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Theoretical studies on the initiation of alkylation pathway by aziridine ring of mitomycin C at guanine nucleobase of DNA

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The aziridine ring of mytomycin C interacts with guanine nucleobase of DNA in the alkylation reaction In this context the activation of aziridine ring at the intermediate state may be analysed for understanding the various steps in the overall mechanism when aziridine may target directly the guanine nucleobase or after ring opening. Here, it is shown that aziridine ring opening is an important step in alkylation reaction.

Keywords: Aziridine ring, DNA, *ab initio*, mitomycin C, mitomycin A, alkylation

Mitomycin C (MCC) is a promising antitumour drug among the drugs of mitomycin family. In general, the research on designing new mitomycins attempts to enhance the biological activity from the distinguished physical and chemical properties of these drugs[1-10]. As such the biological properties are analysed by statistical correlation between the observed physical or chemical properties and the nature of the arbitrarily chosen substituents despite of considering the precise knowledge of the factor that control the biological properties[8-18]. At most, the chances of getting effective drug are less, and sometimes the new drug might possess adverse properties that lead to disappointing results.

There are three major factors that control the biological properties in the alkylation of DNA by mitomycin C, namely, (a) cell penetration, (b) bio-activation and (c) DNA binding ability. However all these factors are strictly correlated, and if the cell penetration is difficult then the bio-activation and DNA binding ability might be inefficient[12-19]. In the attempt of designing highly biologically active drugs, researchers have synthesized mitomycin A, mitomycin B, mitomycin C and mitomycin Z.

It has been known that the quinone reduction process is one of the important reaction steps occur in alkylation of DNA by MCC, and this process significantly depend on the physio-chemical properties of drug molecule. However the DNA binding ability of this drug can be looked with respect to the reactivity of aziridine ring of MCC, because this molecular fragment binds with $–NH_2$ of guanine nucleobase. Also the reduction of quinone part in MCC as well as subsequent involvement of carbamate substituent in the reaction has been highlighted[10-14]. Hence, it is essential to explore the reactivity of aziridine ring at the initial reaction step of alkylation.

The selectivity of $-NH_2$ group of guanine nucleobase in the alkylation mechanism by aziridine ring of MCC has been known[10-17]. In this context, the additional factor in the alkylation pathways is role of the acid and base as catalysts, where the ions present in solution may take part to alter the reactivity of this aziridine ring. Although, the $-NH_2$ group of guanine participates in the alkylation mechanism, the rate of alkylation alters if the cytosine of GC base pair is replaced by a modified cytosine nucleobase[18].

Alternatively the acid–base catalyzed alkylation by aziridine ring of MCC would be important for controlling alkylation rate where the reactivity of this molecular part at $–NH_2$ of guanine can be analysed[7-18]. It is important to examine the stability of aziridine ring in different reaction conditions for understanding the alkylation mechanism initiated by this aziridine molecular fragment.

The energetics of a three member ring and its affinity for metal ions are generally known, but no direct study on the ionic effect on aziridine ring of MCC are known. The three membered aziridine ring might associate with proton and metal ions at the lone

pair electrons of nitrogen atoms or with other atomic sites producing change in ring strain. In many cases the binding ability of ions with the tricyclic ring have been focused on the study of aromatic and anti-aromatic behavior of the ring[19]. At most, the proton affinities of many organic molecules are extensively studied and also the metal ion affinities are systematically analyzed to correlate with proton affinities[19]. It is now felt that the ion binding ability of aziridine part in MCC should also be given importance in the systematic investigation of alkylation reaction by MCC at guanine nucleobase.

So far, studies on the chemical reactivity of aziridine part, and the influence of ions on this ring have not been carried out in spite of many studies on the binding of MCC with DNA. Therefore, for better understanding the chemical reactivity of aziridine ring as well as the acid catalysed ring opening reaction step, it is proposed to carry out various investigations on the reaction steps of MCC with $–NH_2$ of guanine **(Figure 1)**.

In several theoretical studies on the various bonding aspects in DNA base pairs and stacking interaction of nucleobases, *ab initio* methods have been extensively used[20-22]. The applications of quantum mechanical methods for studying hydrogen bonds in base pairs and in other small biological molecules have already been tested[7,20]. One of the reliable methods to date is the inclusion of electron correlation and large basis sets. However, *ab initio* HF level has been used for medium size systems. Hence, *ab initio* and MP2 methods may also be used for analysing the mechanism of alkylation analyzed.

Methodology

Ab initio calculations were carried out to obtain the completely optimized geometries of drugs at 6-31G basis set, and the interaction energies of drug-guanine complexes (ΔE) were determined[23]. The interaction energies of drug-ions at HF/6-31G** and MP2/6-31G were found from the single point calculations on the HF/6-31G optimized geometries of drugs and ion-drug complexes. The values of ΔE are obtained from the difference between the energies of interacted complexes, and the sum of the energies of the individual molecule and ion.

Mitomycin + Guanine $\rightarrow$ Mitomycin-Guanine

Mitomycin + Ion $\rightarrow$ Mitomycin-ion

(E_1) (E_2) (E_3)

Interaction energy = $\Delta E = E_3 - (E_1+E_2)$

Where E_1, E_2 and E_3 are the energies of mitomycin, guanine or ion and mitomycin-guanine or mitomycin-ion respectively.

Population analysis has been carried out by using Mulliken population analysis (MPA), and the net charges on various atomic sites have been obtained.

