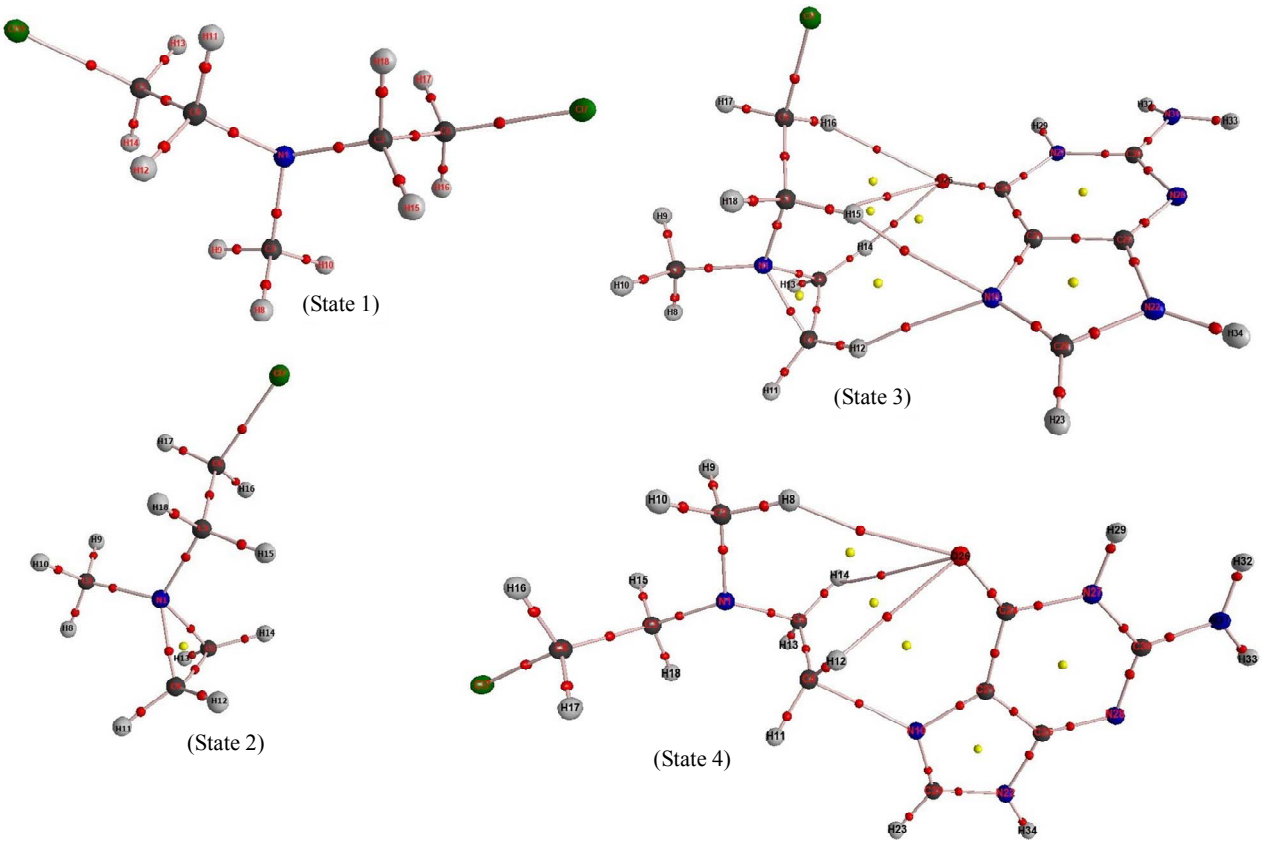

Figure 3. Structures of the 4 reaction states with the critical points obtained by the QTAIM theory. The red critical points are the Bond Critical Points (BCP), and the yellow critical points are the Ring Critical Points (RCP).

Table 3. Electron density of states 1, 2, 3 and 4 of the critical points NACP (Nuclear Atractor Critical Point) (3,−3) and of the critical points BCP (Bond Critical Points) (3,−1) obtained by QTAIM with wavefunction B3LYP/6-31G (d, p).

