CHAPTER 1

Introduction to Organometallic Binuclear Ruthenium complexes with their biological activities

*An overview about Ruthenium complexes as an **anti-cancer agent***

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Cancer: A worldwide epidemic: Need for novel metal based anticancer drugs:

Cancer is the uncontrolled growth of anomalous cells in the body [1]. Around 8.2 million people die of cancer within five years of diagnosis making it the leading cause of death worldwide. According to a WHO report, it is expected that annual cancer cases will rise to 26 million within the next two decades [2]. There have been tremendous efforts to combat cancer with current available drugs. Metal scaffolds play an important role in medicinal chemistry and drug design after the serendipitous discovery and development of platinum compounds [3]. Metallotherapeutics exert their action by inhibiting cancer cell division, trigger apoptosis by inducing DNA damage and disrupting DNA repair process [4]. The platinum-based drug cisplatin is one of the most common and effective drugs used by the oncologists in the treatment of numerous forms of human cancers. However, its effectiveness and therapeutic value is limited by serious side effects and observed drug resistance [5]. Many researchers are actively involved in the search for other alternative transition metal compounds, and new ruthenium compounds have been reported as promising antitumor metallotherapeutics [6].

1.1 Ruthenium complexes as anticancer agent:

Ruthenium-based anticancer metallotherapeutics are alluring alternatives over platinum-based therapeutics because they were found to have certain merits and different modes of action [7]. Several reviews on the anticancer ruthenium compounds have been published in the recent years [8-11]. Ruthenium compounds (i) have low side effects due to their higher selectivity for cancer cells compared with normal cells, (ii) are active against some cisplatin resistant cell lines, (iii) have higher selectivity for their targets which may be due to selective uptake by the malignant tissues and (iv) can mimic iron in binding to some biomolecules [12]. These favourable properties make these ruthenium scaffolds attractive alternatives for medicinal application. Ruthenium therapeutics has inherently low toxicity though ruthenium's ability to mimic iron is often erroneously linked with its low toxicity [13]. Ruthenium belongs to the same group as iron in the periodic table, which is reflected by its high affinity for transferrin and by its activation by reduction in cells [12, 14]. Some ruthenium compounds are excellent candidates for clinical development, due the low cytotoxicity and genotoxicity, different ligand exchange kinetics, facile transport, activation mechanisms, and high biological activity. Many medicinal chemists have designed new Ru (II) scaffolds that are being investigated in preclinical studies at various stages of

development. In the past few decades several patents of antineoplastic ruthenium complexes with a range of different scaffolds have been reported [15].

1.1.1 Non-arene ruthenium complexes with anticancer properties:

1.1.1 .1 Ruthenium ammine-chlorido complexes:

Anticancer Ru complexes proposed by Clarke and co-workers in 1980s, were the chlorido-ammine Ru(II) and Ru(III) complexes which inspired by cisplatin, were thought to act primarily by binding to DNA [16]. Several ammine and chlorido ligands were coordinated to Ru(II) and Ru(III) to form complexes with general formula $[Ru(NH_3)_{6-x}Cl_x]^{n+}$. Those complexes in which the oxidation state of the ruthenium ion was (+3) were expected to bind to DNA in an analogous way to cisplatin [17]. Interestingly, both *cis*-$[Ru(III)(NH_3)_4Cl_2]^+$ and especially *fac*-$[Ru(III)(NH_3)_3Cl_3]$ displayed a comparable antitumour activity to that of cisplatin in a few selected cell lines [18] (*Fig. 1.1*). It has been hypothesized that these complexes, once inside the cell, are reduced to less inert Ru(II) species, which bind to DNA after hydrolysis [19].

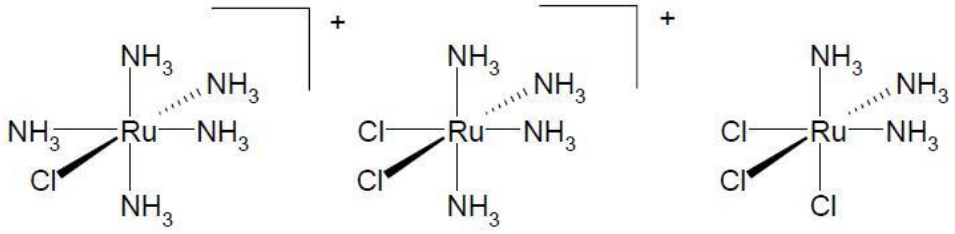

Fig. 1.1: *Ammine-chlorido derivatives. From left to right,* $[Ru(II)(NH_3)_5Cl]^+$*,cis-*$[Ru(III)(NH_3)_4Cl_2]^+$*and fac-*$[Ru(III)(NH_3)_3Cl_3]$

1.1.1.2 Ruthenium dimethylsulfoxide complexes:

Alessio, Sava and co-workers studied the highly water-soluble Ru(II) chlorido-dmso complexes (where dmso is an S-bound dimethylsulfoxide ligand) [20], another important class of ruthenium compounds. The *trans*-$[RuCl_2(dmso)_4]$ complex showed enhanced cytotoxicity compared to its cis-counterpart, which was explained by means of differences in kinetics [21].The higher cytotoxicity and the efficacy to overcome cisplatin resistance for certain cell lines (as observed in the case of the P388 leukaemia) together with the fact that *trans*-$[Ru(II)Cl_2(dmso)_4]$ exhibits a good antimetastatic activity [22], suggests that the *trans*-

ruthenium complex could be an appealing alternative to cisplatin, by acting through a different mechanism of action. Furthermore, it was also established that some Ru (II) chlorido-dmso complexes possessed anti-metastatic activity (particularly in non-small cell lung cancer) but are relatively inactive against primary tumours [23]. Developments in this area include the syntheses and biological investigations of Ru(II)–dmso complexes like Na{*trans*-[Ru(III)Cl_4(dmso)(Him)]}, (Him = imidazole), nicknamed NAMI, and the more stable [H_2Im][*trans*-Ru(III)Cl_4(dmso)(Him)], known as NAMI-A (*Fig.1.2*) [20].