The interaction energies were calculated in many ways. Initially the model for finding the interaction between $–NH_2$ of guanine (alkylation site) and aziridine of drugs were constructed. In this case the C1 of aziridine ring was placed at the vicinity of $–NH_2$ of guanine, and the interaction energies were calculated by optimizing the drug-gaunine complexes at various intermolecular distances. The distance corresponding to minimum energy was computed. The geometrical relaxation of aziridine ring in MCC due to the influence of guanine (alkylation site) at this optimum distance was determined for analyzing the activation of aziridinium ring. The reactivity of aziridine ring in quinone form (keto) as well as in reduced forms (enol) of drugs was examined **(Figure 2a-b).**

Again the models of hydrogen atom abstraction from the ring leading to aziridine ring opening *via* carbonium ion formation were explored by applying constraint optimization. In this case the geometries of drugs were optimized at small interaction distances between ion and hydrogen at C1 of aziridine ring. The optimum distance (D) at the minimum interaction energies were found and the relaxation in the geometry of aziridine ring at this optimum interaction distance (D) was examined.

Results and Discussion

In many of the endeavors of studying mechanism of alkylation, proper task has not been tackled how the activation of aziridine ring or the quinone reduction mechanism affects the alkylation ability of mitomycin C. So there are quite numerous examples of alkylation pathway that lead to mono adduct as well as bi-adduct product formation of mitomycin C with DNA sequence through inter-strand and intra-strand binding. Obviously extensive studies for defining the unique pathway in the sequence specific binding by various mitomycin C have been found. On the other hand, studies on the role of the major binding molecular part, the aziridine ring are a must for monitoring the reactivity of this drug. Therefore, the interaction of this molecular part with guanine nucleobase may be analysed in many ways.

Direct interaction between aziridine ring and guanine nucleobase

In general, the alkylation reaction between C1 of aziridine ring in mitomycin (**3a-c**) and $–NH_2$ of guanine occurs after activation of quinone part (**Figure 1,B**). However, the activation of aziridine ring and subsequent binding between amino group of guanine with C1 of aziridine ring can be schematically analysed from the reaction pathways given in **Figure 2**, since such tricylic ring of some other drugs like nitrogen mustards can alkylate DNA directly. Hence, the reactivity of aziridine ring in reduced MCC(enol form) has been examined, and later compared with that of quinone form(keto form) (**Figure 3a**). The optimum interaction distances and corresponding interaction energies between aziridine ring of MCC and $–NH_2$ of guanine are shown in **Table I**. In the interaction profiles involving both the keto and enol forms of MCC leading to intermediate D are not favorable (**Figure 1**). The activation of aziridine ring at this optimum distance can be visualized from the change in the geometrical parameters of ring before and after interaction with guanine (**Tables II** and **III**). Such changes in the geometrical parameters of aziridine are insignificant; hence the aziridine ring does not interact directly with $–NH_2$ of guanine. Similar studies on the interactions of other mitomycins, the MCC1 and MCC2 with $–NH_2$ of guanine have been carried out and the interaction energies are found positive (**3b,c**). These findings reinforce that the reductive activation of aziridine ring of MCC is a must before binding with $–NH_2$ of guanine can take place in the process of alkylation. Therefore, it is necessary to examine the B and C types of activation pathways, and subsequently activation of aziridine part may be analysed as in path A where the ring opening step before alkylation is shown (**Figure 1**). In turn, the reactivity of aziridine in MCC, MCC1 and MCC2 can be compared; herein the alkylation pathway may be analysed either through acid catalysed reaction step or formation of C1 electrophilic center due to hydrogen atom abstraction from the aziridine ring in the activation step (**Figure 1**).

The Mulliken net charges on the atomic centers of aziridine ring were computed, and significant negative charge distribution at various atomic centers of aziridine ring was observed in both the free and guanine-drug complexes **Tables IV** and **V**. Since the reaction between aziridine rings of MCC, MCC1 and MCC2 and guanine might involve the pathways shown in **Figure 1**, it is important to look for proton affinity of nitrogen atom of aziridine ring as a model study for acid catalyzed pathway, and also the role of ions present in reaction environment may be considered in activating aziridine ring. Hence, the proton and metal ion affinities of certain atomic sites in and around aziridine ring of these drugs can be analysed.

Influence of H^+, Li^+, Na^+ and Mg^{+2} on the atomic sites of aziridine in MCC, MCC1 and MCC2 (Scheme I)

A great deal of attention has been devoted to the studies of proton and lithium binding energies of both saturated and unsaturated tricyclic aziridine rings[17,22]. Similarly, the aziridine rings in MCC, MCC1 and MCC2 may acquire affinities for ions that might benefit the activation of this ring (**Figure 3**). Hence, all atomic positions around the aziridine ring of MCC, MCC1 and MCC2 are chosen for studying ion affinities. There are two important modes of interactions between ions and aziridine ring for activating the ring. The ions may interact either with the nitrogen atom or with the other atoms of ring. The optimum interacted structures of MCC, MCC1 and MCC2 with proton and metal ion at certain atomic sites located near aziridine ring are shown in **Figures 3d-f** and **4a-f**. Such interactions might be important for explaining the acid catalyzed ring activation step of aziridine ring before binding with guanine (**Figure 1**, **B** and **C**). Differences between the Na^+ and Mg^{+2} interactions with atomic sites near aziridine ring in spite of having multiple interactions in both cases (**Figures 4a-f**) were observed. The computed ion affinities for the interactions shown in **Figures 4a-f** are given in **Table VI**. However, in the present study, the ion affinity around the aziridine subpart is considered rather than other parts so that acid catalyzed activation of this molecular part may be analyzed. On the other hand, the optimum aziridine-ion complexes show distinguished mode of interaction with different atomic sites, herein Mg^{+2} and Na^{+1} interact with different atomic sites, and also Mg^{+2} affinities are much more than Na^{+1} affinities. The geometrical parameters of aziridine rings in these drugs have been changed due to the influence of these ions, and **Table VII** shows the comparison of geometrical parameters of aziridine in free and ion-aziridine complexes.

