CHAPTER 5

Summary, general conclusion and future perspectives

TABLE OF CONTENTS

5.1 Scope of the present work:

Binuclear ruthenium (II) arene complexes, have shown a promising approach towards development of new anticancer agents because they are known for their remarkable properties such as less toxicity, the potentiality to mimic iron binding to biomolecules (transferrin, albumin), and high affinity towards cancer tissues over normal tissues. Like platinum drugs, some of the Ru compounds also show interaction with DNA at the same initial sites (N7-guanine). However, the wide spectrum of anticancer activities showed by the complexes makes it difficult to understand their mechanism of action. Generally, it has been found that the many of the ruthenium complexes showed comparable or even better anticancer activity than that of cisplatin against large number of human cancer cells, which indicate that the main target of ruthenium compounds is DNA inside the cells [1]. The chelating ligands show stronger binding with ruthenium which appears to be desirable because these ligands offer advantages of structural stability in solution, as a result significantly influence the *in vitro* anticancer activity of the complexes. However, instability and the difficult ligand exchange chemistry has been seen in ruthenium complexes which can be overcome with more stable organoruthenium complexes, in order to enhance their potential as drug candidates [2].

Half-sandwich organometallic binuclear ruthenium (II) arene complexes are emerging as promising candidates for cancer treatment. The stability achieved by the metal centre is mainly due to the presence of aromatic ligand which is attached to the complex, having capacity of occupying three coordination positions, while the remaining coordination sites can be occupied by the ligand as well as bridging ligands that could impart antitumor activity. This architectural friendliness observed in half-sandwich arene ruthenium complexes offers an opportunity to improve their cytotoxic profile [1]. The complexes show an excellent antitumor activity, with IC_{50} values comparable to those obtained for cisplatin. This could indicate that DNA is a one of the important targets of their action. Such results indicate promising compounds with which to tackle the common problem of developed cisplatin resistance, frequently occurring during chemotherapy [3].

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

5.2 Summary of the work presented in the thesis:

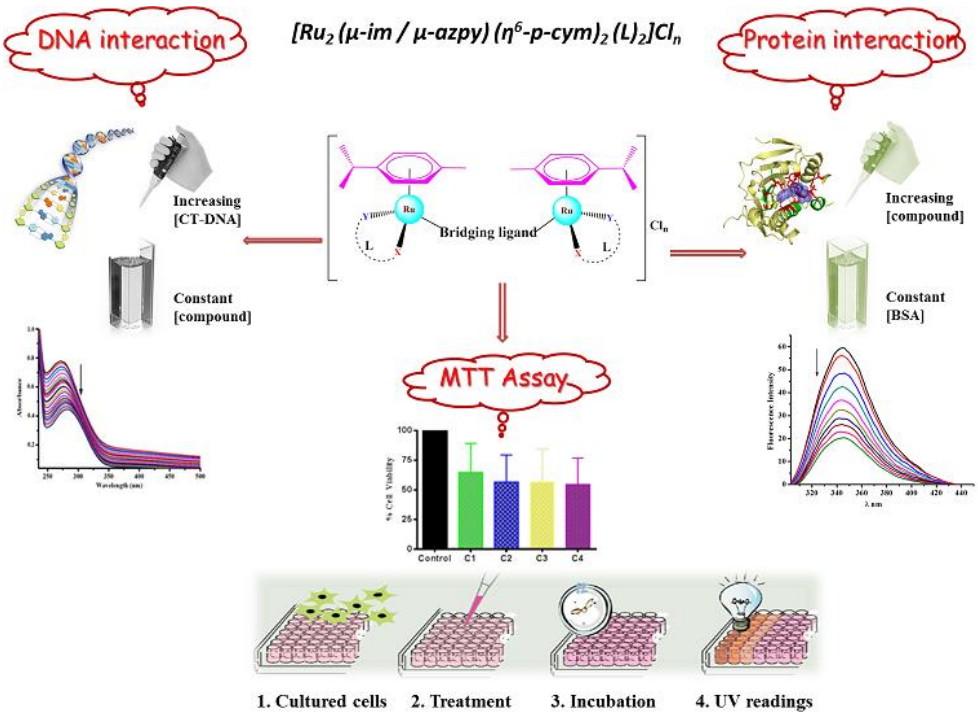

Fig. 5.1: *Pictorial representation of work presented in the thesis*

5.2.1 Design and structural composition of the synthesized Binuclear Ru (II) arene complexes:

Chapters 2 and 3 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 binuclear Ru (II) arene 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 using two different bridging ligands imidazole and 4, 4'- azo pyridine.

- 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 3. This chapter contains the design and synthesis of binuclear 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 and two metal centre were connected with bridging ligands. All the arene complexes have been well characterized by ESI-MS, UV-Visible, FT-IR, and ^{1}H NMR and conductance measurement suggesting electrolytic behaviour. These organometallic complexes were found to be pseudo-octahedral in geometry with three coordination sites taken up by the arene ligand (resonating structure) forming very stable arene-Ru bond that stabilizes ruthenium in its +2 state.

