CHAPTER 6

Summary, general conclusion and future perspectives.

This chapter summarizes the findings of research work presented in this thesis as well as throws light on the future perspectives of the kind of work presented here.

6.1 Scope of the present work:

Ruthenium(II) compounds, offer a promising approach to the development of new anticancer agents because they show remarkable features such as low general toxicity, the ability to mimic iron binding to biomolecules (transferrin, albumin), and stronger affinity for cancer tissues over normal tissues. Some of the compounds interact with DNA at the same initial sites (N7-guanine) as platinum compounds. However, the broad spectrum of anticancer activities displayed by the complexes makes it difficult to deduce their mechanism of action. Generally, anticancer activity of the ruthenium compounds is comparable or even better than that of cisplatin against a range of human cancer cells, thereby indicating that, for these types of ruthenium compounds, DNA is one of the targets of their action inside the cells. The use of chelating ligands with stronger binding to ruthenium appears to be desirable because these ligands offer advantages of structural stability in aqueous solution, thereby influencing *in vitro* anticancer activity of the complexes quite significantly. Furthermore, aqueous solubility of these ruthenium complexes gives rise to promising antitumor activities and high selectivity while also rendering them suitable for oral administration. However, instability and the difficult ligand exchange chemistry of inorganic ruthenium complexes present setbacks which can only be overcome with more stable organoruthenium complexes, in order to enhance their potential as drug candidates.

Half-sandwich organometallic ruthenium(II) arene complexes are emerging as promising candidates for cancer treatment. The aromatic ligand attached to the complex occupies three coordination positions, thereby offering stability to the metal centre, while the remaining coordination sites can be occupied by the ligand that could impart antitumor activity together with ligands that can control electronic properties at the ruthenium centre. This architectural friendliness observed in half-sandwich arene ruthenium complexes offers an opportunity to improve their cytotoxic profile. The compounds possess excellent antitumor activities, with IC_{50} values comparable to those found for cisplatin. This could indicate that DNA is one of the targets of their action.

All these findings suggest that further development of ruthenium compounds may contribute to the improvement of future chemotherapeutic protocols.

6.2 Summary of the work presented in the thesis:

6.2.1 Design and structural composition of the synthesized mixed ligand ruthenium(II) complexes:

Chapters 2, 3 and 4 describe the design, synthesis and characterization of the compounds studied upon and presented as PhD work in this thesis.

- In Chapter 2 light has been thrown upon the biological importance of heterocyclic compounds. Looking at the versatility of heterocyclic compounds in natural and synthetic medicines, a variety of heterocyclic compounds have been synthesized, characterized and employed in further synthesis of mixed ligand ruthenium(II) complexes with an aim to enhance the bioactivity of the already known and reported ligands as bioactive, specifically anticancer, agents. Two different sets of ruthenium complexes were synthesized: one containing a polypyridyl compound as the ancillary ligand and the other containing an arene moiety as the ancillary ligand.
- DNA is one of the important targets of anticancer agents and polypyridyl complexes are known to interact with DNA and stop further replication causing cell death. Following this insight ruthenium(II) polypyridyl complexes has been worked upon and presented in chapter 3. Here mixed ligand ruthenium(II) complexes of 1,10-phenanthroline has been synthesized and obtained as perchlorate salts. The presence of perchlorate as counter anion has been confirmed by ESI-MS, FTIR and conductance measurement suggesting 1:1 electrolytic behavior. UV-Vis spectroscopy was also supportive of the proposed structure wherein distinct metal centered d-d bands were observed in the spectra of all the synthesized phenanthroline complexes that are peculiar to the absorption properties of ruthenium(II) polypyridyl complexes [1]
- The virtues of organometallic ruthenium(II) complexes have been explored from a perspective of possible anticancer agents and their synthesis and characterization have been presented in chapter 4. This chapter contains the design and synthesis of mixed ligand ruthenium(II) complexes of *p*-cymene where the arene occupies three coordination sites of the ruthenium(II) metal centre leaving the other three coordination sites for a chloride and a bidentate ligand lending an overall 'piano stool' type structure to the half sandwiched ruthenium(II) arene complex. All the arene complexes have been well characterized employing different spectroscopic techniques. The presence of *p*-cymene as the arene ligand has been confirmed from the well defined peaks obtained in the ^{1}H NMR assignable to the *p*-cymene and also from the FTIR spectra of the complexes.

6.2.2 Bio-application of the test compounds - Anti-cancer via apoptosis:

Chapter 5 envisages the bio applicability of the ruthenium(II) complexes *in vitro* as well as *in cellulo.*