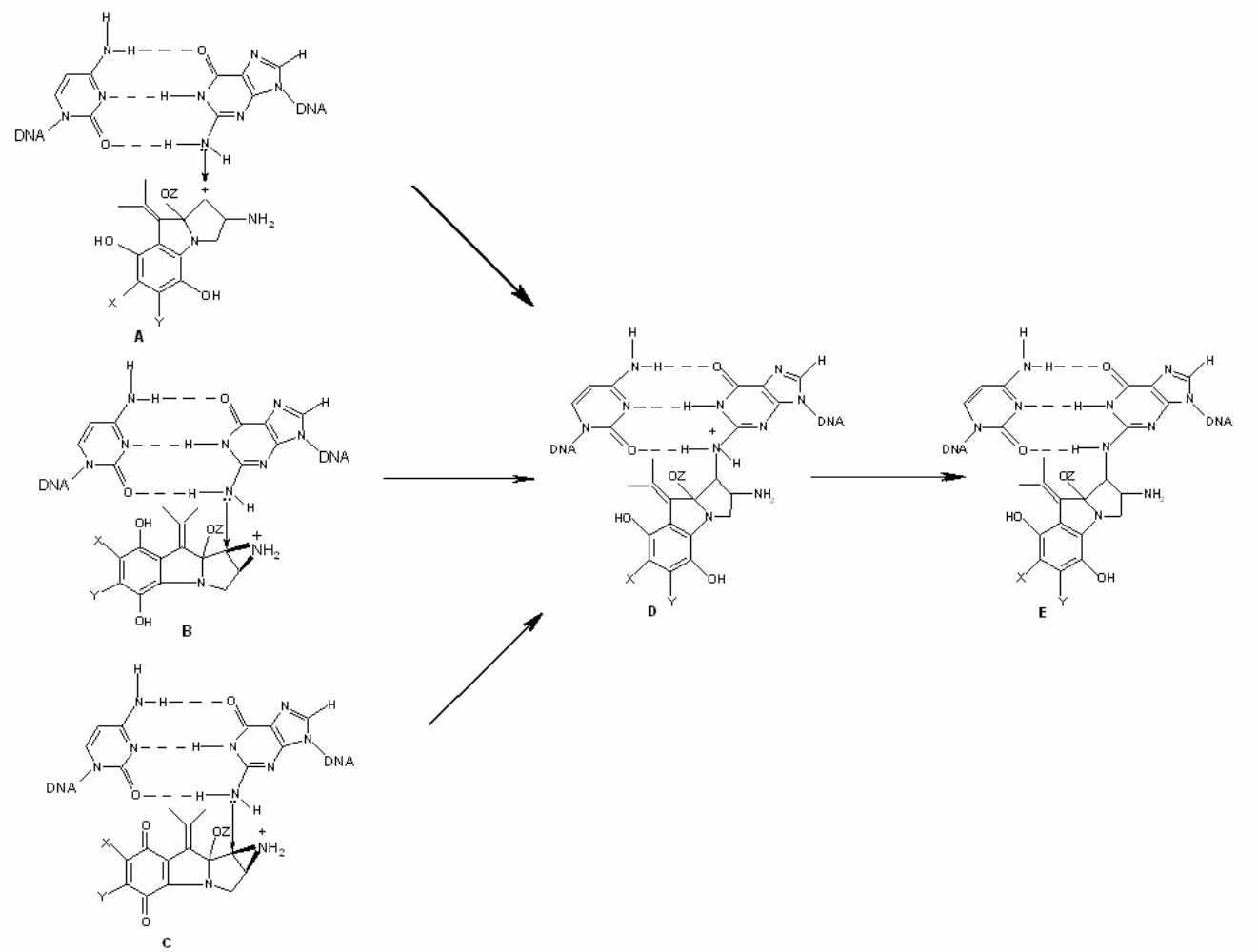

Figure 1 — The alkylation reaction between C1 of aziridine ring in mitomycin and $–NH_2$ of Guanine; Pathway-A is the ring opening step before alkylation Pathway-B and Pathway C are the alkylation step before ring opening for both enol and keto form of drug respectively.

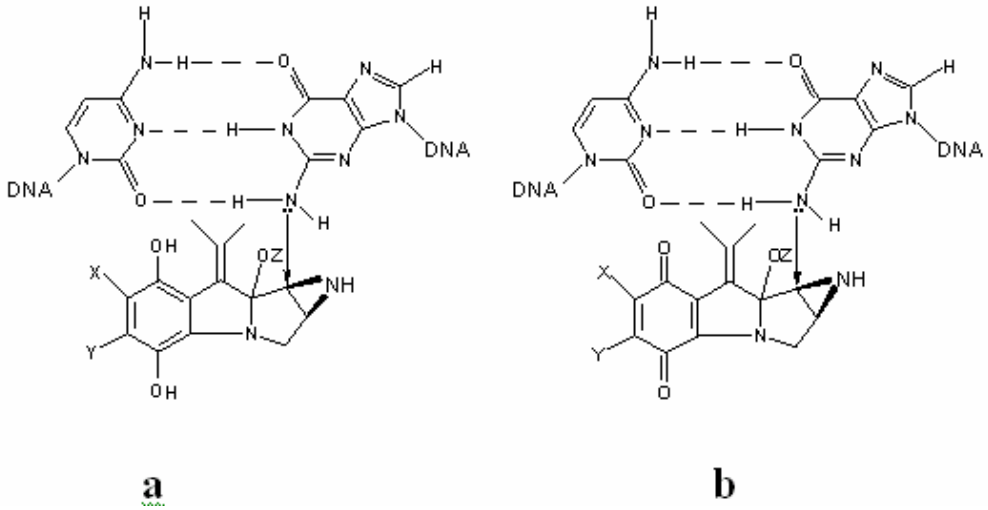

Figure 2 — Model of interaction between C1 of mitomycin and $–NH_2$ of guanine: (a) in enol form, (b) in keto form of mitomycin).

