CHAPTER 4

STUDY OF REACTIVITY OF A FEW AZIRIDINIUM ION INTERMEDIATES
4.1 INTRODUCTION

The reactivity/stability of intermediate/s formed in bio-chemical, organic, inorganic reactions are found to be dependent on reaction environment [1]. Different factors like temperature, nature of solvent and substrate etc., govern the rate of a reaction. As for example, a nucleophilic substitution reaction may be $S_N2$ in gas phase while $S_N1$ or $S_N2$ in solution phase [2]. Interestingly, the rate of DNA alkylation reaction as well as anticancer activity of aliphatic nitrogen mustards (N-mustards) is different from that of aromatic N-mustards [3]. Experimental and theoretical studies have been made to understand the effect of various factors such as strength of nucleophile, pH, temperature and common ion effect on the reaction pathway, as well as hydrolysis and dynamics of DNA alkylation by N-mustards [4].

Solvent exerts enormous impact on nucleophilic substitution reactions and bond heterolysis [5]. A reaction can accelerate or slow down by a factor of $10^{20}$ depending upon presence/absence of solvent. Moreover, the solvent not only harbours a chemical reaction but also gets intimately involved in it and solvent effect can be more potent than any other factors like steric effect. The influence of various factors on the mechanism of action of a few bis-alkylating N-mustard anticancer drugs has already reported [6,3-4].

Cancerous cells/tissues exposed to high temperature (313 K-320 K) experience enhanced tumour sensitivity to both radio- and chemotherapy [7]. Cytotoxicity of antitumor drugs reportedly increases if temperature is raised from 313 K to 318 K [8,10(b),10(f)]. Alkylating agents like melphalan shows to be the most effective at moderately elevated temperatures (313 K-316 K) while cisplatin
or oxaliplatin was less pronounced towards antitumor activity at high temperature [9]. Elevated body temperature enhances the sensitivity of tumour cells to anticancer drugs like doxorubicin [10,11,14], vincristine [11] and carboplatin [12]. It is also reported that elevation of heat slightly influences the efficacy of cytotoxic anticancer drug etoposide [13].

It was mentioned earlier that the reactivity of aziridinium (Az\(^+\)) ion intermediate may be affected by different factors; such as external electric field, structural variation of the intermediate species etc. [14]. Herein, an investigation is carried out with the objective to study the effect of nature of solvent (polar/non-polar) and temperature on the reactivity/stability of a few Az\(^+\) ion intermediates, formed during alkylation of DNA by N-mustards.

4.2 THEORETICAL AND COMPUTATIONAL DETAILS

Global reactivity descriptors (GRDs), specifically chemical potential (\(\mu\)), global hardness (\(\eta\)) and electrophilicity (\(\omega\)) are calculated in gas phase as well as in three different solvent phases (ranging from polar to non-polar). Apart from that, basis set superposition error (BSSE) [15] corrected interaction energy between Az\(^+\) ion intermediates and guanine-cytosine (GC) base pair is also computed using supermolecular approach [16] \(\Delta E_{\text{int}} = E_{Az^+\text{+GC}} - E_{Az^+} - E_{GC}\).

Enthalpy and Gibbs free energy of formation of Az\(^+\) ion intermediates are analysed at different temperatures and solvents. Further, the natural bond orbital (NBO) [17] analysis is also performed.

The gas phase geometrical minima of the species are optimized using 6-31+G(d) basis set with Becke three parameter exchange and Lee, Yang and Parr
correlation functional, B3LYP [18] and are confirmed by absence of any imaginary frequency. Single point calculations are performed at the same level of theory at different temperatures (ranging from 77 K to 315 K) and different solvents with different di-electric constants (n-octanol, $\varepsilon = 9.86$; 1,2-ethanediol, $\varepsilon = 40.24$; and water, $\varepsilon = 78.35$), using polarizable continuum model (PCM) [19]. The di-electric constant of the fluids in different parts of human body are different, varying from non-polar to a polar one and hence, three solvents having a range of di-electric constants are chosen. The GRDs (chemical potential, global hardness and electrophilicity) are calculated as defined within the framework of DFT [20]. All calculations are performed using Gaussian09 [21].

4.3 RESULTS AND DISCUSSION

Reactivity/stability of the $Az^+$ ion intermediates of six anticancer drugs belonging to the N-mustard family is analysed using DFT based reactivity descriptors. Impact of solvent media on global hardness, electrophilicity, change in enthalpy ($\Delta H$) and Gibbs free energy ($\Delta G$) are computed. Thermodynamic feasibility of $Az^+$ ion intermediate formation is observed at different temperatures and gas as well as solvent phases. The optimized structure of the drug intermediates, obtained at B3LYP/6-31+G(d) level of theory are shown in Figure 4.1.