NACP (3,−3)	ρ (u.a.) State 1	ρ (u.a.) State 2	ρ (u.a.) State 3	ρ (u.a.) State 4	$\Delta\rho$ (u.a.) State (2-1)	$\Delta\rho$ (u.a.) State (3-2)	$\Delta\rho$ (u.a.) State (4-3)
N_1	2.1728	2.1726	2.1727	2.1730	−0.0002	0.0001	0.0003
C_2	1.9459	1.9466	1.9466	1.9462	0.0007	0.0000	−0.0004
C_3	1.9452	1.9455	1.9458	1.9454	0.0003	0.0003	−0.0004
C_4	1.9452	1.9464	1.9465	1.9422	0.0012	0.0001	−0.0043
C_5	1.9444	1.9464	1.9465	1.9468	0.0020	0.0001	0.0003
C_6	1.9444	1.9447	1.9448	1.9446	0.0003	0.0001	−0.0002
Cl_7	7.9550	7.9549	7.9549	7.9550	−0.0001	0.0000	0.0001
H_8	0.1273	0.1258	0.1262	0.1256	−0.0015	0.0004	−0.0006
H_9	0.1279	0.1256	0.1260	0.1270	−0.0023	0.0004	0.0010
H_{10}	0.1279	0.1251	0.1256	0.1257	−0.0028	0.0005	0.0001
H_{11}	0.1272	0.1249	0.1259	0.1268	−0.0023	0.0010	0.0009
H_{12}	0.1284	0.1248	0.1221	0.1259	−0.0036	−0.0027	0.0038
H_{13}	0.1278	0.1248	0.1259	0.1268	−0.0030	0.0011	0.0009
H_{14}	0.1273	0.1249	0.1207	0.1257	−0.0024	−0.0042	0.0050
H_{15}	0.1284	0.1267	0.1250	0.1277	−0.0017	−0.0016	0.0027
H_{16}	0.1278	0.1263	0.1250	0.1269	−0.0015	−0.0013	0.0019
H_{17}	0.1272	0.1266	0.1273	0.1271	−0.0006	0.0007	−0.0002
H_{18}	0.1284	0.1258	0.1267	0.1262	−0.0026	0.0009	−0.0005
BCP (3,−1)	ρ (u.a.) State 1	ρ (u.a.) State 2	ρ (u.a.) State 3	ρ (u.a.) State 4	$\Delta\rho$ (u.a.) State (2-1)	$\Delta\rho$ (u.a.) State (3-2)	$\Delta\rho$(u.a.) State (4-3)
N_1-C_2	1.3472	1.2342	1.2446	1.2483	−0.1130	0.0104	0.0037
N_1-C_3	1.3497	1.2418	1.2083	1.2794	−0.1079	−0.0335	0.0711
N_1-C_4	−	1.0124	1.0036	−	1.0124	−0.0088	−1.0036
N_1-C_5	1.3487	1.0170	0.9985	1.5739	−0.3317	−0.0185	0.5754
C_4-C_5	1.2499	0.9859	0.9922	1.5367	−0.2640	0.0063	0.5445
C_3-C_6	1.2167	1.2561	1.2479	1.2525	0.0394	−0.0082	0.0046
Cl_7-C_6	0.9888	1.0330	1.0215	1.0175	0.0442	−0.0115	−0.0040
C_2-H_8	0.9687	0.9715	0.9777	0.9692	0.0028	0.0062	−0.0085
C_2-H_9	0.9997	0.9784	0.9788	0.9900	−0.0213	0.0004	0.0112
C_2-H_{10}	0.9998	0.9495	0.9556	0.9529	−0.0503	0.0061	−0.0027
C_3-H_{15}	1.0128	0.9977	0.9688	1.0067	−0.0151	−0.0289	0.0379
C_3-H_{18}	0.9856	0.9658	0.9813	0.9681	−0.0198	0.0155	−0.0132
C_4-H_{11}	0.9855	0.9487	0.9605	0.9747	−0.0368	0.0118	0.0142
C_4-H_{12}	1.0128	0.9502	0.9299	0.9814	−0.0626	−0.0203	0.0515
C_5-H_{13}	1.0004	0.9463	0.9602	0.9699	−0.0541	0.0139	0.0097
C_5-H_{14}	0.9676	0.9530	0.9147	0.9577	−0.0146	−0.0383	0.0430
C_6-H_{16}	0.9677	0.9691	0.9679	0.9703	0.0014	−0.0012	0.0024
C_6-H_{17}	1.0001	0.9884	0.9926	0.9940	−0.0117	0.0042	0.0014