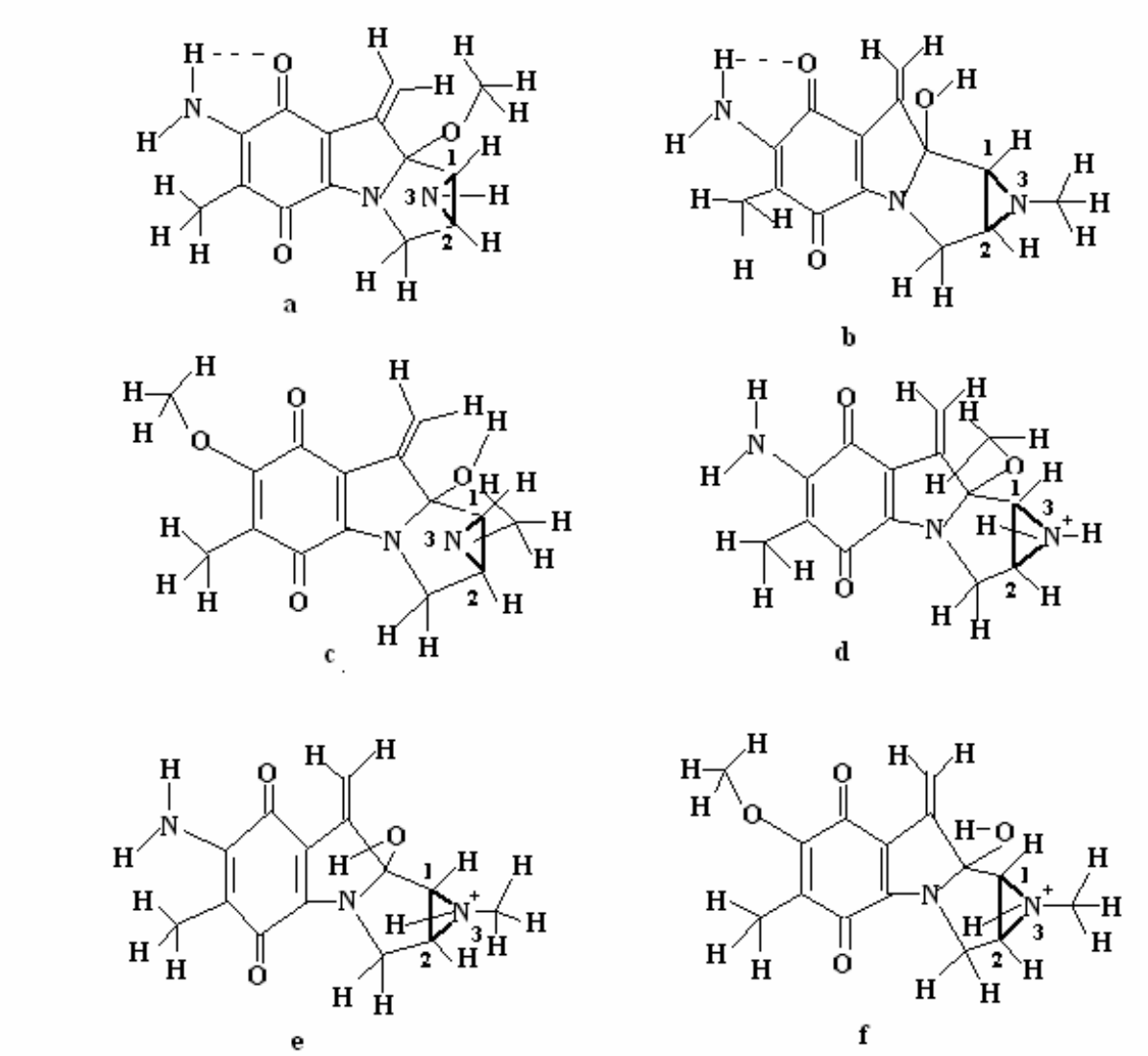

Figure 3 — Structures of mitomycins (a) MCC (b)MCC1, and (c) MCC2, Protonated structures of (d) MCC (e) MCC1, and (f) MCC2 at the N atom of aziridine ring

Table I — Computed interaction energies (HF/STO-3G) of mitomycin and guanine (G) at the optimum distances between C1 of aziridine ring and $-NH_2$ of guanine (both for keto and enol form of drug).

Complexes	Interaction Energies(kcal/mol) for		Optimum interaction distances for (Å)	
	keto form	enol form	Keto form	Enol form
MCC-G	53.45	31.75	4.61	4.61
MCC1-G	3.90	31.12	4.65	4.61
MCC2-G	50.87	31.64	4.61	4.61

Again, the interactions of proton and metal ions with the nitrogen atom in aziridine ring have been chosen for study. These ions interact preferably with nitrogen atom of aziridine ring of MCC, MCC1 and MCC2, and their respectivhe ion complexes are shown in **Figures 5a-5i**. The N atomic site of aziridine ring acquires strong affinity for proton, and the Li^+ and Na^+ interact less favorably with this

Table II — Computed geometrical parameters of aziridine ring (keto and enol forms) in free mitomycin.