- The *in vitro* biomolecular interactions of the compounds have brought about some interesting conclusions. The two main biomolecules used in the present work are DNA and serum albumin which are used by and large for these types of studies owing to their said reasons. The *in vitro* binding studies were carried out with the help of UV-Vis and fluorescence spectroscopy. Looking at the binding constants it can be concluded that the ruthenium(II) phenanthroline complexes came out to be good DNA binders / intercalators mostly owing to the planar 1,10-phenantroline rings that can stack in between the base pairs of the DNA double helix. It has been reported that DNA damaging agents can activate the intrinsic pathway of apoptosis involving the release of cytochrome c and other mitochondrial apoptogenic factors [2] and trigger autophagy simultaneously as a self-defense mechanism [3,4].
- Ruthenium(II) arene complexes showed to be better serum albumin binders than their phenanthroline counterparts. This result is speculative of hydrophobic interactions of the arene moiety with the hydrophobic pockets of the protein. Moreover the crystallographic experiments of RAPTA-C with the nucleosome core showed that proteins are the primary target of the compound [5,6]. Binding interactions with biomolecules was the first and foremost step of venturing into the bioactivity studies of the synthesized compounds. This is a very preliminary study giving an idea regarding the extent of interactions of the compounds with general cellular targets for anticancer activities.
- The general *in cellulo* cytotoxicity of the complexes was checked using MTT assay. The IC_{50} values so obtained were much lower to those of NAMI-A and RAPTA ruthenium complexes which are under clinical trials. This provided a thrust for further studies into the mechanistic aspects of the cytotoxicities of the tested compounds.
- The facs analysis and other staining techniques revealed that the cell death is mostly being caused by apoptosis (programmed cell death) which is the preferred mode of cell death.
- Apoptosis is usually brought about through a series of chain of reactions and occurrences which have been broadly categorized into the extrinsic and intrinsic pathways. In order to get further insights into the mechanism being followed to bring about apoptosis in the cancer cell lines by the test compounds, gene expression studies were carried out. Bax (a pro-apoptotic gene) and Bcl-2 (an anti-apoptotic gene) expressions in presence and absence of the test compounds were evaluated. The

results clearly indicate that the expression of Bax was enhanced in presence of the test compounds.

6.3 General conclusion and future perspectives:

The versatile synthetic chemistry of ruthenium opens up a wide range of complexes with a variety of ligands that could provide an arsenal of compounds for clinical uses. Several research groups have developed different types of Ru anticancer drugs, including NAMI-A,[7] KP1019 [8] and Ru(II)–arene complexes [9,10]. However, recent trends point to the likelihood of common pathways of biological activation for all types of Ru complexes (*Fig. 6.1*) that include competing processes of extracellular protein binding and cellular uptake. Unlike Pt complexes, where binding to extracellular proteins is thought to cause the loss of biological activity, binding of Ru complexes to extracellular matrix proteins or to the cell surface is likely to be responsible for the anti-metastatic properties of NAMI-A and of some Ru(II)–arene complexes [7,11]. The main factors that determine the mode of action of a Ru complex appear to be its lipophilicity (favouring cellular uptake) and the presence of labile ligands such as chlorido or carboxylato (ligands that favour extracellular binding).

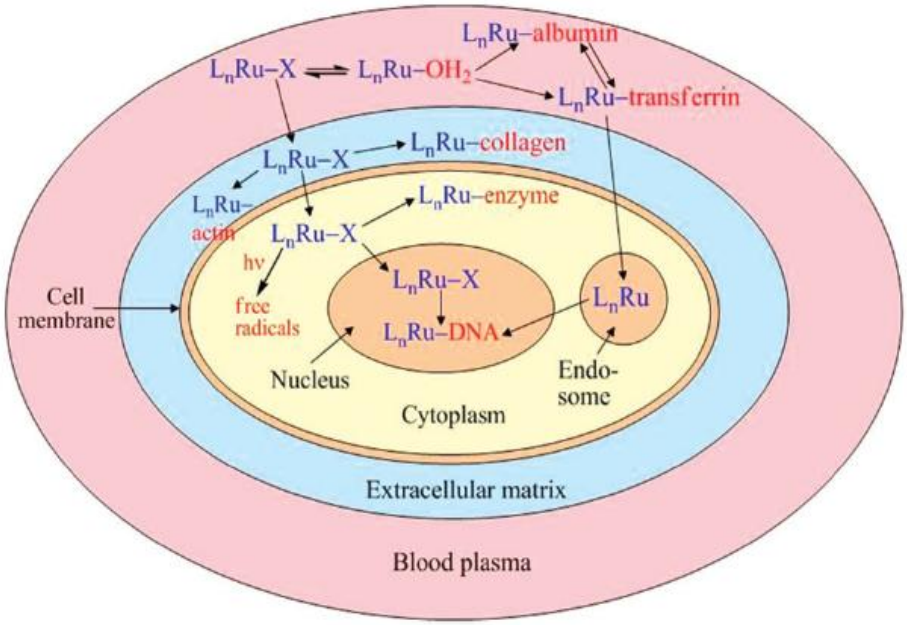

Fig. 6.1: *Proposed generalised pathways of action of Ru anticancer drugs (L are the tightly bound ligands and X is the leaving group) [12].*

The rich selection of ruthenium(II) compounds with a multitude of innovative modes of action has added new flavor to the field of ruthenium-based anticancer agents and holds great promise for future drug development.

The recent growth in molecular sciences and the advances in genomics and proteomics have generated several potential new drug targets, leading to changes in the paradigms of anticancer drug discovery toward molecularly targeted therapeutics. Both small and large

molecular compounds continue to be investigated as anticancer agents. Successful drug treatment in human disease requires an adequate therapeutic index reflecting the treatment's specific effects on target cells and its lack of clinically significant effects on the host. In cancer, the therapeutic goal is to trigger tumor-selective cell death. The mechanisms responsible for such death are of obvious importance in determining the efficacy of specific treatments. The importance of apoptosis as a pathogenic mechanism with a causative or contributing role for many diseases has become increasingly evident. The identification of the genes and gene products that regulate apoptosis, together with an increased knowledge about their mechanisms of action, has laid the foundation for the discovery of new drugs targeting apoptosis. The process of apoptosis is controlled at multiple molecular levels, each of which is influenced by different pro- and antiapoptotic proteins. Further mechanistic insight is also required to help focus design strategies in the future. The various decision points of life and death do not only provide an exciting multitude of molecular targets, but also offer a variety of therapeutic options.

6.4 References:

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