5.2.2 Bio-application of the test compounds-Anti-cancer activity via MTT assay:

Chapter 4 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) arene complexes with bridging ligands imidazole / azopyridine came out to be good DNA binders / intercalators mostly owing to the planarity, positive charge on the complexes, less steric hindrance between the two metal centres, all this factors increase the binding efficiency of complexes between the base pairs of the DNA double helix. Moreover, the nature of the plots (hypochromism) and viscosity measurements indicate that the complexes bind to DNA through non-covalent interactions (intercalation and hydrophobic interactions). There may also be weak electrostatic interactions with the cationic complexes. The DNA binding constants ($\mathbf{K_b}$) and the Stern-Volmer quenching constants ($\mathbf{K_{sv}}$) for the ligands are in the range of $10^3 - 10^4$ M^{-1} whereas the $\mathbf{K_b}$ and **Ksv** values for the complexes are in the range $10^3 - 10^5$ M^{-1}. Nature of the bridging ligand does not seem to have any substantial impact on the binding of the complexes.

- Binuclear Ruthenium (II) arene complexes showed to be better serum albumin binders. 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 [4, 5]. 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. Furthermore quenching of emission in the presence of the compounds (λ vs FI plot) may be owing to a variety of molecular interactions, collisional quenching, energy transfer, ground state complex formation and molecular rearrangements. These interactions cause a change in the micro environment around the protein molecule thereby causing quenching of the tryptophan residues of BSA. The linear nature of the Stern – Volmer plots reveals that only one quenching mechanism (either static or dynamic) is operative in the quenching process. The binding constant ($\mathbf{K_b}$) values for the ligands and the complexes are in the range of 10^3 -10^7 M^{-1}. There is only one binding site (n ~ 1) on the macromolecule for binding of the compounds.

- The general *in cellulo* cytotoxicity of the complexes against HeLa cancer cell line was checked using MTT assay. The cytotoxicity of the synthesized complexes was found to be in the order of 4.2 – 120 μM. The complexes are less toxic than cis-platin except few but more toxic than the Ru-complexes 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. An important feature of the mechanism underlying anti-tumour effects of DNA binding metal based compounds is repair of their DNA adducts. The level of DNA repair synthesis induced by the DNA adducts of Ru-arene complexes was found to be markedly higher than that induced by cis-platin. This implies that the adducts of Ru-arene compounds are removed from DNA more efficiently than cis-platin. This explains the lesser cytotoxicity of these complexes compared to cis-platin

5.3 Current Developments in Ruthenium Anticancer Agents and Future Perspectives:

The antitumor activity of ruthenium complexes has led to congenial achievements and recognition of some promising antitumor compounds [6]. Researchers who are involved in the design of anticancer agents should learn from successes and difficulties faced during the development of previous complexes to understand the desirable physical, chemical, and biological properties associated with a successful future Ru drug candidate. Moreover, the

results obtained from the clinical studies should be considered in determining the reasons for the failure of the clinical investigations of NAMI-A and KP1019, which could lead to design drugs with less side effects, greater selectivity, and higher bioavailability. For example, KP1339 is the sodium salt of KP1019, which is recently under clinical studies, has better water solubility and trans-membrane absorption efficiency than compare to KP1019 [7]. The Ru (II) complex TLD1433, act as a photosensitizer, significantly enhanced the efficacy of phototherapy and also produced less toxicity in vitro and in vivo studies [8]. Even more interesting is observing the escalation of novel approaches regarding targeting strategies and mechanism of action involved in the anticancer activity of Ru complexes, all of which are fuelled by our increased understanding of the complexes' fates at the cellular level. Disruption of protein-protein interactions, [9] enzymatic inhibition [10,11], and redox modulation [12], as well as chromatin [13] and histone [14] targeting, are only a few examples of cellular events being used as means for antiproliferative activity, with in-cell catalysis taking advantage of well-established reactions in the chemistry of materials field [15]. The existing research achievements should be combined with molecular biology and nanomaterials, applying the advantage of existing tools and methods to develop antitumor drugs with better therapeutic effects, based on these complexes. Although structure activity relationships are not easily established, current and further developments of analytical and cellular techniques are still needed. Before the ruthenium complex can be used clinically, there are numerous problems need which are to be address, including strategies to improve the hydrolysis of ruthenium complexes to achieve effective absorption and better metabolism, as well as enhance their cellular penetration to achieve targeted tumor cell death. Furthermore, methods to avoid and alleviate the side effects of ruthenium complexes, enhance their efficacy via synergism, and overcome drug resistance are imperative. The solution to these problems would provide a promising direction for the design and screening of ruthenium complexes, which are of great significance for their use in clinical diagnosis and therapy of tumors [6].

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