Drugs	Bond lengths(Å)of			Bond angles(in degrees) of		
	N-C1	C1-C2	C2-N	N-C1-C2	C1-C2-N	C2-N-C1
MCC	1.485	1.488	1.485	59.9	59.9	60.2
	(1.485)	(1.486)	(1.485)	(59.9)	(60.0)	(60.1)
MCC1	1.481	1.491	1.482	59.8	59.8	60.4
	(1.481)	(1.488)	(1.482)	(59.9)	(59.8)	(60.3)
MCC2	1.481	1.490	1.482	59.8	59.8	60.4
	(1.481)	(1.488)	(1.482)	(59.9)	(59.8)	(60.3)

( ) bracketed values are for enol

Table III — Computed geometrical parameters of aziridine ring (keto and enol forms) in mitomycin after interaction with -NH_2 group of guanine

Complex	Bond lengths(Å)of			Bond angles(in degrees) of		
	N-C1	C1-C2	C2-N	N-C1-C2	C1-C2-N	C2-N-C1
MCC-G	1.463	1.488	1.471	59.8	59.3	60.9
	(1.485)	(1.486)	(1.485)	(59.9)	(60.0)	(60.1)
MCC1-G	1.481	1.490	1.482	59.8	59.8	60.4
	(1.481)	(1.488)	(1.482)	(59.9)	(59.8)	(60.3)
MCC2-G	1.456	1.489	1.464	59.6	59.1	61.3
	(1.481)	(1.488)	(1.482)	(59.9)	(59.8)	(60.3)

( ) bracketed values are for enol

Table IV — Computed net charges on different atoms of aziridine ring(keto and enol) in drug-guanine complex (HF/STO-3G)

Form of Complex	Net charges on different atoms of aziridine in								
	MCC			MCC1			MCC2		
	C1	C2	N	C1	C2	N	C1	C2	N
Keto	-0.015	0.001	-0.337	-0.016	-0.008	-0.256	-0.007	-0.001	-0.273
Enol	-0.023	-0.007	-0.305	-0.014	-0.008	-0.254	-0.016	-0.117	-0.256

Table V — Computed interaction energies of atomic sites near aziridine ring with metal ions.

Ions	Interaction energies(kcal/mol) of drugs					
	HF/6-31G**			HF/6-31G/MP2		
	MCC	MCC1	MCC2	MCC	MCC1	MCC2
Na^+	-49.05	-40.37	-36.82	-53.96	-46.23	-40.32
Mg^{2+}	-213.60	-201.52	-198.00	-223.82	-213.36	-209.55

atomic site. As can be seen in **Figures 4f-4i**, the sodium and magnesium ions can interact with the other atomic sites rather than N atom of aziridine ring. Unlike metal ion, the proton interacts preferably with N atom of aziridine in MCC, MCC1 and MCC2. The geometrical relaxations as well as net charges are shown in **Tables VIII** and **IX**. It is seen that the C1 carbon atom in protonated aziridine acquires significant positive charge unlike free molecule.

In this way N atom in aziridine ring is found to be the important site for interaction with proton and ions, which might be useful for demonstrating acid catalyzed ring opening of aziridine.

Electrophilic pathway or formation of carbocation at C1

It can be seen that the metal ions and proton acquire large affinity for N atom of aziridine ring