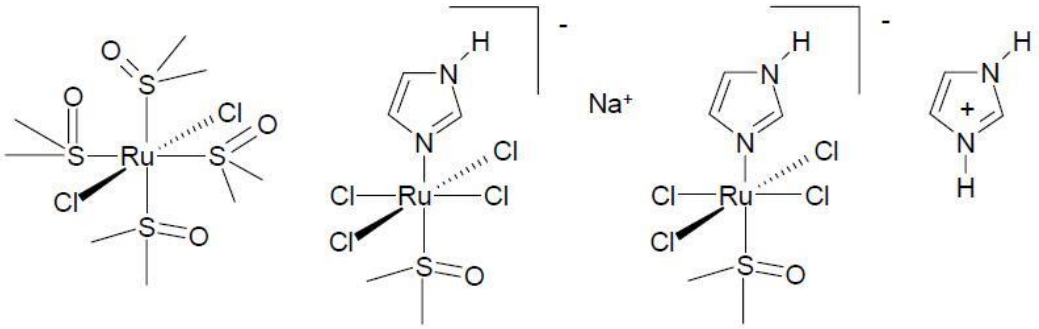

Fig. 1.2: *Dimethylsulfoxide complexes. From left to right, trans-[Ru(II)(dmso)$_4$Cl$_2$], Na{trans-[Ru(III)Cl$_4$(dmso)(Him)]} (NAMI) and [H$_2$Im]{trans-[Ru(III)Cl$_4$(dmso)(Him)]} (NAMI-A).*

The ruthenium therapeutic, imidazolium(imidazole)(dimethylsulfoxide) tetrachlororuthenate (III) (NAMI A) originally synthesized by the groups of Alessio and Sava, was the first ruthenium based complex to reach human clinical investigations [24, 25]. In the preclinical investigations in several tumor animal models, NAMI-A appeared to lack cytotoxic actions but exhibited inhibition of tumour metastases [26, 27]. Compared to cisplatin, NAMI-A has a wide variety of biological targets, most of which are extracellular rather than DNA-based and unlike cisplatin , the main mechanism of action of both NAMI and NAMI-A is probably, not directly related to their DNA binding [28-30]. Though NAMI A succeeded in phase I clinical studies, but in phase II clinical studies showed only limited efficacy which prevented its further clinical development [31]. Many analogues of NAMI-A (which includes Os (III) complexes) have been well synthesized and characterised, but no major improvements have been seen in their anti-metastatic activity compared with the parent drug have been reported yet [32].

*1.1.1.3 **Ruthenium** Complexes with other heterocyclic ligands:*

Contemporary with the development of NAMI-A by Sava and co-workers, Keppler *etal* synthesized a group of so-called “Keppler-type” compounds. These are anionic ruthenium

(III) complexes with monodentate heterocyclic nitrogen donor ligands, the most successful of which have the formula *trans*-$[RuCl_4(L)_2]^-$, where L is imidazole (KP418) or indazole (KP1019 and KP1339), and the counterion $(LH)^+$ or Na^+ (*Fig.1.3*) [33].

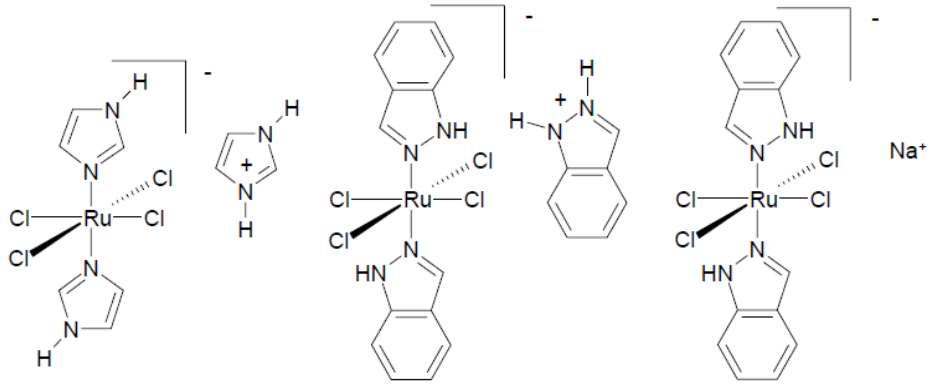

Fig. 1.3: *Molecular formula of the ruthenium (III) complexes. From left to right, (KP418), (KP1019) and (KP1339)*

