Theoretical study on the mechanism of alkylation at N-7 of guanine by few nitrogen mustards

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The interaction of aziridinium ion with N-7 of guanine is considered the common reaction step in the alkylation of DNA by nitrogen mustards. However, the involvement of another intermediate, carbonium ion is expected in some nitrogen mustards. The study on the energies of forming these intermediates indicates that carbonium ion is generated at different energy levels in the transition state and energetically less favourable, which might indirectly explain the experimentally observed lower alkylating abilities of some nitrogen mustards. These drugs acquire ability of generating aziridinium ion but substitution at N-4 might not enhance the reactivity of nitrogen mustards. Some drugs may generate another energetically less favourable carbonium ion intermediate.

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Alkylation at N-7 of guanine in DNA has been considered the favoured mechanism of alkylating agents like nitrogen mustards. The mechanism of alkylation has been studied both experimentally and to some extent theoretically. In general, alkylating agents produce unstable aziridinium ion intermediate and follow similar mechanism but the chemical properties of these drugs are different. Some drugs like uracil and quinacrine mustards follow similar alkylation mechanism but possess quite different anticancer properties. In fact, the exact nature and origin of such differences can’t be trapped. Thus, the energetic of the intermediates may be necessary for understanding the extent of major disruption in DNA, and also might reveal useful information for addressing the differences of drug properties.

In some cases, alkylation labialises the imidazole ring resulting ring opening, which damages DNA drastically. So, it is necessary to control certain mechanism for checking the cytotoxic effects of drugs. Ultimately, in some reaction pathway strong binding of drug with thymine may occur, and in such conditions it recognizes AT base pair for a GC. In addition to covalent bond formation with thymine, alkylated guanine may recognize other bases that would benefit for converting DNA miscoding in mutagenic processes. But, the common mechanism of alkylation involves covalent binding with N-7 of guanine through aziridinium ion intermediate (Figure 1). Thus, the reactivity of aziridinium ion is the most crucial factor for determining the alkylating abilities of drugs and also for deciding the related biological properties like potency and cytotoxicity.

The potency and cytotoxicity have been analysed on the basis of their affinities for N-7 of guanine or for the region containing a number of GC sequences. Moreover the clinically useful nitrogen mustards (Figure 2) are well studied on the basis of their interstrand cross-linking abilities through aziridinium ion, and subsequent theoretical studies for relating the biological properties to interaction abilities of these nitrogen mustards specifically at N-7 of guanine are not reported. Alternatively, the importance of aziridinium ions of different drugs that contribute strong interaction with N-7 of guanine has not been directly demonstrated and this aspect is equally important in determining mechanistic origin of sequence specificity or any differences in drug properties.

![Figure 1](image-url) — Alkylation mechanism of nitrogen mustards at N-7 of guanine
properties. Thus, we have taken some useful drugs such as mustine, melphalan, chlorambucil and bendamustine to analyze the explicit description of intermediates that could interact with N-7 of guanine in the intermediate reaction pathway.

On the other hand, the preferred mechanism of alkylation through aziridinium ion than the carbonium ion in some nitrogen mustards has been reported\textsuperscript{1-3}. The possibility of generating carbonium ion before attacking N-7 of guanine is yet to be explored. Thus, if these two mechanisms exist, analysis can be made on the energetic of these intermediates before binding with N-7 of guanine. For instance, the studies on the selected drugs are used for understanding both the mechanisms. We now analyze the energies as well as the structural description of intermediates in the transition state of different drugs. The study might be useful for understanding the differences of properties among nitrogen mustards.

**Method**

\textit{ab-initio} Computational method for determining the intermediates and transition state (TS) geometries of aziridinium and carbonium ions\textsuperscript{21} has been used. Complete geometry optimizations are carried out using 6-31G* basis sets for obtaining the geometries of these intermediates. Gaussian programme code for all the calculations\textsuperscript{21} has been used.

In order to obtain the optimum geometries of aziridinium ion, the correlation plots between reaction coordinates of forming cyclic aziridinium ions versus total energies are examined. The optimum geometries of aziridinium ions are obtained from the minimum energy in the potential energy curves (\textbf{Figures 3a-d}) and the geometries of aziridinium ions (intermediate) as shown in \textbf{Figure 3e} are further relaxed to obtain the geometries of transition state. The geometries are shown in \textbf{Figures 3a-d}.
corresponding to the maximum and minimum potentials in the reaction-path are shown in Figure 4. The model of transition state expected in the drug-guanine adduct is shown in Figure 5.

**Figure 4** — Energy profile of alkylation

**Figure 5** — Transition state feature of aziridinium ion in drug-guanine adduct

**Results and Discussion**

The energies required for the formation of aziridinium ions in the intermediate state as in reaction step I (Figure 1) are given in Table I. We observe narrow differences in the energies among these drugs except for bendamustine, which forms aziridinium ion at slightly high energy level. Again the energy barrier (E_a) for changing the aziridinium ion from intermediate to transition state is also computed to analyze the possibility of direct interaction by aziridinium ion intermediates at N-7 of guanine (Figure 4). The energy barriers (E_a) are found to be in the decreasing order of drugs, mustine < melphalan < chlorambucil < bendamustine (Table I). The small variation in these energies indirectly show that the energetic of alkylation by most of the nitrogen mustards are equal, however, bendamustine requires slightly more energy to form aziridinium ion than those of other nitrogen mustards. Thus, the alkylation of bendamustine may affect by the slow process in reaction step I (Table I). Hence, it might cause limitation in pharmacological efficiency of this drug by deactivating the aziridinium ion in the surrounding water and metal ions1a (Table II).