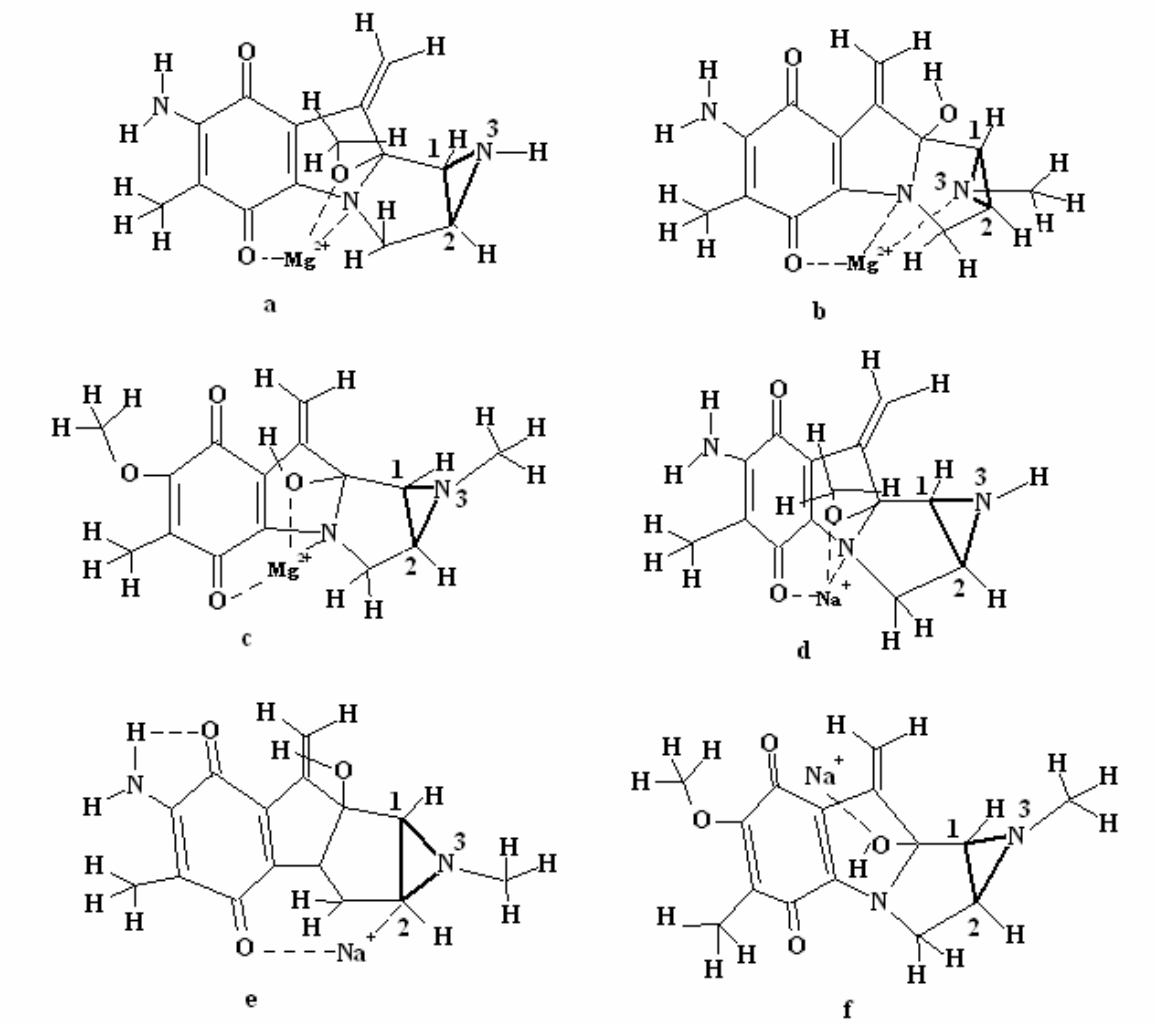

Figure 4 — Optimum structures of (a) MCC-Mg^{+2} complex (b) MCC1-Mg^{+2} complex (c) MCC2-Mg^{+2} complex (d) MCC-Na^{+1} complex (e) MCC1-Na^{+1} (f) MCC2-Na^{+1} complex.

Table VI — Computed interaction energies at nitrogen of aziridine ring with proton and metal ions (HF/6-31G**)

Ions	Interaction energies(kcal/mol) of drugs		
	MCC	MCC	MCC2
H^+	-244.25	-241.51	-240.08
Li^+	-59.19	-56.16	-54.73
Na^+	-42.12	-39.25	-40.41
Mg^{2+}	-184.44	-175.13	-188.87

(**Tables VI**). However, the interaction of ion with H atom at C1 in aziridine ring can be analysed for representing H atom abstraction pathways in alkylation, because such tricyclic rings are prone to interact with ions. It is assumed that Na^{+1} interacts with the lone pair electron of nitrogen atom as well as with the H atom attached to C1 in the ring. A model study has been carried out involving hydrogen atom abstraction from the C1-H sites of aziridine part in MCC, MCC1 and MCC2. The sets of interaction energy values obtained from the optimum complexes of Na^+ with H-C1 of aziridine ring in mitomycin are summarized in **Table IX**. Most of these complexes are found to be quite stable, and the optimum complexes are found from the minimum energy in the curve shown in **Figures 6a-c**. The relaxation of aziridine ring due to ions that lead to subsequent ring opening before alkylation with $-NH_2$ of GC base pair are being explored. The formation of C1 electrophilic reaction pathway from the conventional reaction steps given in **Figure 1** can be explained from the model studies of hydrogen atom abstraction. The Mulliken net charges in the Na^+-HC1 (aziridine ring in

Table VII — Computed geometrical parameters of aziridine ring in the protonated drugs and ion interacted drugs.

Drugs-Ion Complex	Bond lengths (Å) of			Bond angles (in degrees) of		
	N-C1	C1-C2	C2-N	N-C1-C2	C1-C2-N	C2-N-C1
MCC-H^+	1.510	1.469	1.528	61.7	60.4	57.8
MCC1-H^+	1.499	1.473	1.518	61.4	60.1	58.5
MCC2- H^+	1.499	1.473	1.518	61.4	60.2	58.4
MCC-Li^+	1.490	1.476	1.503	60.9	60.0	59.1
MCC1-Li^+	1.483	1.477	1.494	60.6	59.9	59.5
MCC2-Li^+	1.483	1.477	1.494	60.6	59.9	59.5
MCC-Na^+	1.486	1.479	1.497	60.6	59.9	59.4
MCC1-Na^+	1.456	1.490	1.464	59.6	59.1	61.4
MCC2-Na^+	1.477	1.477	1.494	60.8	59.6	59.6
MCC-Mg^{2+}	1.515	1.465	1.550	62.7	60.2	57.1
MCC1-Mg^{2+}	1.505	1.469	1.533	62.0	60.1	57.8
MCC2-Mg^{2+}	1.505	1.464	1.545	62.7	60.0	57.4