Compared to NAMI A, KP1019 is more stable toward aquation and hydrolysis, readily taken up by cells [34-36] and also shows remarkable activity against primary cisplatin-resistant colorectal tumours with no pronounced anti-metastatic activity. The mechanism of action of these complexes is thought to differ considerably from that of cisplatin. The involvement of the "activation-by-reduction" process and the transferrin-mediated transport into the cells seem to play a very important role in the efficiency of the "Keppler-type" complexes as in the case of NAMI-A [34, 37]. Subsequently, indazolium *trans*-tetrachloridobis (1H-indazole)ruthenate-(III)] KP1019 entered clinical trials and successfully completed phase I clinical trials in 2005 [38]. But its low solubility limited its further development and its more soluble sodium salt, sodium *trans*-tetrachloridobis(1H-indazole)ruthenate-(III)] KP1339, is currently undergoing clinical trials. The phase I trial of **KP1339** was very well used in a dose-escalation study for the treatment of advance solid tumors. **KP1339** was particularly very effective against neuroendocrine tumors and exhibited limited side effects [39-41].

1.1.1.4 Ruthenium polypyridyl complexes:

It has been more than 67 years since the biological activity of Ru(II) polypyridyl complexes was first reported by Dwyer *et. al.* [42]. They demonstrated that the enantiomeric $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$ complexes have different biological activities. The Ru(II) polypyridyl complexes are lipophilic in spite of being cationic and usually contain chelating ligands such as polypyridine, 1,10-phenanthroline and their derivatives (*Fig. 1.4*) [43]. These

coordinatively saturated Ru(II) tris(bidentate) complexes with octahedral geometry have been demonstrated to possess a variety of biological properties [44]. Ji *et. al.* synthesized a number of Ru(II) tris(polypyridyl) complexes as potential anticancer drugs *and* systematically investigated the interactions between the complexes and DNA molecules [43]. Being positively charged, most Ru (II) tris(polypyridyl) complexes interact electrostatically with various biomolecules. In addition, intercalation of the aromatic rings within the base pairs of DNA is a classical and frequent mechanism of anticancer activity of the Ru(II) polypyridyl complexes [45]. In addition, a number of Ru(II) polypyridyl complexes are used as photosensitizers in cancer photodynamic therapy (PDT) [46] because of various photophysical properties endowed to them like large Stokes shift, long luminescence lifetime, significant two-photon absorption and photostability. A Ru(II)-polypyridyl complex TLD1433 (*Fig 1.5*) entered phase 1 and phase 2a clinical trials for bladder cancer treatment with photodynamictherapy (PDT) [47].

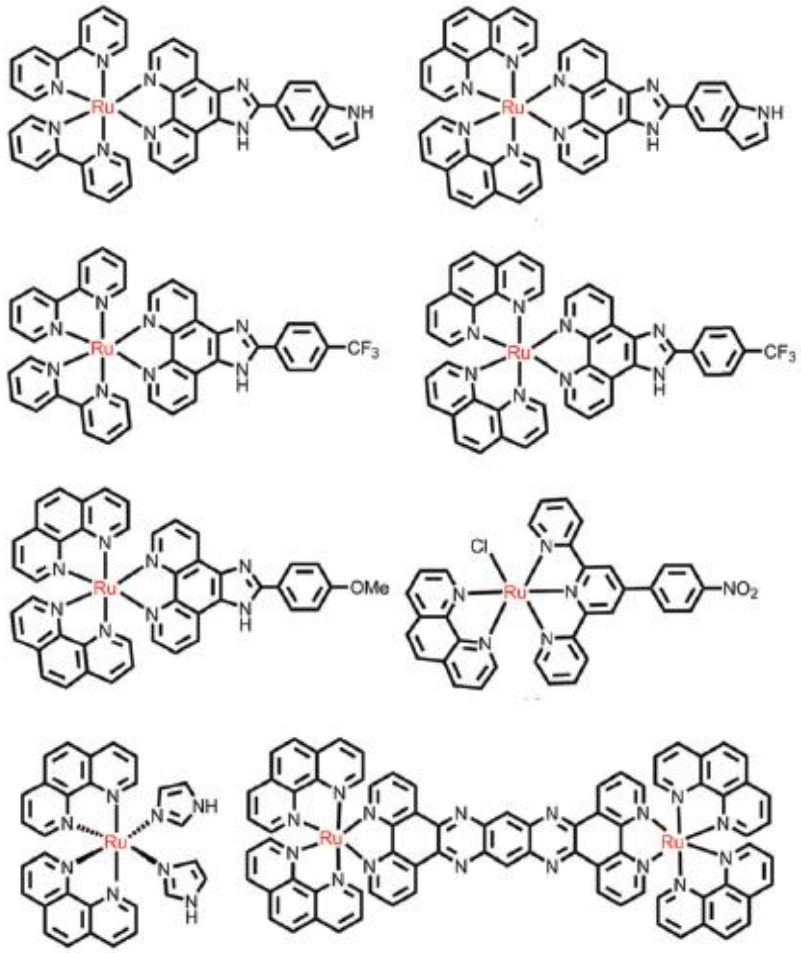

Fig. 1.4: *Structures of selected Ru (II) polypyridyl compounds as anticancer drugs*