4.3.1 Variation of Reactivity Descriptors:

The reactivity/stability of $Az^+$ ion intermediates is monitored using GRDs: global hardness and electrophilicity. The gas phase global hardness and electrophilicity are calculated at B3LYP/6-31+G(d) level of theory. Further, to observe the effect of solvent on the reactivity pattern, the calculations are repeated
In three different solvents at the same level of theory; variations are shown in Figure 4.2.

In gas phase, the variation of global hardness (in au) follows the order: mustine (0.1475) > spiromustine (0.1176) > chlorambucil (0.1087) > uracil mustard (0.1000) > bendamustine (0.0980) > melphalan (0.0816) and changes to: mustine (0.1548) > spiromustine (0.1220) > chlorambucil (0.1140) > uracil mustard (0.1026) > melphalan (0.1009) > bendamustine (0.1000) in aqueous phase, Figure 4.2 (a). On the other hand, electrophilicity (in au) is in the order: uracil mustard (0.5110) > spiromustine (0.4130) > melphalan (0.4080) > chlorambucil (0.3509) > bendamustine (0.3410) > mustine (0.2980) in gas phase and changes to: uracil mustard (0.1638) > melphalan (0.1175) > bendamustine (0.1140) > chlorambucil (0.1071) > spiromustine (0.0990) > mustine (0.0871) in aqueous phase, Figure 4.2 (b). It is important to note that, as we move from gas phase to a solvent phase, global hardness of the $Az^+$ ion intermediates do not change much, Figure 4.2 (a). On the contrary, electrophilicity (in au) shows a dramatic drop in their values on moving from gas phase to n-octanol, indicating low reactivity of the $Az^+$ ion intermediates in solvent phases, Figure 4.2 (b). Hence, it is clear that the electrophilicity of $Az^+$ ion intermediates decreases with incorporation of polar solvent. This is because of the interaction between the polar solvent and the positively charged $Az^+$ ion intermediates. Low electrophilicity of the $Az^+$ ion intermediates might effect the acceptance of electron density from N7 centre of guanine (in DNA), as shown in Figure 4.3. This, in turn weaken the tendency (measured in terms of interaction energy between the two species) of the
$Az^+$ ion intermediates to bind with guanine (in DNA) in solvent phases. To verify this, we calculated the interaction energy between $Az^+$ ion intermediates and GC base pair.

4.3.2 Interaction Energy:

The BSSE corrected interaction energies are reported in Table 4.1 and are calculated using super-molecular approach, $\Delta E_{\text{int}} = E_{Az^+GC} - E_{Az^+} - E_{GC}$ (for the sake of simplicity, we considered only one GC base pair instead of the DNA chain).

Interaction energy values clearly show a sharp drop as we move from gas to aqueous phase Table 4.1. Trend of interaction energy (in kcal/mol) in gas phase is in the order: uracil mustard (-56.97) > spiromustine (-52.71) > chlorambucil (-50.40) > mustine (-48.64) > melphalan (-46.76) > bendamustine (-44.56). However, gas phase interaction energy (in kcal/mol) variation does not tally with the aqueous phase order and is observed to be: melphalan (-34.96) > uracil mustard (-25.20) > chlorambucil (-25.03) > bendamustine (-24.26) > mustine (-23.08). This indicates that the extent of solvation varies from species to species. Uracil mustard possesses highest interaction energy (in kcal/mol) in gas phase (-56.97) while the aqueous phase interaction energy of melphalan (-34.96) is maximum.

4.3.3 Thermochemical Properties:

The second part of the study pertains to the influence of temperature and polarity of the solvent (di-electric of medium) on thermochemistry. Thermodynamic feasibility of the $Az^+$ ion intermediate formation ($drug \rightarrow Az^+$
+ Cl), is observed from ΔH and ΔG values involved in the process. They are calculated using supermolecular approach (e.g., ΔH = (H_{Az^+} + H_{Cl^-}) – H_{drug}).

