CONCLUSION

NAMI-A is the first anticancer ruthenium complex that has successfully entered human clinical trials. It shows a remarkable activity against lung metastases of solid tumors, but is not effective in the reduction of primary cancer. The success of NAMI-A as an efficient antimetastatic agent has stimulated the present researcher for searching new NAMI-A analogue with equal or greater antitumor activity and lower toxicity. In addition ruthenium(II) complexes have received great interests now a days due to their strong DNA binding affinity. In this thesis, we have taken NAMI-A type and ruthenium(II) polypyridyl complexes for study, with two main purposes: finding new potential anticancer metallodrugs and getting some insight in their mechanism of action. This chapter gives a brief overview of all the work described so far and it provides a number of suggestions for further research.

The structure and reactivity of of imidazolium [*trans*-$RuCl_4$ (3H-imidazole) (DMSO-S)] (NAMI-A) (**I**) and its five derivatives such as 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**) have been described. DFT based reactivity descriptors: global hardness, electrophilicity, chemical potential and local philicity etc. are evaluated to study the reactivity of some selected ruthenium complexes. These reactivity descriptors reveal the highest reactivity of complex **II** in the gas phase. Inclusion of solvent effect changes the reactivity trend of all the complexes with respect to their gas phase trend and complex **IV** is found to be the most reactive in solvent medium. Simple linear regression analysis is carried out to build a quantitative structure-activity relationship (QSAR) model by using DFT derived reactivity descriptor namely, local philicity (ω^+) for the ruthenium complexes against HT-29 colon carcinoma cell line.

Two highly reactive ruthenium(III) complexes such as **II** and **IV** obtained so far are then subjected to hydrolysis in order to understand their mechanism of action at the molecular level. Our calculations provide a picture of the hydrolysis of complexes **II** and **IV** with stepwise loss of chloride and DMSO ligands up to second aquation.

Analysis of the result indicates that Cl^- dissociation is more favorable than DMSO dissociation. Further, the most stable products that are formed during Cl^- hydrolysis reaction is monoaqua and *cis*-diaqua complexes.

To examine the stability and binding affinity of monoaqua and *cis*-diaqua form of ruthenium(III) 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, inside the protein environment, we have studied their interaction with human serum albumin (**HSA**) by molecular docking and two layer QM/MM hybrid methods. Molecular docking simulation shows that diaqua adducts (**Ib** and **IVb**) exhibit higher binding affinity compared to monoaqua adducts (**Ia** and **IVa**). 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**. Thus molecular docking and QM/MM results show that ruthenium complexes interact with the protein receptor more rapidly after their second hydrolysis.

Interaction mechanism of monoaqua and *cis*-diaqua form of ruthenium(III) complexes **IIa** and **IIb** with histidine and cysteine has been discussed. Calculated activation energy values for the ligand exchange reaction of **IIa** and **IIb** with histidine and cysteine reveal that interaction of **IIa** with histidine and **IIb** with cysteine are thermodynamically and kinetically favoured.

The hydrolysis and nitrosylation reaction mechanism of NAMI-A (**I**) inside the protein albumin (**HSA**) environment have been studied in detailed. 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 agrees experimental results. Nitrosylation reaction of **Ia-HSA** adduct is found to be thermodynamically more favorable in solvent medium compared to gas medium. This investigation also 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.

Nitrosylation of hydrolyzed **Ia-HSA** adduct with nitric oxide provides a detailed understanding related to the antimetastatic activity of complex **I** (NAMI-A).

DNA has been reported to be a primary intracellular target for antitumor drug molecules because drug -DNA adduct can cause DNA damage, blocking its cell division followed by death of cancer cells. Detection of single base pair mismatches in DNA will provide a new path for the development of better diagnostics and chemotherapeutics. Therefore, we have investigated the interaction of normal and mismatch base pairs and their adducts with monoaquated ruthenium complexes **I** and **IV** to understand the nature of DNA damage. Both the complexes have been found to interact strongly with the mismatch base pair **GG** than the normal base pairs. Interaction energy evaluated by DFT suggests higher stability of **GG-Ia**, **GG-IVa**, **GC-Ia** and **GC-IVa** adducts as compared to other adducts. However, reactivity of complex **IVa** is found to be higher than complex **Ia** when comes to DNA environment. It is also observed that both the complexes prefer to bind N7 site of adenine and guanine, O2 site of cytosine and O4 site of thymine. Results of NBO analysis show that the interaction (e.g. Ru^{3+}—N7, Ru^{3+}—O2, $Ru3^{+}$—O4) in complex-base pair adducts are electrostatic in nature where charge transfer phenomenon occurs from base pair to ruthenium complexes.

Ruthenium(II) polypyridyl complexes have received a great deal of attention because of their stability, ease of construction, chirality, opto-electronic properties, strong binding affinity to DNA and luminescence characteristics. We have focused on interaction of ruthenium(II) polypyridyl 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 in order to evaluate the information regarding the intercalative binding mode of the complexes with DNA receptors. Interaction of the complexes has been computationally investigated by the molecular docking and two layer QM/MM hybrid method. Docking simulation shows that ruthenium complexes bind with DNA base pairs via intercalative mode. Further, these complexes prefer to bind $d(ATATAT)_2$ sequences rather than $d(GCGCGC)_2$ sequence. In addition, docking simulation have exhibited the greater binding affinity of complex **III** toward the DNA sequences compared to complexes **I** and **II** suggesting methyl substituent

effect. The stability order for ruthenium(II) complexes with $d(ATATAT)_2$ and $d(GCGCGC)_2$ sequences obtained from two layer QM/MM method are as follows: complex **III**> complex **II**> complex **I**. Thus molecular docking and QM/MM results express that intercalating ligand having substituent significantly increases the DNA binding affinity of the metal complexes.

All the work presented in this thesis leads to their respective extensions that will strengthen their impact and role in real life applications. The desired and expected extensions are termed here as the future scope of the reported work which can be outlined as

1. Interaction mechanism of ruthenium(III) complexes at different sites of DNA bases.
2. QM/MM studies of ruthenium(III) complexes with DNA sequence.
3. Molecular docking and QM/MM studies of polypyridyl ruthenium(II) complexes with protein.