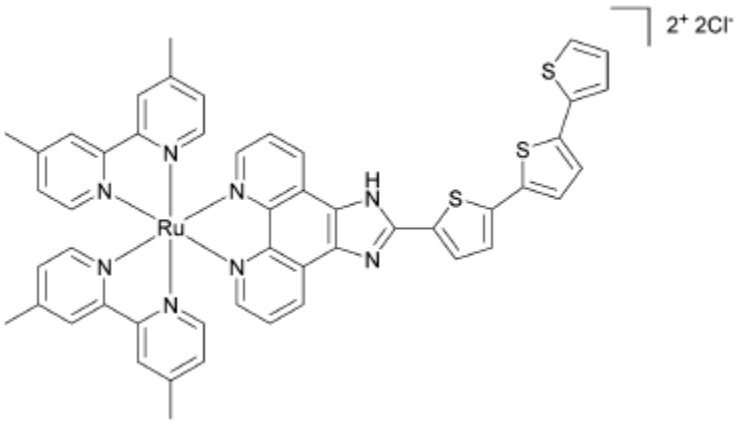

Fig. 1.5: *Structure of Ru-polypyridyl complex TLD1433*

1.1.2 Arene ruthenium (II) complexes as anticancer agent:

The use of Ru^{II} arene complexes as potential anticancer agents has been explored given that they often possess good aqueous solubility and the arene ligand is relatively inert towards displacement under physiological conditions [48]. Sadler and Dyson are the pioneers in the field of anticancer ruthenium (II)-arene complexes [49, 50] also called the piano stool complexes, where the arene forms the seat of the piano stool and the ligands resemble the legs [51]. In particular, the discovery of the biological activity of organoruthenium complexes of the type $[(\eta^6\text{-arene})Ru(X)(Y)(Z)]^{n+}$, where the common arene rings include benzene (ben), methyl isopropyl benzene (cym), biphenyl (bip) and dihydroanthracene (dha), the ligands X and Y can be two monodentate ligands or one bidentate ligand, and Z is usually a leaving group, such as a halide ion, [52] as shown in *Fig. 1.6* has opened up a new route towards the design and synthesis of novel anticancer agents. Although their mode of action is not yet completely understood, few studies suggest that DNA might be an important target [52].

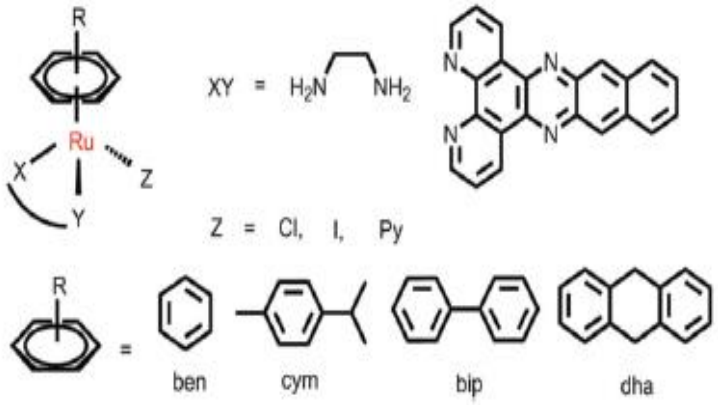

Fig. 1.6: *Common structures of $[(\eta^6$-arene)Ru(X)(Y)(Z)]*

A particularly attractive feature of Ru^{II} arene anticancer complexes is the possibility of modifying their basic structural framework. This provides considerable scope for the optimisation of their design in terms of mechanisms of action, selection of target sites, and modulation of possible side effects [53-55].

1.1.2.1 Structural Features of Ru (II) Arene Complexes for Anticancer Activity:

1.1.2.1.1 The Arene

The arene moiety is regarded as the core component of the Ru (II) arene complexes with the general formula $[(\eta^6\text{-arene})Ru(X)(Y)(Z)]^{n+}$. It stabilizes Ru in its 2+ oxidation state and determines the electron distribution at the Ru (II)-centre which affects the stability of the complexes. The more extended coordinated arenes such a biphenyl or tetrahydroanthracene, could provide a hydrophobic face that might assist the passage of the Ru^{II} drug across cell membranes [54] and facilitate interactions with potential targets [53]. It has been observed that the cytotoxic activity of Ru^{II} arene complexes appears to increase with the size of the coordinated arene [56]. When tested against the human ovarian cancer cell line (A2780), the 50% inhibitory concentrations (IC_{50} values) of a series of Ru(II) - arene- ethylenediamine (en) complexes were found to decrease in the order: benzene (17μM) > *para*-cymene (10 μM) > biphenyl (5 μM) > dihydroanthracene (2 μM) > tetrahydroanthracene (0.5 μM) [57]. The enhanced cytotoxicity in these compounds and other related Ru(II) extended arene complexes is assumed to arise from the ability of the arenes to intercalate into DNA, resulting in medium-to-strong π-stacking interactions that cause a significant distortion of the structure of DNA [58]. The effect on cytotoxicity and DNA binding of Ru(II) complexes of structural isomers of the terphenyl ligand (*para*-, *meta*-, and *ortho*-terphenyl) as shown in *Fig 1.7* have been explored [55].

