ABSTRACT

The present dissertation entitled “DFRT STUDIES ON THE REACTIVITY OF A FEW DNA ALKYLATING NITROGEN MUSTARDS” describes the reactivity/stability of a few aziridinium ($Az^+$) ion intermediates and their corresponding adducts during alkylation of DNA by nitrogen-mustards (N-mustards) using density functional theory (DFT) and density functional reactivity theory (DFRT).

The findings of the research work are presented systematically into seven Chapters including general introduction and computational methodology. This abstract contains the summary of each Chapter.

Chapter 1: Introduction

This Chapter includes a brief introduction to DFT, DFRT, N-mustards and their derivatives.

DFT plays a fundamental role in explaining and understanding the basic problems of chemical interest. Reactivity descriptors defined within the framework of DFRT are used to explain reactivity/stability of a species. These descriptors are classified into two; global reactivity descriptors (GRDs) and local reactivity descriptors (LRDs). GRDs such as global hardness, chemical potential, softness, electrophilicity, etc., are used to describe the reactivity/stability of chemical systems as a whole. LRDs are used to characterize the local reactivity and site selectivity in different chemical systems. Fukui function, local softness, local philicity, etc., are examples of LRDs.
N-mustards are being used in cancer chemotherapy for several decades. This class of drug molecules preferentially alkylate N7 centre of guanine leading to the formation of both intra- and inter-strand cross-linking in DNA, which eventually produces mono- and cross-linked adducts. N-mustards have been studied and clinically used for last few decades and the mode of action on genomic DNA is well understood. However, they still provide an area of extremely intense and progressive investigation. The mechanism of drug action and research works carried out on it are discussed in this Chapter.

Chapter 2: Computational Methodology

DFT based reactivity descriptors are handy tool to analyse the reactivity/stability of chemical species, to obtain molecular structures, total energy of a system, reaction pathways, thermochemistry etc. Details of the descriptors are explained in this Chapter. Uses of Self-Consistent Reaction Field (SCRF), Polarizable Continuum Model (PCM), and Solvation Model Density (SMD) are also illustrated.

**Reactivity descriptors:** The reactivity descriptors that are used in the study are:

**(A) Global Reactivity Descriptors:**

Chemical potential ($\mu$), global hardness ($\eta$), softness ($S$), electrophilicity ($\omega$) explain the reactivity of a chemical system as a whole.

**(B) Local Reactivity Descriptors:**

Local philicity ($\omega^\alpha_\alpha$), Fukui function ($f^\alpha_\alpha$), local softness ($s$) explain the reactivity of the reactive site of a chemical system.
Solvent effect is estimated using PCM and SMD models. All the calculations in this research work are carried out using Gaussian09.

**Chapter 3: Structural Variation in Aziridinium Ion Intermediates Facilitates DNA Alkylation**

It is expected that during the course of DNA alkylation, $Az^+$ ion intermediates undergo some structural changes.

![Diagram](image)

**Figure 1:** Change of $\angle NCC$ bond angle of aziridinium ($Az^+$) ion intermediate during alkylation of DNA.

Variation of DFT based reactivity descriptors (chemical potential, global hardness, electrophilicity, Fukui function, and local electrophilicity) has been analysed when the tricyclic ring flips apart during DNA alkylation. Applicability of maximum hardness principle and minimum electrophilicity principle (MHP and MEP) are also analysed.

This study clarified that the structural variation of the $Az^+$ ion intermediate during alkylation of DNA by N-mustards is an important factor for the alkylation reaction to occur. Our study also confirms the applicability of MHP and MEP.
Chapter 4: Study of Reactivity of a Few Aziridinium Ion Intermediates

With a view to estimate the effect of solvent phase, nature of solvent, temperature etc., on the reactivity/stability of $Az^+$ ion intermediates, we carried out an investigation on the reactivity/stability of a few $Az^+$ ion intermediates, formed during alkylation of DNA by N-mustards in three different solvent phases with different dielectric constants and also observed the impact of variation in temperature (80 K to 317 K) on the thermochemistry of $Az^+$ ion intermediate formation process. It is found that, reactivity of the $Az^+$ ion intermediates decreases in polar solvent compared to gas phase. In polar solvent, the electrophilicity of the $Az^+$ ion intermediates drop sharply which makes them more stable. High temperature and polarity of solvents favour $Az^+$ ion intermediate formation. Natural bond orbital (NBO) analysis shows that substitution of the methyl group of mustine by electron withdrawing groups at the ‘N’ atom may slow down the rate of $Az^+$ ion intermediate formation. The substitution at the ‘N’ atom becomes successful in withdrawing electronic charges from ‘N’ atom, which is necessary to slow down the rate of $Az^+$ ion formation.