Variation of ΔG and ΔH in different solvents at 298.15 K are summarised in Figure 4.4. It is interesting to note that, as the di-electric constant of the medium increases (moving from gas phase to aqueous phase), ΔG and ΔH values drop suddenly (still exhibiting positive values). For example, the gas phase ΔG (in kcal/mol) of mustine is 119.67 and drops suddenly to 12.16 in n-octanol, which further decreases to 3.56 and 2.17 in 1,2-ethanediol and water respectively. Similarly, for uracil mustard the order of ΔG (in kcal/mol) values in different phases is: gas phase (126.97) > n-octanol (21.77) > 1, 2-ethanediol (12.42) > aqueous phase (10.89). Az^+ ion intermediates of melphalan, chlorambucil, bendamustine and spiromustine also follows the same trend, Figure 4.4 (a). Similar result is also obtained in case of ΔH values. In gas phase, ΔH (in kcal/mol) value of mustine is 126.09 while it decreases to 19.73 in n-octanol, 10.51 in 1,2-ethanediol and 9.06 in aqueous phase. This clearly indicates the effect of solvent polarity on both the parameters, Figure 4.4 (a) and Figure 4.4 (b). It is conclusive to comment that the Az^+ ion intermediate formation is comparatively favourable in solvent phase as that of gas phase. A positive value of ΔH indicates the absence of thermodynamic driving forces for Az^+ ion intermediate formation.

Our next step is to analyse the effect of temperature on the thermodynamic parameters. ΔG and ΔH for the Az^+ ion intermediate formation are calculated at
five different temperatures in gas and aqueous phases and are shown in Figure 4.5.

It is evident from Figure 4.5 that, as temperature increases, gas as well as aqueous phase $\Delta G$ drops significantly (Figures 4.5 (a-b)) whereas $\Delta H$ decreases slightly (Figures 4.5 (c-d)). At 77 K, $\Delta G$ (in kcal/mol) and $\Delta H$ (in kcal/mol) values of uracil mustard are 131.71 and 133.36 respectively while at 315 K these values are 126.61 and 133.30 in gas phase. It is to be worth mentioning that spiromustine $Az^+$ ion intermediate exhibit exceptional behaviour in gas as well as in aqueous phases. $\Delta H$ of spiromustine $Az^+$ ion intermediate is 122.95 (13.25) kcal/mol at 77 K and increases slightly with the increase of temperature; 124.52 (15.23) kcal/mol at 315 K in gas (aqueous) phase. Our observations suggest that the $Az^+$ ion intermediate formation is favoured at high temperature and polar solvents. As cytoplasm is a polar medium, this observation implies that the $Az^+$ ion intermediate formation is favoured in cytoplasm at normal body temperature (310 K).

4.3.4 NBO Analysis:

In order to have a better understanding on the reactivity/stability of $Az^+$ ion intermediates, it is important to calculate the charge density on the reactive centres of the intermediates. NBO analysis is helpful to study the distribution of electron density in atomic and molecular orbitals [22]. Therefore, NBO analysis is performed at B3LYP/6-31+G(d) level of theory.

The effect of substitution of the methyl group of mustine by electron withdrawing groups is observed from the calculated NBO charges on the carbon
(C) and nitrogen (N) centres of the $Az^+$ ion intermediates as shown in Figure 4.3. The NBO charges at the ‘N’ centre of the drug is in the order: mustine (-0.5553) > bendamustine (-0.5545) > uracil mustard (-0.5491) > spiromustine (-0.5226) > chlorambucil (-0.5081) > melphalan (-0.4878). It is worth noting that, all the drug molecules possess lesser negative charge on the ‘N’ centre compared to mustine. Thus, substitution at the ‘N’ centre becomes successful in withdrawing electronic charges, which is necessary to slow down the rate of $Az^+$ ion intermediate formation.

4.4 CONCLUSION

In this Chapter, an effort was made to study the reactivity/stability, interaction energy, and thermochemistry of $Az^+$ ion intermediate formation. This study reveals that reactivity of the $Az^+$ ion intermediates is lowered on incorporation of solvent media, which in turn reduces the capability of the species to accept electron density from N7 centre of guanine. Exceptional drop in electrophilicity of the $Az^+$ ion intermediates in polar solvent make them stable.