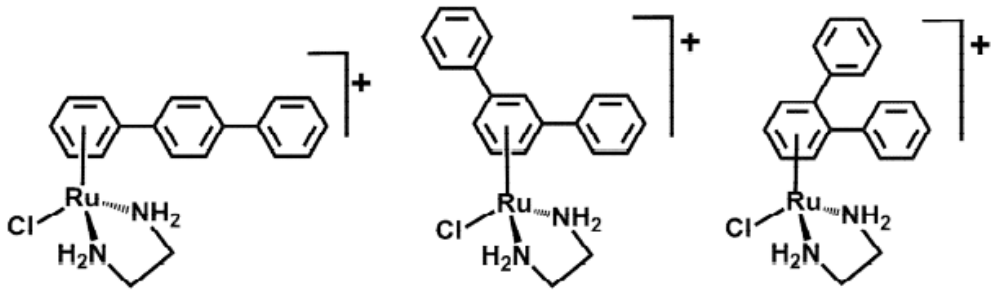

Fig. *1.7:* *Structures of $[(\eta^6\text{-arene})Ru(en)Cl]^+$ complexes. From left to right, para-terphenyl, meta- terphenyl and ortho-terphenyl*

The complex containing *para*-terphenyl as the arene ligand, exhibits increased cytotoxicity in several human tumour cell lines, including those resistant to cisplatin. In contrast, complexes containing *meta*- or *ortho*-terphenyl are comparatively less cytotoxic. The results also revealed that the DNA binding mode of the Ru^{II} *para*-terphenyl complex involves combined intercalative and monofunctional coordination binding which explains its relatively high cytotoxicity. In contrast, the *ortho*-terphenyl complex was found to bind to DNA only through monofunctional coordination to DNA bases.

1.1.2.1.2 The X and Y Monodentate Ligands

Introducing bifunctional reactivity into Ru(II) arene complexes can be achieved by synthesising derivatives of the form $[(\eta^6\text{-arene})Ru(X)(Y)(Z)]^{n+}$ where X, Y, and Z are all monodentate ligands. The use of this approach is expected to increase interactions with potential targets such as DNA, in a comparable way to cisplatin. The synthesis and activity of the complex $[(\eta^6\text{-}p\text{-cym})Ru(NH_3)_2Cl]^+$ have been investigated [59]. Despite its constitutional similarity to cisplatin, the complex exhibits much lower cytotoxicity and displays an IC_{50} value *ca.* 500 times larger than that of the platinum drug under the same conditions. The lesser activity probably arises from its instability in solutions. In contrast the compound $[(\eta^6\text{-}p\text{-cym})Ru(en)Cl]^+$ was found to be almost a hundredfold more cytotoxic than its structural analogue. The substitution of at least one of the NH_3 groups by a sterically more demanding ligand, usually a heterocyclic amine, a pyridine or phosphine, has also been tried. RAPTA compounds initially from Dyson's laboratory are characterized by a monodentate phosphane ligand pta (1,3,5-triaza-7-phospha-tricyclo-[3.3.1.1]decane) and a η^6-arene ligand bound facially to the metal centre, with the general formula $[(\eta^6\text{-arene})Ru(pta)(Y)(Z)]$, where Y and Z are most commonly chloride [60].The hydrophilic pta ligand has good aqueous solubility and is preferentially protonated in a low pH environment. RAPTA derivatives (RAPTA-C, RAPTA-T and RAPTA-B) (*Fig. 1.8*) containing two chloride ligands were susceptible to hydrolysis in a low chloride environment [61].

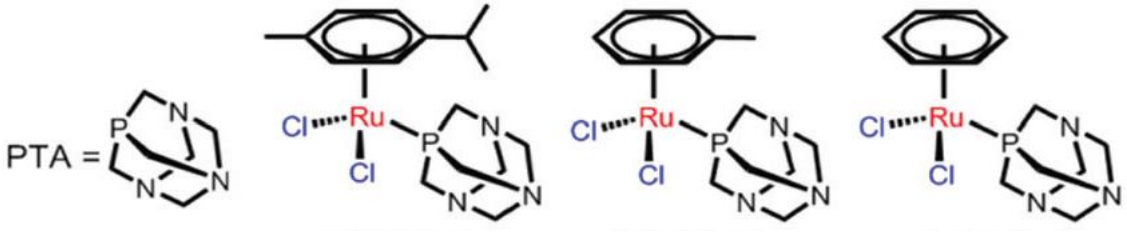

Fig. 1.8: *Structural formula of (RAPTA complexes. From left to right, (RAPTA-C), (RAPTA-T) and (RAPTA-B)*

The complexes [(η^6-p-cym)Ru(II)pta)Cl_2] (RAPTA-C) and [(η^6-toluene)Ru(II)(pta)Cl_2] (RAPTA-T) exhibit only a low activity in vitro but are active in vivo, where they inhibited lung metastases in Cytometric Bead Array (CBA) mice bearing MCa mammary carcinoma [62]. Initially it was postulated that the RAPTA derivatives target DNA of the host cells primarily. RAPTA-C was claimed to exhibit pH-dependent DNA damage; the pH, at which the damage was the greatest, significantly correlated with the pH of the cancer cells [60]. Dyson and Davey *et. al.* observed for the first time that RAPTA-T can combine with auranofin synergistically for effective killing of cancer cells [63]. The RAPTA derivatives were highly cytotoxic in A2780 and A2780cisR cell lines wherein the complexes with curcuminoid ligands exhibited more potency than cisplatin [64]. In addition, the RAPTA complexes can readily react with proteins and inhibit enzymes like glutathione transferase, lysozyme, cathepsin B (Cat B) and TrxR but there is no significant correlation between these functions and toxicity in cancer cells. Dyson *et. al.* postulated that the RAPTA derivatives have multiple modes of action in tumours which trigger cell death [65].