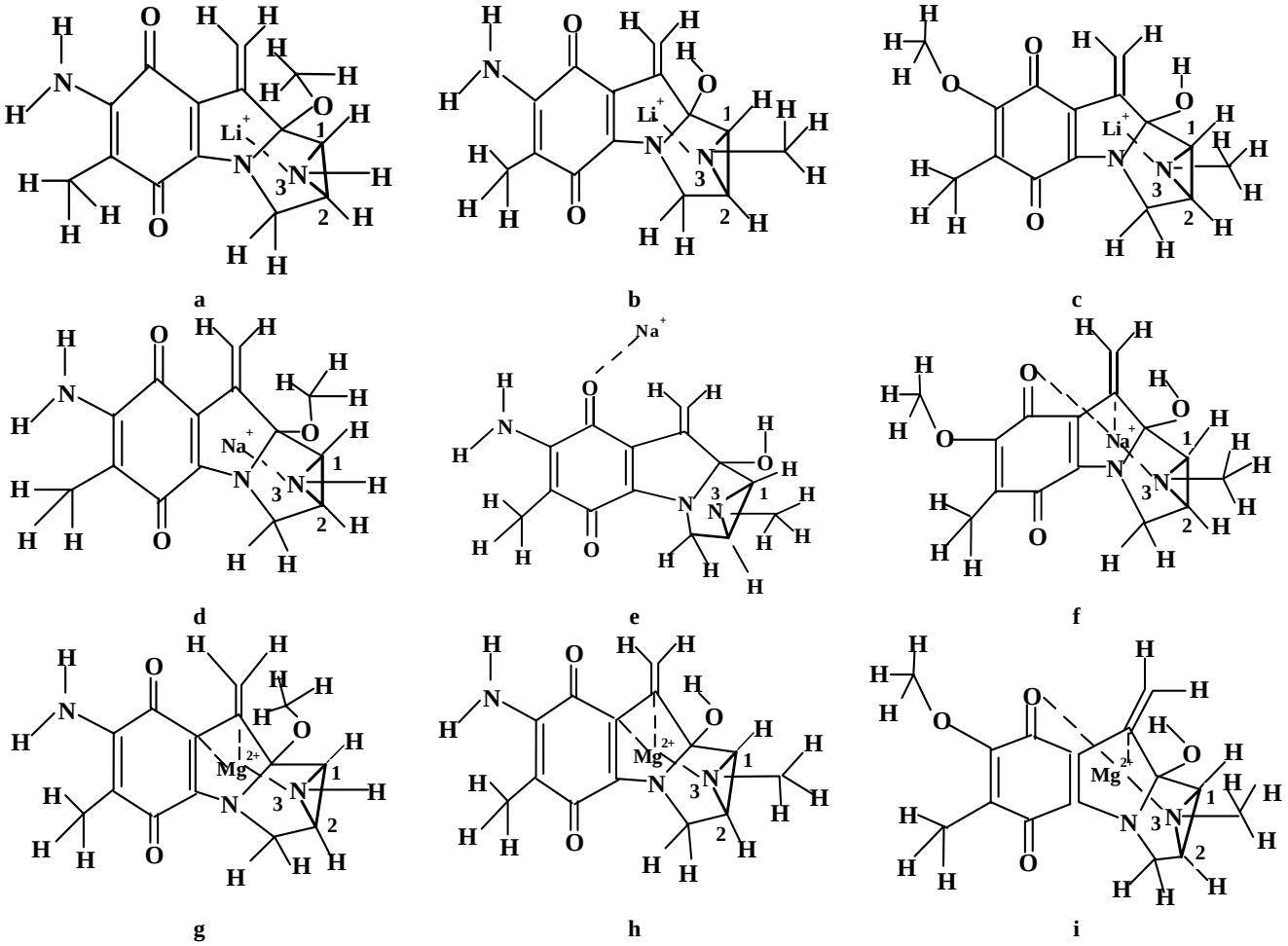

Figure 5 — Metal ion interacted structures of MCC, MCC1 and MCC2 at N of aziridine: (a)-(c) with Li^{+1}, (d)-(f) with Na^{+1}, (g)-(i) with Mg^{+2} .

Table VIII — Computed net charges on different atoms of aziridine ring in free drug, protonated and ion interacted drug at nitrogen.

Interacting ions	Net charges on different atoms of aziridine in								
	MCC			MCC1			MCC2		
	C1	C2	N	C1	C2	N	C1	C2	N
Free drug	-0.10	-0.11	-0.61	-0.04	-0.09	-0.57	-0.04	-0.08	-0.57
H^+	0.01	-0.04	-0.81	0.03	-0.03	-0.79	0.03	-0.02	-0.79
Li^+	-0.08	-0.04	-0.83	-0.03	-0.03	-0.79	-0.03	-0.03	-0.79
Na^+	-0.09	-0.06	-0.79	-0.05	-0.08	-0.58	-0.06	-0.04	-0.69
Mg^{2+}	-0.03	0.01	-1.03	-0.01	0.02	-0.98	-0.04	0.03	-0.94

Table IX — Optimum distances, interaction energies and net charges on different positions of the aziridine ring in the model of H atom abstraction (at C1) by Na^+

Drugs	Optimum distances (Å)	Interaction energies(kcal/mol)	Net charges in		
			C1	C2	N
MCC	1.6	-26.61	-0.034	0.001	-0.277
MCC1	2.0	-60.34	-0.027	-0.004	-0.245
MCC2	2.6	-40.05	-0.056	-0.020	-0.229