The $Az^+$ ion intermediates of the prototype drugs exhibit significant interaction energy with GC base pair in gas as well as in aqueous phases and are important key for their cytotoxicity. High temperature and polarity of the solvent favours the thermochemistry of the $Az^+$ ion intermediate formation. Thus, normal body temperature (310 K) and polarity of cytoplasm allows the formation of $Az^+$ ion intermediate. NBO analysis shows that substitution at the ‘N’ centre of mustine may slow down the rate of $Az^+$ ion intermediate formation process.
Figure 4.1: Optimized structure of the aziridinium ($Az^+$) ion intermediates obtained at B3LYP/6-31+G(d) level of theory (colour code: white = hydrogen, grey = carbon, blue = nitrogen, red = oxygen, green = chlorine).
Figure 4.2: Variation of global hardness (in au) and electrophilicity (in au) of the aziridinium ($Az^+$) ion intermediates from gas to different solvent phases at B3LYP/6-31+G(d) level of theory (Entry No.: 1 = mustine-$Az^+$ ion, 2 = melphalan-$Az^+$ ion, 3 = chlorambucil-$Az^+$ ion, 4 = bendamustine-$Az^+$ ion, 5 = spiromustine-$Az^+$ ion, 6 = uracil mustard-$Az^+$ ion; □ = gas phase, ○ = n-octanol, ▼ = 1,2-ethanediol, □ = water; connecting lines do not mean any average).
Figure 4.3: Transfer of electron density from guanine to aziridinium ($Az^+$) ion intermediate during alkylation.
Figure 4.4: Variation of Gibbs free energy ($\Delta G$, in kcal/mol) and enthalpy ($\Delta H$, in kcal/mol) involved in aziridinium ($A_z^+$) ion intermediate formation process from different drug molecules at B3LYP/6-31+G(d) level of theory (Entry No.: 1 = mustine-$A_z^+$ ion, 2 = melphalan-$A_z^+$ ion, 3 = chlorambucil-$A_z^+$ ion, 4 = bendamustine-$A_z^+$ ion, 5 = spiromustine-$A_z^+$ ion, 6 = uracil mustard-$A_z^+$ ion; $\square$ = gas phase, $\circ$ = n-octanol, $\Delta$ = 1,2-ethanediol, $\nabla$ = water; connecting lines do not mean any average).
(a) Variation of $\Delta G$ in gas phase

(b) Variation of $\Delta G$ in aqueous phase

(c) Variation of $\Delta H$ in gas phase
Figure 4.5: Variation of Gibbs free energy ($\Delta G$, in kcal/mol) and enthalpy ($\Delta H$, in kcal/mol) of aziridinium ($A_2z^+$) ion intermediates with temperature (in Kelvin) in gas and aqueous phases at B3LYP/6-31+G(d) level of theory ($\nabla$ = spiromustine-$A_2z^+$ ion, $\Diamond$ = uracil mustard-$A_2z^+$ ion, $\triangleleft$ = melphalan-$A_2z^+$ ion, $\nabla$ = bendamustine-$A_2z^+$ ion, $\circ$ = chlorambucil-$A_2z^+$ ion, $\Delta$ = mustine-$A_2z^+$ ion; connecting lines do not mean any average).
Table 4.1: BSSE corrected interaction energy (in kcal/mol) in gas and aqueous phases obtained at B3LYP/6-31+G(d) level of theory.

<table>
<thead>
<tr>
<th>Drug molecule</th>
<th>Interaction energy (in kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gas phase</td>
</tr>
<tr>
<td>Uracilmustard</td>
<td>-56.97</td>
</tr>
<tr>
<td>Spiromustine</td>
<td>-52.71</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>-50.40</td>
</tr>
<tr>
<td>Mustine</td>
<td>-48.64</td>
</tr>
<tr>
<td>Melphalan</td>
<td>-46.76</td>
</tr>
<tr>
<td>Bendamustine</td>
<td>-44.56</td>
</tr>
</tbody>
</table>
REFERENCES

   (d) S. Winstead and R. E. Buckles, *J. Am. Chem. Soc.*, 1943, **65**, 613;

   (b) A. A. Mohamed and F. Jensen, *J. Phys. Chem. (A)*, 2001, **105**, 3259;

   (b) C. Williams and B. Witten, *Cancer Res.*, 1967, **27**, 33;
(e) W. L. Hase, Science, 1994, 266, 998;
(f) L. Sun, K. Song and W. L. Hase, Science, 2002, 296, 875;
(g) D. J. Mann and W. L. Hase, J. Phys. Chem. (A), 1998, 102, 6208;


(b) M. Urano, M. Kuroda and Y. Nishimura, *Int. J. Hyperthermia*, 1999, 15, 79;
(c) M. H. Falk and R. D. Issels, *Int. J. Hyperthermia*, 2001, 17, 1;
(d) S. Z. Fradkin, E. A. Zhavrid and Y. P. Istomin, in Book of Abstracts, 23rd Annual Meeting of the European Society for Hyperthermic Oncology, Berlin, 2006;


(b) P. K. Bhattacharyya and R. Kar, Comput. Theoret. Chem., 2011, 967, 5;


(f) J. E. Carpenter and F. Weinhold, J. Mol. Struct. THEOCHEM., 1988, 46, 41;