1.1.2.1.3 The XY Chelating Ligand

It seems to be highly advantageous for anticancer activity and aqueous solution stability of the [(η^6-arene)Ru(X)(Y)(Z)]$^{n+}$ complexes, that the monodentate X and Y ligands are replaced by a bidentate chelating group, usually represented as XY. Generally, complexes containing chelating ligands tend to be more active than those containing only monodentate ligands [57]. Several studies on structure activity relationships (SARs) with regard to changes in the nature of the donor atoms in the XY chelating ligand have been carried out. The chelate ligand is also known to determine the rate of binding to DNA nucleobases and more dramatically, to change the selectivity for them. This feature is believed to be directly related to the ability to inhibit cancer cell growth *in vitro*. The stabilising effect of the bidentate ligand seems to be essential in the case of Ru(II)–arene complexes.

1.1.2.1.3.1 Arene Ru(II) complexes with N,N-, N,O-, O,O-, S,O-, C,N- and N,S- chelating ligands

Arene ruthenium complexes containing N, N-chelating ligands have been studied systematically by Sadler and co-workers. The group reported that variation in the arene ring, the N,N-chelating ligand and the leaving group, can have a significant effect on the chemical and biological activity of the compounds [66]. A change from ethylenediamine (en) to the

anionic O,O-chelating acetylacetonate (acac) ligand, has been found to increase the rate and extent of the hydrolysis of the leaving group (Z) considerably [67].The chelate ligands also determine the rate of binding to DNA nucleobases and more specifically, change the preference for a specific base. For example, when the chelating ligand is acac, the overall affinity for adenine bases (A) appears to be greater than that for guanine (G) bases, as opposed to the affinity for G when the chelating ligand is en [68]. This reveals the imperative role the N–H groups play in the stabilisation of adducts with G bases via H-bonding, just as the oxygen atoms in acac can form favourable H-bonds with the C_6NH_2 group of A bases [69]. Similar H-bonding is also assumed to play a role in the recognition of DNA by cisplatin [70]. Moreover, SAR studies for Ru^{II} arene complexes containing N, N' chelating ligands have revealed that the species containing en showed an enhanced cytotoxicity whereas complexes containing tetramethylethylenediamine (TMEDA) were inactive [67].Steric effects of the methyl groups as well as no possibility of H-bonding may contribute to the loss of activity of the TMEDA derivative. In general, good cytotoxic activity is observed when en is replaced by aromatic amines such as 1, 2-diaminobenzene (dab); the IC_{50} values determined for a series of Ru^{II} complexes were in the range between 7 and 32 μM. Furthermore, other non-amine N,N'-chelating ligands have also been investigated and the results show that 2,2'-bipyridine (bpy) complexes tend to be non-cytotoxic towards A2780 cells. A group of ruthenium (II) arene complexes containing diamine moieties in the coordination sphere has been reported. The complexes contain chelating bidentate ligands such as paullones [71] and staurosporine (where the π-arene is a cyclopentadienyl, Cp) inserted into the ruthenium scaffolds (*Fig. 1.9*) [69, 72-75].

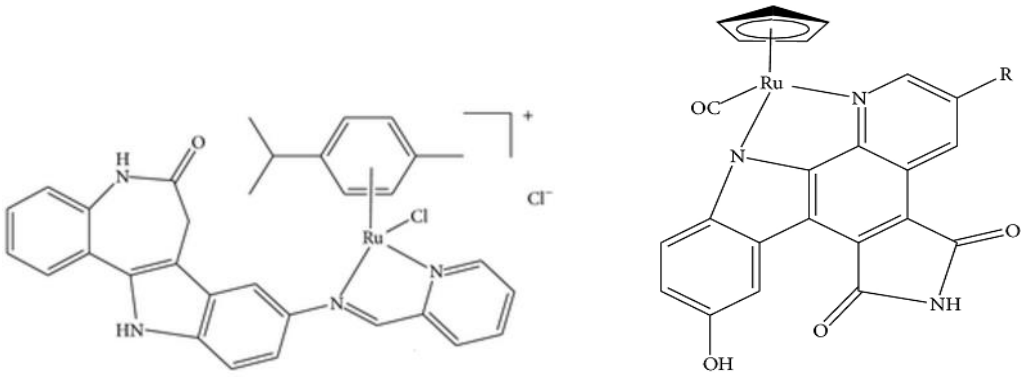

Fig. 1.9 *Paullone and Staurosporine-type arene ruthenium(II) complexes.*

Recently, antitumor activity of organoruthenium complexes with chelate aromatic ligands, derived from1,10-phenantroline (*Fig. 1.10*) were synthesized to study their structure-activitiy

relationship and reveal the changes in biological activity resulting from small structural modifications of the four Ru(II)complexes [76]. Based on the IC_{50}values, it was stated that minor changes in dppz ligand, like the introduction of a strongly electron-withdrawing $-NO_2$ or a weakly electron-withdrawing -Cl substituents were not sufficient to induce important changes in the biological activity, compared to complexes without substituents on the ligand.