(3,−3), which means the atomic nuclei, this variation is very low. The exception is the critical points that correspond to the carbon atoms 4 and 5, when the system passes from state 1 to state 2. The positive signal of $\Delta\rho$ indicates that these atoms had an electron density increase when the triangular cycle C_4-N_1-C_5 was formed, probably because of the N_1 higher electronic sharing with these atoms. The variation of electron density of the Hatoms usually have an order of magnitude around de $\sim 10^{-3}$ u.a. because of its high polarization capacity, not being important to the analysis of the alkylation reaction mechanism.

It is interesting to note that the $\Delta\rho$ values between states 1 and 2 of the BCPs (Bond Critical Point) are much higher than the values at of the critical points (3,−3). It also can be noticed that the sum of the $\Delta\rho$ values for the critical points (3,−1) with the negative signal results in −1.0772 u.a., a close value to the determined value of ρ for the bond critical point N_1-C_4 of 1.0124 u.a. This result maybe can suggest that there is an electron delocalization of these BCPs at the formation process of the bond σ N_1-C_4 when the triangular cycle C_4-N_1-C_5 is formed.

Figure 4 shows that when the aziridinium ion of mechlorethamine is close to the guanine molecule, there is the formation of hydrogen bonds (3,−1): three bonds of hydrogen with the carbonyl oxygen (H_{16}-O_{26}, H_{15}-O_{26}, H_{14}-O_{26}) and two bonds of hydrogen with the guanine N_7 (H_{12}-N_{19} e H_{15}-N_{19}). Even more noticeable are the $\Delta\rho$ data that corresponds to the change from state 2 to state 3. All the negative values of $\Delta\rho$ that correspond to the BCPs of the aziridinium ion are related to the critical points that are placed in a frontal position to the guanine molecule approach. The positive values of the ion BCPs correspond to the critical points that are in the opposite direction to the nitrogenous base.

According to these results, it is believed that the guanine approach causes an electron density polarization of the aziridinium BCPs, making the frontal part of the more susceptible to the nucleophilic attack, while the critical points at the opposite direction present a gain in electron density. When the $\Delta\rho$ from states (3) and (4) are compared, the triangular bond σ N_1-C_4 is broken, probably shifting the electron density of the region between these two atoms to the critical points N_1-C_5 and C_4-C_5, since these two BCPs had an expressive gain in the electron density of 0.5754 and 0.5445 u.a. respectively. The sum of these two $\Delta\rho$ values being 1.1200 u.a. is very close to the N_1-C_4 BCP $\Delta\rho$ value of −1.0036 u.a., a good indication that this electron density is distributed between the triangular cycle atom at the transition state.

