ABSTRACT

The subject of this thesis is the study of structure and reactivity of ruthenium anticancer complexes as well as their interaction with DNA and proteins. NAMI-A and its derivatives are selected for our investigation.

The content of entire thesis is distributed over eight chapters.

Chapter 1

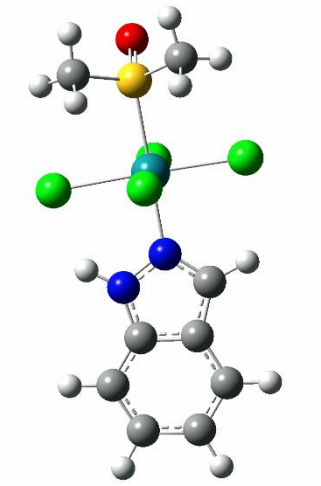
The role of ruthenium in the field of anticancer metallodrugs cannot be understand until a proper description of platinum chemistry is not given. With this in mind, some historical background to cisplatin is given in Chapter 1, followed by an explanation of its mode of action. Ruthenium chemistry has been presented as a possible alternative to platinum therapy. A brief introduction to ruthenium complexes with regards to property and classification and their possible mechanisms of action are discussed in Chapter 1.

Chapter 2

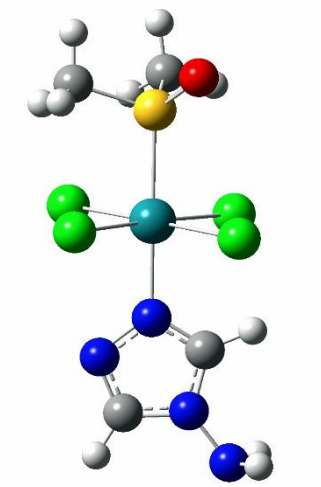
The theory of several molecular modeling methods adopted in this thesis are discussed in detail in Chapter 2. The methods described include molecular mechanics (MM), quantum mechanics (QM), quantum mechanics/molecular mechanics method (QM/MM) and molecular docking simulation.

Chapter 3

In Chapter 3 we have analyzed the structure and reactivity of six ruthenium(III) anticancer complexes namely imidazolium[*trans*-$RuCl_4$ (3H-imidazole) (DMSO-S)] (NAMI-A) (**I**), indazolium [*trans*-$RuCl_4$ (2H-indazole) (DMSO-S)] (**II**), 1,3,4-triazolium [*trans*-$RuCl_4$ (4H-1,3,4-triazole) (DMSO-S)] (**III**), 4-amino-1,2,4-triazolium [*trans*-$RuCl_4$ (4-amino-1,2,4-triazole) (DMSO-S)] (**IV**), 4-methyl-1,3,4-triazolium [*trans*-$RuCl_4$ (4-methyl-1,3,4-triazole) (DMSO-S)] (**V**) and imidazolium[*trans*-$RuCl_4$(3H-imidazole)$_2$] (**VI**) using density functional theory based reactivity descriptors such as global hardness, electrophilicity, chemical potential and local philicity. These reactivity descriptors reveal the highest reactivity of complex **II** in the gas phase and complex **IV** in the solvent medium.