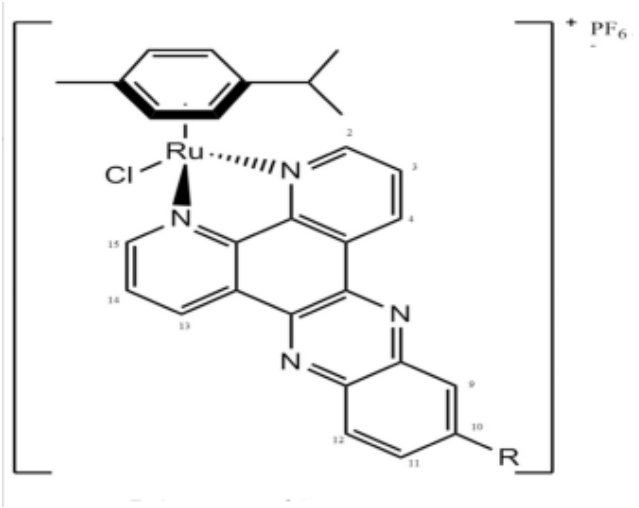

Fig. 1.10*: Ruthenium (II)-cymene complexes of 1,10-phenantroline derivatives*

Reports by Betanzos-Lara et al indicated that arene Ru(II) complexes [(p-cym)Ru(bpm)(py)][PF_6]$_2$ (where p-cym= para-cymene, bpm= 2,2'-bipyrimidine and py = pyridine / pyridine derivatives) can selectively photodissociate a monodentate ligand (py) when excited by visible light [77]. The relationship between the photoactivity and structure of the arene Ru (II) pyridine and pyridine-derivative complexes with N, N-chelating ligands as shown in Fig. 1.11 was also investigated. It was found that more electron-donating substituents in the 4- position of the py ring moderately increased the extent of photoinduced hydrolysis whereas more electron-donating substituents on the arene ring increased both the extent and the rate of photoinduced hydrolysis. Increasing the aromatic character of the N, N-chelating ligands decreased the extent of photoinduced hydrolysis, though no effect on rate was observed [78]. Wang *et. al.* reported the facile photodissociation of the ferrocenyl pyridine ligand from the [(pcym)Ru(bpy)(py-Fc)]$^{2+}$ complex (*Fig. 1.11*) [79].

Sadler *et. al*. studied the structure–activity relationships of arene Ru (II) complexes containing N,O-, and O,O-chelating ligands in context to their cytotoxicity. Their results indicated that the amino acidato (N, O-) complexes were inactive in A2780 cells, with IC_{50}

values 4100 mM. However, the O, O-chelated arene Ru(II) complexes were efficacious in A2780 cells [67].

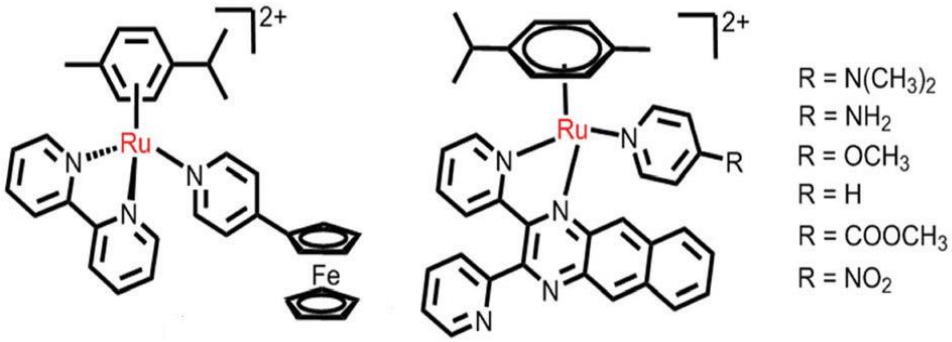

Fig. 1.11: *The N, N-ligand arene Ru(II) compounds with good photoactivity*

Chelopo *et. al*. determined the anticancer efficacy of several arene Ru(II) complexes containing 1,2,3,4- tetrahydroisoquinoline amino alcohol ligands (*Fig. 1.12*) in the human cancer cell lines MCF-7, A549, and MDAMB-231 [80]. The other N, O-Chelating ligands investigated include tetrahydroisoquinoline and selected amino acids whereas the O, O-ligands, is common β-diketonate and pyrone ligands [81].

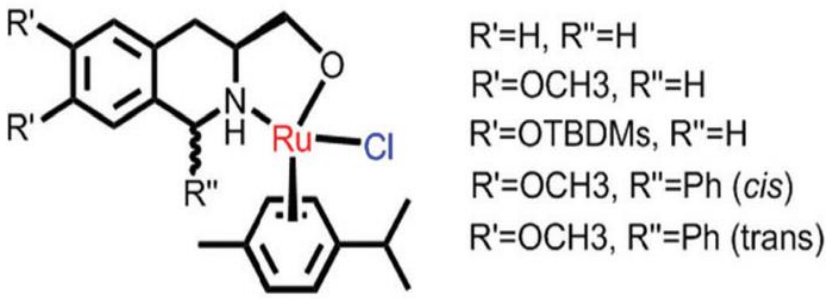

Fig. 1.12: *Structural formula of Ru(II) arene complexes bearing N,O-chelating ligands*

The thiopyrones (S, O) were reported to be more lipophilic than their pyrone analogues. Antiproliferative activities of the pyrone and thiopyrone compounds (*Fig. 1.13*) were investigated against the colon carcinoma SW480 and ovarian carcinoma CH1 cancer cell lines, and the thiopyrone complexes were observed to be more active compared to their pyrone analogues. DFT calculations show that thiopyrones have stronger binding to ruthenium compared to pyrones, and the different stabilities of these compounds may be responsible for the observed influence of the donor atoms on *in vitro* anticancer activity [81].