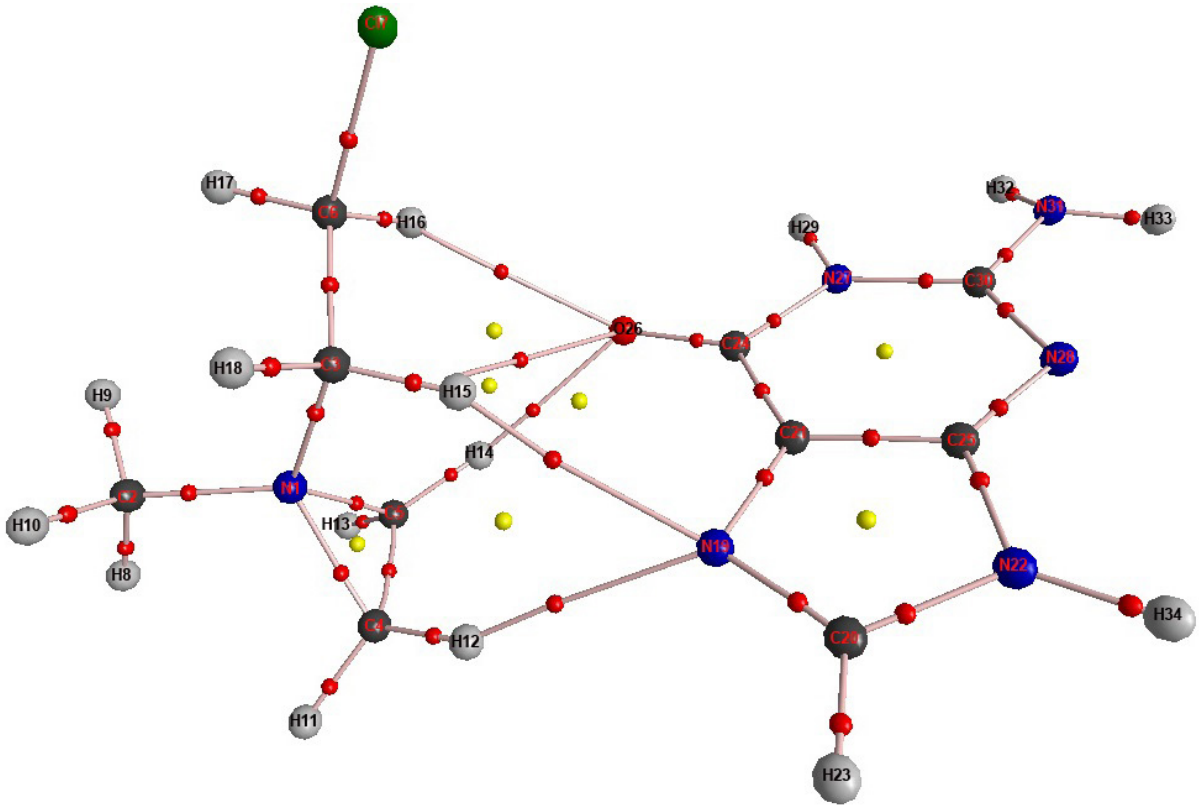

Figure 4. Structure at state (3) showing the mechlorethamine approach to the guanine. The picture shows that the BCPs H_{16}-O_{26}, H_{15}-O_{26}, H_{14}-O_{26}, H_{12}-N_{19} and H_{15}-N_{19} are hydrogen bonds.

In the **Table 4** that refers to the Laplacian of the electron density, it is noticed that at the aziridinium ion of mechlorethamine (state 2), there is a low asymmetry between the $\Delta^2\rho$ values of the carbon 4 and 5 (3.3838 and

Table 4. Laplacian of the Electron density of states 1, 2, 3 and 4 of the critical points NACP (Nuclear Attractor Critical Point) (3,−3) and the critical points BCP (Bond Critical Points) (3,−1) obtained by QTAIM with wave function B3LYP/6-31G (d, p).