II



IV

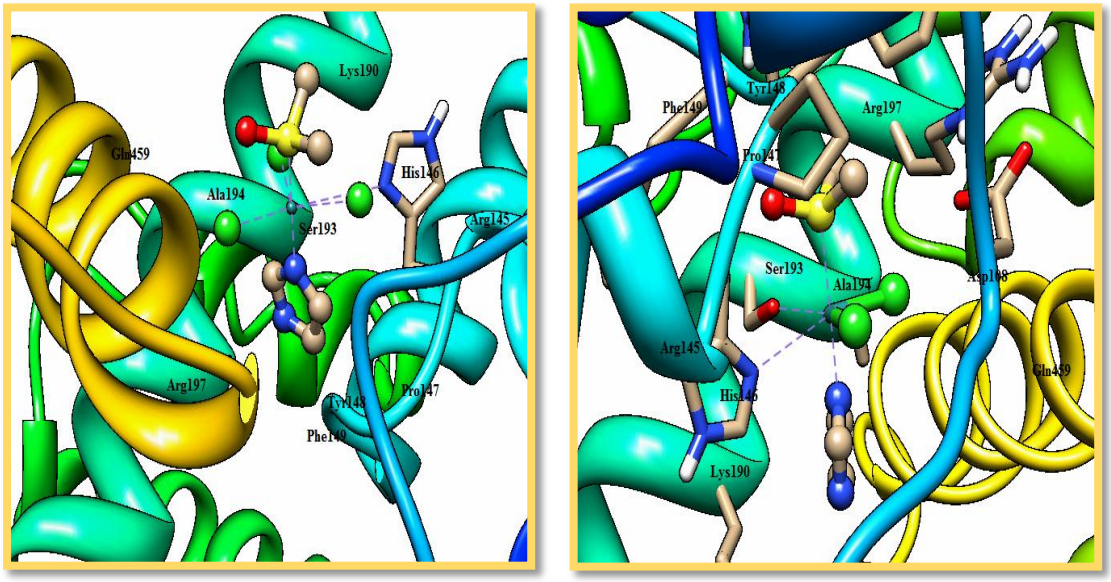
Chapter 4

The hydrolysis mechanism of two highly reactive ruthenium complexes **II** and **IV** is investigated in Chapter 4. The stationary points for the hydrolysis mechanism of these complexes have been fully optimized and characterized. For the first hydrolysis step, Cl^{-1} dissociation is found to be more favorable than DMSO dissociation in aqueous solution. For the second step of hydrolysis, different results are obtained for complex **II** and **IV**. For complex **II**, Cl^- dissociation is found to be favorable while for complex **IV**, DMSO dissociation is found to be favorable. For the second Cl^- hydrolysis step, the formation of *cis*-diaqua products preferred thermodynamically over the *trans* isomers.

Chapter 5A

Chapter 5A incorporates the interaction processes of ruthenium(III) complexes with protein receptor. Monooaqua and diaqua ruthenium complexes such as, [*trans*-$RuCl_3(H_2O)$(3H-imidazole)(DMSO-S)] **Ia**, [*trans*-$RuCl_2(H_2O)_2$(3H-imidazole)(DMSO-S)]$^{1+}$ **Ib**, [*trans*-$RuCl_3(H_2O)$(4-amino-1,2,4-triazole)(DMSO-S)] **IVa** and [*trans*-$RuCl_2(H_2O)_2$(4-amino-1,2,4-triazole)(DMSO-S)]$^{1+}$ **IVb**, which are formed after intracellular aquation of their respective complexes, with human serum albumin (**HSA**) has been investigated in this chapter by molecular docking and two layer QM/MM hybrid methods. The computed binding energies of monoaqua adduct, **Ia-HSA** and **IVa-HSA** evaluated by docking simulation are found to be -4.52 kcal mol^{-1} and -4.58 kcal mol^{-1} whereas the binding energy of diaqua adducts **Ib-HSA** and **IVb-HSA** are evaluated to be -4.74 kcal mol^{-1} and -4.91 kcal mol^{-1}, respectively. Docking results also show that the ruthenium atoms of all the complexes are actively

involved in coordination with histidyl nitrogen atom in the active site of protein. In addition, in order to probe the stabilities of monoaqua and diaqua ruthenium complexes in the active site of protein, we have calculated their energetics by two layer QM/MM method. QM/MM study suggests higher stability of diaqua adduct, **Ib-HSA**. The stability of adducts varies in the order: **Ib-HSA**> **IVb-HSA**> **Ia-HSA**> **IVa-HSA**. Binding energy values of all the complexes increase with the incorporation of solvent effect. Thus molecular docking and QM/MM results show that ruthenium complexes interact with the protein receptor more rapidly after their second hydrolysis. Hence, docking as well as ONIOM results will be highly beneficial for providing insight into the molecular mechanism of ruthenium complexes with protein receptor.



Monoaqua adduct **Diaqua adduct**

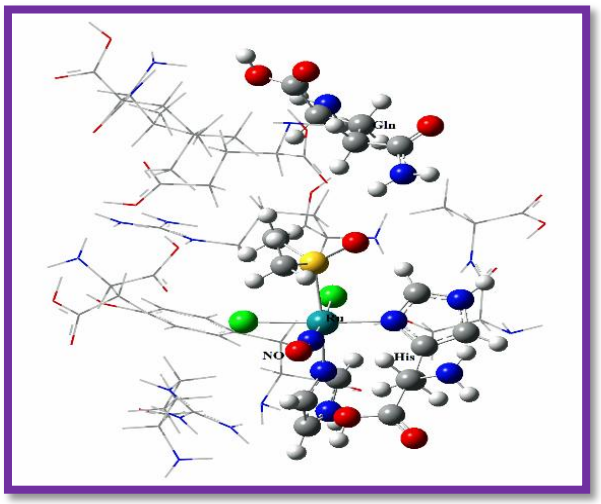
Chapter 5B

Mode of action of substitution of aqua ligand from monoaqua [*trans*-$RuCl_3(H_2O)$(2H-indazole)(DMSO-S)] (**IIa**) and diaqua [*trans*-$RuCl_2(H_2O)_2$(2H-indazole)(DMSO-S)]$^{+1}$ (**IIb**) complexes with histidine and cysteine are discussed in Chapter 5B. Activation energy values for the ligand exchange reaction of monoaqua ruthenium(III) complex with histidine and cysteine in aqueous medium are found to be 21.24 and 32.94 kcal mol^{-1}, respectively, while the corresponding values for diaqua complex are evaluated to be 32.88 and 21.15 kcal mol^{-1}. Calculated aqueous medium activation energy values are found to be lower than their corresponding gas phase values, indicating profound solvent effect on the ligand exchange reactions. Rate