Moreover, one could consider the negative charges on the atoms of aziridinium ions, which are the prominent sites of attack by the surrounding cations and protons (Table III). This may lead to direct ring opening of aziridinium ion before attacking the N-7 of guanine. These results may be useful for analyzing experimentally observed differences in the chemical properties of these drugs5-14 (Table II).

On the other hand, the possibility of generating carbonium ion for proceeding to different reaction paths may be speculated. Alternatively, the computed net charges on the atoms of the aziridinium ions can be considered for understanding preferential atomic site for binding at N-7 of guanine. The net charges on different atoms of aziridinium ions are shown in Table III. We observe large negative charges on atoms of aziridinium ions of all drugs but the

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<th>Table I — Comparison of stabilities of aziridinium ions and carbonium ions of drugs</th>
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<td>Drugs</td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Mustine</td>
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<tr>
<td>Melphalan</td>
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<td>Chlorambucil</td>
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<td>Bendamustine</td>
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<th>Table II — The energy barrier for the aziridinium ions of different drugs in transition state</th>
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<td>Bendamustine</td>
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Table III — Geometrical parameters of the aziridinium ions for different drugs

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<tr>
<th>Drugs</th>
<th>( \theta )</th>
<th>( r_1 )</th>
<th>( r_2 )</th>
<th>( r_3 )</th>
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<tbody>
<tr>
<td>Mustine</td>
<td>60.410</td>
<td>1.5070</td>
<td>1.4913</td>
<td>1.5085</td>
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<tr>
<td>Melphalan</td>
<td>60.305</td>
<td>1.5144</td>
<td>1.4885</td>
<td>1.5085</td>
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<tr>
<td>Chlorambucil</td>
<td>60.341</td>
<td>1.5093</td>
<td>1.4897</td>
<td>1.5071</td>
</tr>
<tr>
<td>Bendamustine</td>
<td>60.336</td>
<td>1.5085</td>
<td>1.4896</td>
<td>1.5069</td>
</tr>
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aziridinium ions of chlorambucil and bendamustine acquire unequal net charges at C-6 and C-7. Hence, the nature of the electron densities on respective atoms of aziridinium ions appending a probe on the mechanism that involved carbonion (carbocation) in transition state. It has been reported that the abilities of cross-linked interstrand formation by these intermediates correlate with drug cytotoxicity\(^{15c}\). But the narrow differences in the energies of aziridinium ions in transition state cannot definitely confirm the variation in anticancer properties of drugs. We observe that carbonium ion is generated at higher energy level than that of aziridinium ion (Table I). The ability of forming carbonium ion at slightly high energy level does not suggest direct interaction by this intermediate with N-7 of guanine. But in chlorambucil and bendamustine we observe narrow differences between the energies of carbonion and aziridinium ion. Thus, these drugs are expected to undergo alkylation through aziridinium as well as carbonium ion (Table I). Here, the efficacy of generating aziridinium and carbonium ion might control the alkylation reaction. Such variation of energies is large for mustine and melphalan. Hence the alkylation only through aziridinium ion is expected in these drugs. The results highlight the lack of pharmacological efficiencies of drugs like chlorambucil and bendamustine despite of their anticancer properties or any other special property\(^{14}\). And such molecules are generally good agent to form mono adduct with DNA and also acquire carcinogenic property.

The interesting feature in these drugs is the relatively small differences in the energies of forming the aziridinium ions (Table I), which indeed may be due to the attainment of a stable aziridinium ring (saturated structure). Again we observe very less differences in the geometrical parameters of the aziridinium ions (Figure 3e). Since these tricyclic rings are believed to possess aromaticity and probably in most of the drugs, the aziridinium ions might attain such stable structure that simply cannot further induce. The findings do not support the synthetic chemists’ approach of designing nitrogen mustards by modifying N-4 substituents. The study also focuses on the essential conditions of studying the mechanism of alkylation by characterizing the transition state rather than the intermediate state.

### Conclusion

The findings obtained from the theoretical models of structures and energies of aziridinium and carbonium ions in the alkylation reaction can be summarised as follows:

(i) The nitrogen mustards have equal abilities to form aziridinium and drugs like chlorambucil and bendamustine may also generate carbonium ion in the transition state.

(ii) The energies of forming carbonium ions in the transition state are higher than that of aziridinium ion, but small differences between the energies of these two types of intermediates are observed in chlorambucil and bendamustine. The results certainly lend support for the differences in the chemical properties among nitrogen mustards.

(iii) It is likely that some nitrogen mustards might undergo alkylation reaction through carbonium ion, but substituent at N-4 of nitrogen mustard cannot enhance binding ability of aziridinium ion.

### Acknowledgement

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### References