NACP (3,−3)	$\Delta^2\rho$ (u.a.) State 1	$\Delta^2\rho$ (u.a.) State 2	$\Delta^2\rho$ (u.a.) State 3	$\Delta^2\rho$ (u.a.) State 4	$\Delta(\nabla^2\rho)$ (u.a.) State (2-1)	$\Delta(\nabla^2\Delta\rho)$) (u.a.) State (3-2)	$\Delta(\nabla^2\rho)$ (u.a.) State (4-3)
N_1	2.4213	2.4235	2.4248	2.4250	0.0022	0.0013	0.0002
C_2	3.3851	3.3858	3.3833	3.3857	0.0007	−0.0025	0.0024
C_3	3.3832	3.3832	3.3853	3.3859	0.0000	0.0021	0.0006
C_4	3.3839	3.3838	3.3830	3.3627	−0.0001	−0.0008	−0.0203
C_5	3.3824	3.3836	3.3829	3.3864	0.0012	−0.0007	0.0035
C_6	3.3828	3.3878	3.3882	3.3854	0.0050	0.0004	−0.0028
Cl_7	25.1423	25.1530	25.1589	25.1635	0.0107	0.0059	0.0046
H_8	0.2475	0.2448	0.2458	0.2442	−0.0027	0.0010	−0.0016
H_9	0.2499	0.2440	0.2450	0.2475	−0.0059	0.0010	0.0025
H_{10}	0.2498	0.2429	0.2443	0.2452	−0.0069	0.0014	0.0009
H_{11}	0.2489	0.2421	0.2447	0.2462	−0.0068	0.0026	0.0015
H_{12}	0.2511	0.2420	0.2344	0.2429	−0.0091	−0.0076	0.0085
H_{13}	0.2489	0.2420	0.2447	0.2472	−0.0069	0.0027	0.0025
H_{14}	0.2475	0.2421	0.2306	0.2440	−0.0054	−0.0115	0.0134
H_{15}	0.2510	0.2466	0.2417	0.2493	−0.0044	−0.0049	0.0076
H_{16}	0.2489	0.2457	0.2428	0.2469	−0.0032	−0.0029	0.0041
H_{17}	0.2489	0.2464	0.2480	0.2474	−0.0025	0.0016	−0.0006
H_{18}	0.2510	0.2446	0.2468	0.2461	−0.0064	0.0022	−0.0007
BCP (3,−1)	$\Delta^2\rho$ (u.a.) State 1	$\Delta^2\rho$ (u.a.) State 2	$\Delta^2\rho$ (u.a.) State 3	$\Delta^2\rho$ (u.a.) State 4	$\Delta(\nabla^2\rho)$ (u.a.) State (2-1)	$\Delta(\nabla^2\Delta\rho)$ (u.a.) State (3-2)	$\Delta(\nabla^2\rho)$ (u.a.) State (4-3)
N_1-C_2	−0.7180	−0.7079	−0.7014	−0.6660	0.0101	0.0065	0.0354
N_1-C_3	−0.7388	−0.7514	−0.7232	−0.7027	−0.0126	0.0282	0.0205
N_1-C_4	-	−0.5191	−0.5182	-	−0.5191	0.0009	0.5182
N_1-C_5	−0.7373	−0.5172	−0.5129	−0.9286	0.2201	0.0043	−0.4157
C_4-C_5	−0.5391	−0.2799	−0.2855	−0.6744	0.2592	−0.0056	−0.3889
C_3-C_6	−0.5080	−0.5379	−0.5305	−0.5367	−0.0299	0.0074	−0.0062
Cl_7-C_6	−0.5648	−0.5726	−0.5808	−0.5702	−0.0078	−0.0082	0.0106
C_2-H_8	−0.1888	−0.1724	−0.1778	−0.1714	0.0164	−0.0054	0.0064
C_2-H_9	−0.2020	−0.1749	−0.1758	−0.1876	0.0271	−0.0009	−0.0118
C_2-H_{10}	−0.2020	−0.1499	−0.1557	−0.1632	0.0521	−0.0058	−0.0075
C_3-H_{15}	−0.2245	−0.2033	−0.1746	−0.2156	0.0212	0.0287	−0.0410
C_3-H_{18}	−0.2068	−0.1694	−0.1854	−0.1828	0.0374	−0.0160	0.0026
C_4-H_{11}	−0.2069	−0.1389	−0.1464	−0.1509	0.0680	−0.0075	−0.0045
C_4-H_{12}	−0.2245	−0.1401	−0.1292	−0.1634	0.0844	0.0109	−0.0342
C_5-H_{13}	−0.1970	−0.1360	−0.1462	−0.1674	0.0610	−0.0102	−0.0212
C_5-H_{14}	−0.1667	−0.1419	−0.1153	−0.1586	0.0248	0.0266	−0.0433
C_6-H_{16}	−0.1668	−0.1701	−0.1682	−0.1715	−0.0033	0.0019	−0.0033
C_6-H_{17}	−0.1968	−0.1854	−0.1887	−0.1900	0.0114	−0.0033	−0.0013

3.3836 u.a. respectively), besides there is also an asymmetry to the BCPs N_1-C_4 and N_1-C_5 (−0.5191 and −0.5172 u.a.).