constant (k) values for substitution of aqua ligand from **IIa** with histidine and cysteine in aqueous medium are evaluated to be 1.67×10^{-3} s^{-1} and 4.45×10^{-12} s^{-1}, respectively, while, the corresponding rate constant values for **IIb** are found to be 4.91×10^{-12} s^{-1} and 1.97×10^{-3} s^{-1}. Calculated rate constant values correlate well with the available experimental results. These parameters reveal that interaction of monoaqua ruthenium(III) complex with histidine and diaqua complex with cysteine are thermodynamically and kinetically favoured.

Chapter 5C

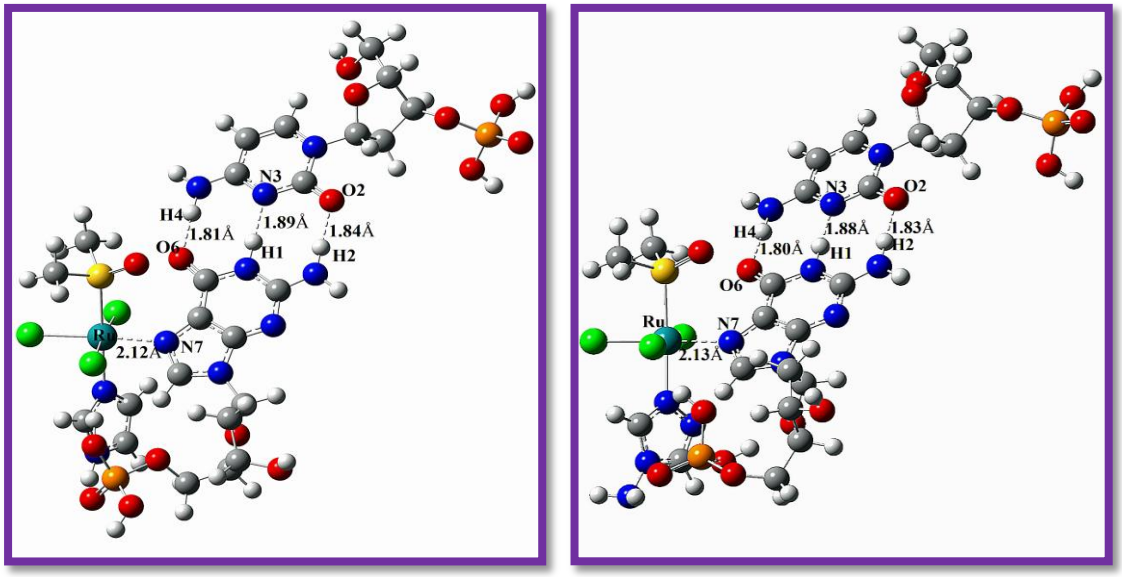
Hydrolysis of adduct **Ia-HSA** and nitrosylation of hydrolyzed **Ia-HSA** adduct have been investigated in detailed in Chapter 5C. It has been observed that the chloride exchange reaction with water in the **Ia-HSA** adduct follow an interchange dissociative mechanism passing through an unstable heptacoordinated activated complex. The computed free energy of activation (ΔG) and rate constant (k) for the hydrolysis process in aqueous medium is observed to be 24.97 kcal mol^{-1} and 3.09×10^{-6} s^{-1}, respectively, in agreement with experimental results. Nitrosylation of hydrolyzed **Ia-HSA** adduct with nitric oxide provides a detailed understanding related to the antimetastatic activity of complex **I**. Nitrosylation of hydrolyzed **Ia-HSA** adduct with nitric oxide is found to be thermodynamically more favorable with the incorporation of solvent effect. The activation free energy and rate constant for the nitrosylation reaction in solvent medium is found to be 9.11 kcal mol^{-1} and 1.29×10^{6} s^{-1}, respectively. This investigation shows that nitric oxide coordinate linearly to **Ia-HSA** adduct leading to the reduction of ruthenium(III) to more active ruthenium(II), with the reduction potential of -2.32V.