Chapter 5: Effect of External Electric Field on Aziridinium Ion Intermediate and Mustine-Guanine Adduct

The effect of external electric field on $Az^+$ ion intermediate of mustine and its corresponding adduct with guanine has been studied in this Chapter.

It is seen that the shape and energy of the Lowest Unoccupied Molecular Orbital (LUMO) varies with the variation of external electric field.
Figure 2: Structure of (a) $A_{2}^{+}$ ion intermediate of mustine and (b) mustine-guanine adduct.

Figure 3: Variation of LUMO shape in (a) absence and (b) presence of external electric field.

A cytoplasmic environment may therefore shifts the LUMO significantly towards the ring carbon Figure 3; which would facilitate DNA alkylation. MHP as well as MEP are also observed to obey. Moreover, stability of the mustine-guanine adduct have been observed in presence of external electric field. High magnitude of electric field leads to a very high interaction energy. External electric field applied in particular direction favours $\Delta G$ and $\Delta H$ of the mustine-guanine adduct formation process.
Chapter 6: DNA Alkylation by Nitrogen Mustards

Analysis of the reactivity/stability of both $Az^+$ and $Az^{2+}$ ion intermediates as well as mono- and cross-linked adducts formed during alkylation of DNA by N-mustards are presented in this Chapter. Hence, DFT based global and local reactivity descriptors are used to compare the reactivity/stability of $Az^+$ and $Az^{2+}$ ion intermediates, and their corresponding mono- and cross-linked adducts.

Figure 4: Reaction of $Az^+$ and $Az^{2+}$ ion intermediates with guanine during alkylation of DNA.

Propensity of cross-linked adduct formation by uracil mustard is observed to be highest compared to other members. Moreover, because of higher charge
(+2), cross-linked adducts acquire more stability in aqueous phase compared to mono-adducts.

Chapter 7: Understanding the Effect of Variation of Internal Coordinates on the Reactivity and Thermochemistry of Mustine-Guanine Adduct

This Chapter contains the study of the effect of internal coordinate variation (bond length ($l$, distance between N2 guanine and C3 of $Ae^+$ ion intermediate), bond angle ($\theta$, $\angle N2C3C4$) and dihedral angle ($\phi$, $\angle C1N2C3C4$, numbering of atoms shown in Figure 5) on the reactivity of mustine-guanine adduct. In summary, it can be concluded that the internal coordinates such as bond length, bond angle and dihedral angle impart significant influence on the reactivity of the mustine-guanine adduct.

**Figure 5:** Structure of mustine-guanine mono-adduct (numbering in adduct is not in accordance with IUPAC, but an arbitrary one).

HOMO and LUMO energies are also effected to a considerable extent, whereas, the thermochemical parameters are not affected.
**Summary and Future Perspective:**

Though the mode of action of the N-mustard drugs is well understood, the mechanism associated with their biological effectiveness remains contentious. Various research groups are studying more effective N-mustards with improved selectivity against tumour cells. Thus, analysis of the reactivity pattern of these drug molecules bears importance.

Our results reveal that $Az^+$ ion intermediates must undergo some structural changes to facilitate DNA alkylation. Interestingly incorporation of solvent phase lowers the reactivity of $Az^+ / Az^{2+}$ ion intermediates. Moreover high temperature and polarity of solvents favour aziridinium ion formation. Because of solvation, solvent phase interaction energy between the guanine cytosine (GC) base pair and aziridinium ion intermediates is lower than that of gas-phase.

External electric field produced by the presence of ionic species in cellular environment effect the reactivity/stability of the drug intermediate and drug-DNA adduct. It is observed that aziridinium ion intermediate would be more reactive in presence of stronger electric field.

Free energy of solvation ($AG_{sol}$) of $Az^{2+}$ ion intermediate is higher than that of $Az^+$ ion intermediate. This suggests that $Az^{2+}$ ion intermediates are more stable than $Az^+$ ion intermediates in polar solvent. The variation of internal coordinates does not change the electrostatic nature of a molecular system rather it highly effects the chemical behaviour of the system.

Despite of lots of computational works done on N-mustards, still there are more to explore at the molecular level for the development of more efficient N-mustards.