Can also be observed in the **Table 4** that the state (3), with the presence of the guanine, an increase on the Laplacian difference is seen between the BCPs N_1-C_4 and N_1-C_5 for states (2) and (3), being now $\Delta\nabla^2\rho = 0.0053$ u.a.. It is also observed at state (3), a reduction in the Laplacian of the electron density of C_4 with respect to the state (2), and when states (3) and (4) are compared, this reduction is even greater ($\Delta\left(\nabla^2\rho\right) = -0.0203$ u.a.. This demonstrates that the guanine approach increases the hole factor at the C_4 (**Figure 5**), and that the nucleophilic attack, based on the lump-hole concept, makes the region of this atom to become the reaction site for this alkylation.

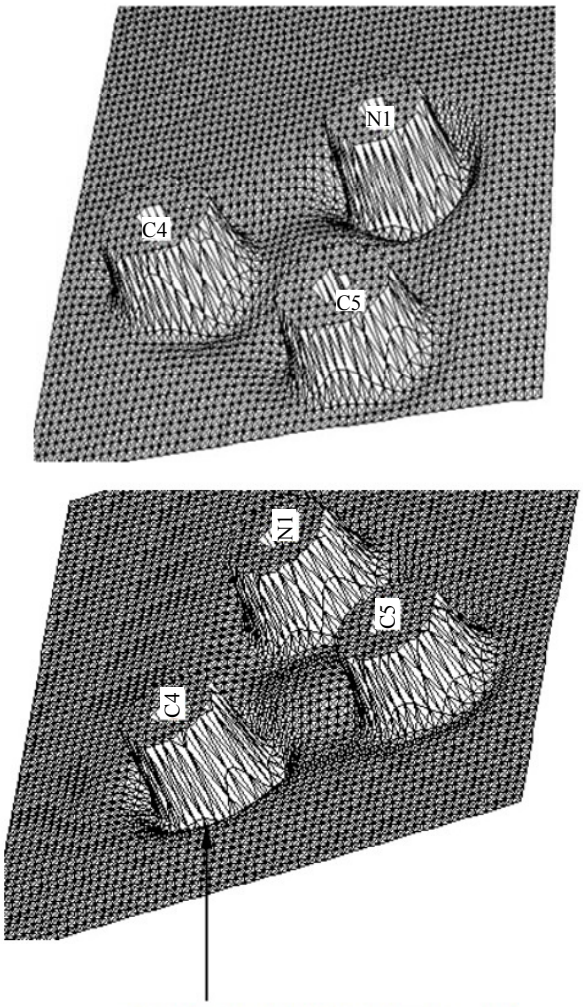

Figure 5. Relief maps of the Laplacian of the electron density of the states 2 (up) and 3 (down). It is noticed a greater hole at carbon 4 at the structure on right, emphasizing that the guanine presence increases the electrophilic carater of this atom.

4. Conclusions

The results of the NBO analysis clearly showed that when the triangular cycle C_4-N_1-C_5 is formed at the cyclization process, there is a kind of electronic resonance among these atoms. It was also noticed a certain asymmetry between the second order perturbation energy of the C_4 and C_5 of the triangular cycle, indicating that the C_4 is more susceptible to a nucleophilic attack. Finally, the NBO analysis showed that the nucleophilic attack occurs through an electronic transition between the guanine N_7 lone pair and the C_4 of the triangular cycle of the aziridinium ion of mechlorethamine transition state, state found by the algorithm QST3.

The data obtained with the Atoms in Molecules (QTAIM) theory showed that when there is a change in the state of the mechlorethamine molecule, the main variation in electron density is at the critical points (3,−1) and not at (3,−3). It was also noticed that the approach of the guanine molecule possibly generates a polarization of the BCPs electron density of the aziridinium ion of mechlorethamine, making that the frontal part of the molecule (related to the guanine position) becomes more deficient in electrons, being more susceptible to a nucleophilic attack.

The values obtained for the Laplacian of the electron density showed that the region around the C_4 of the C_4-N_1-C_5 triangular cycle is the reaction site of the alkylation reaction of the aziridinium ion of mechlorethamine, due to the presence of a greater hole than the C_5.

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