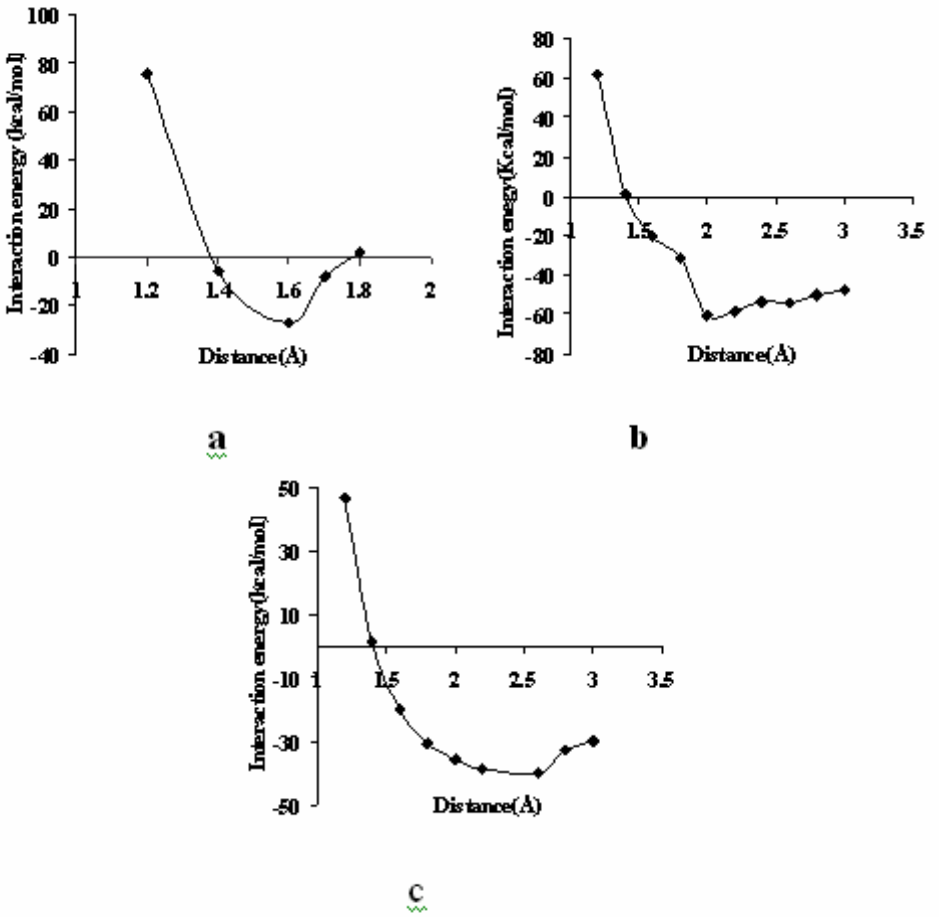

Figure 6 — Plot of interaction distances (D) of Na^{+1} from H of C1 (aziridine ring) in (a) MCC (b) MCC1 (c) MCC2, versus their corresponding interaction energies.

mitomycin) is shown in **Table IX**. The formation of an electrophilic center at C2 is also observed in MCC whereas in other drugs all the atomic centers acquire large negative charge that is contrary to forming an electrophilic center at C1 necessary for undergoing pathway shown in **Figure 1** (**Table IX**) In this way, the abstraction of H atom from the aziridine ring may be proposed as another mechanism of forming electrophilic centre at C1 on the basis that the aziridine ring of mitomycin C may interact with ions as other tricyclic ring.

In this pathway the formation of electrophilic center (carbocation) at C1 is required for interacting with $–NH_2$ of guanine. But the model of hydrogen atom abstraction by Na^+ for generating electrophilic center at C1 as given in **Figure 1** is not observed.

If two pathways are considered: (a) the formation of protonated species at N of aziridine ring, and (b) the formation of C1 electrophilic center due to ions, the pathway (a) might be important since the proton can interact preferably with N than abstracting hydrogen atom from C1 (**Tables VI** and **IX**). Again, the net charge on C1 in the protonated aziridine ring is highly positive, then it can be considered that the formation of protonated species for generating C1 electrophilic center before attacking guanine is important rather than the reaction pathway through H atom abstraction (**Table IX**). Hence, the solution *p*H as well as the effect of ions present in the medium might influence the activation of aziridinium ring. It can be seen that the formation of C1 electrophilic center is not possible through H atom abstraction, and if it is the initial pathway, then an entirely different mechanism may be followed since the net charges on C2 is positive only in MCC (**Table IX**). In addition to this, the H atom abstraction is less favorable than protonation at N of aziridine. Significant differences are observed in the net charges on C1 and C2 centers in the N protonated aziridine ring, where C1 acquires large positive charge for serving as electrophilic center (**Table IX**). Since the nitrogen atom of these drugs acquire strong affinity for H^+, Li^+ and Na^+, the ring opening step might be initiated by these ions. It may be assumed that the formation of protonated aziridine for generating the C1 electrophilic center in MCC, MCC1 and MCC2 can take place at the initial step of drug and guanine interaction. Hence the results justify the preferred alkylation reaction of C1 with $–NH_2$ of guanine. Moreover, the presumed pathways of direct alkylation by aziridine ring at $–NH_2$ of guanine is ruled out, and the aziridine ring opening pathway in the quinone reduction process of species A should occur before binding with guanine. These pathways have been analysed for MCC, MCC1 and MCC2 from the potential energy diagram of forming the activated aziridine ring before attacking the $-NH_2$ of guanine nucleobases (**Figure 2**, **Table I**).

It is possible to modify mitomycin C for enhancing the reactivity of aziridine ring for binding with $–NH_2$ of guanine, but in some cases the specific binding of MCC takes place with guanine present in variety of sequences like CG, GG, TG and AG. In that case, some MCC bind with TG and AG rather than CG and GG (18). Hence, it is crucial to understand the reactivity of aziridine ring in mitomycins and its binding affinity for guanine. However, wide differences have been found between the interaction energies of protonated aziridine ring (at N) of these drugs (MCC, MCC1 and MCC2). At the same time association of ions favorably at the lone pair electron (N), and small affinity of ions on other atomic sites as well as H atom abstraction profile for generating electrophilic C1 center might demonstrate the influence of ions at the initial reaction step of alkylation reaction.

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