NO coordinates to **Ia-HSA** adduct

Chapter 6

In Chapter 6 the interaction between normal (**GC**, **AT**), mismatch base pairs (**GG**, **AA**, **TT**, **CC**) and monoaqua ruthenium complexes **Ia** and **IVa** have been studied in detailed. Isolated normal and mismatch base pairs deviate from their planarity upon interaction with ruthenium complexes. Both the complexes interact strongly with the mismatch base pair **GG** than the normal base pairs. We have evaluated the interaction energies (ΔE) of ruthenium complexes with DNA base pairs in order to analyze their stability. DFT evaluated interaction energy reveals higher stability of **GG-I**, **GG-II**, **GC-I** and **GC-II** adducts as compared to other adducts. N7 of adenine and guanine, O2 of cytosine and O4 of thymine are observed to be the preferred binding site of ruthenium complexes. Results of NBO analysis reveal the interaction (e.g. Ru^{3+}—N7, Ru^{3+}—O2, Ru^{3+}—O4) in complex-base pair adducts as electrostatic in nature where charge transfer phenomenon occurs from base pair to ruthenium complexes.

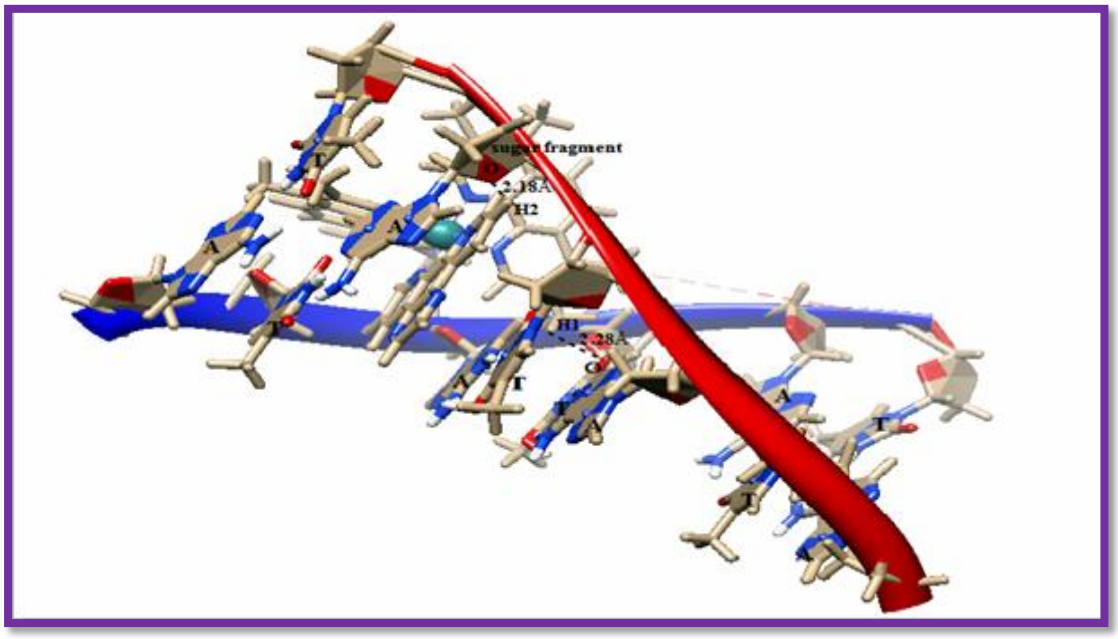


GC-Ia **GC-IVa**

Chapter 7

Interaction of three polypyridyl ruthenium(II) complexes of the type $[Ru(tmp)_2(dpq)]^{2+}$ (**I**), $[Ru(tmp)_2(dppz)]^{2+}$ (**II**) and $[Ru(tmp)_2(11,12\text{-dmdppz})]^{2+}$ (**III**) with two B-DNA hexamers of alternative AT and GC sequences, namely $d(ATATAT)_2$ and $d(GCGCGC)_2$ respectively, has been computationally investigated by the molecular docking and two layer QM/MM hybrid methods in Chapter 7. Docking simulation reveals the intercalative minor groove binding mode of ruthenium complexes with DNA base pairs as well as their preferential binding to $d(ATATAT)_2$

over d(GCGCGC)$_2$. In addition, docking simulation exhibits the greater binding affinity of complex **III** toward the DNA sequences compared to complexes **I** and **II**, which indicates that methyl substituent effect of the intercalating ligand increases the binding affinity. Binding energies of ruthenium complexes with DNA sequences obtained from two layer QM/MM calculation show higher stability of **III**-DNA adduct as compared to the other adducts. Thus molecular docking and QM/MM results reveal that intercalating ligands having substituent interacted with DNA more strongly.



d(ATATAT)$_2$—**I**

Chapter 8

Chapter 8 contains an overall conclusion reflecting the significant findings of the work along with a brief idea on future scope of studies.