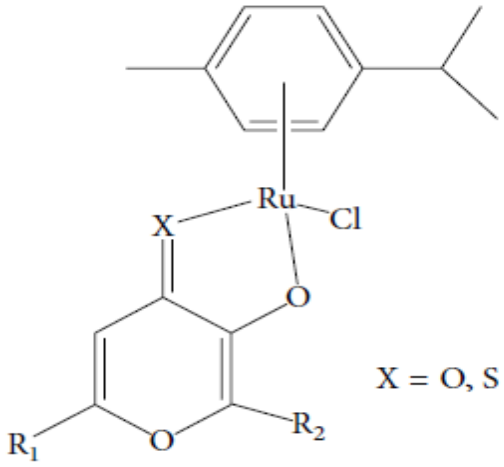

Fig. 1.13: *Pyrone and thiopyrone ruthenium(II)-cymene complexes.*

Jordon Sandland et al synthesized a water-soluble porphyrin-RAPTA conjugate (*Fig. 1.14*) and evaluated its photochemical and biological properties. The photostability of the porphyrin component of the conjugate was found to be good both in white and red light. Biological evaluation revealed that the conjugate is not only a viable photosensitizer, but it also operates as an anticancer agent by controlling cell proliferation in the "dark" at low concentrations and shorter incubation time [82].

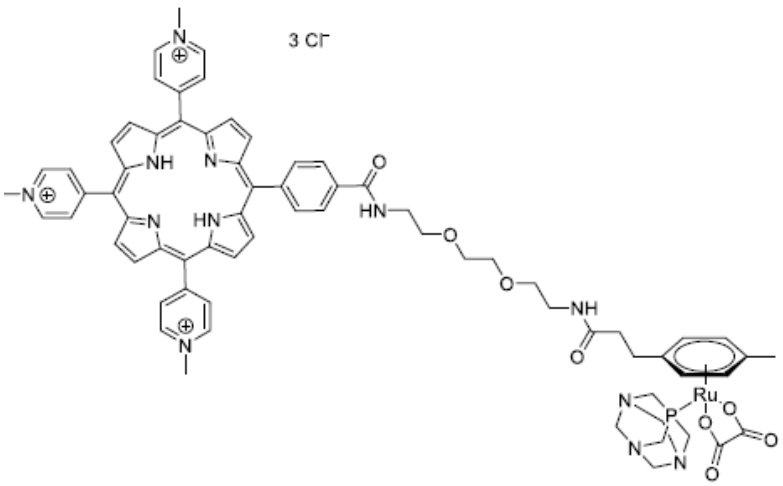

Fig. 1.14*: Cationic RAPTA-Porphyrin Conjugate*

A series of water-soluble iminophosphorane Ru (II) complexes were synthesized by Frik *et. al.* Most of the complexes were found to be more cytotoxic than cisplatin in several human cancer cell lines [83]. Typically, arene Ru (II) complexes with C, N-cyclometalated ligands are more efficacious than cisplatin as anticancer compounds. Yellol and co-workers

synthesized some neutral C, N-cyclometalated arene Ru (II) complexes (*Fig. 1.15*), and these complexes were efficacious in HT29, T47D, A2780 and A2780cisR cancer cell lines [84]. Subsequently, Yellol *et. al*. determined the effects of varying substituents (R = H, Me, F, CF_3, MeO, NO_2, and Ph) in the C-4 position of the phenyl ring of the 2-phenylbenzimidazole chelating ligand on the anticancer efficacy of the complexes [85].

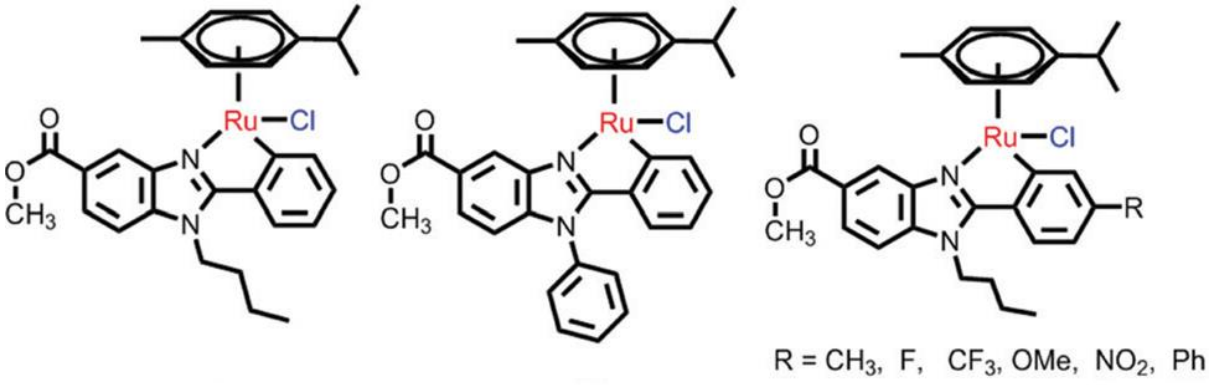

Fig. 1.15: *C, N-Cyclometalated (η^6-p-cymene) Ru(II) complexes*

A series of new Ru(II)-arene complexes with triarylamine−thiosemicarbazone hybrid (N,S-chelating) ligands with higher anticancer activity than cisplatin were reported. A pyrrolidine-attached Ru(II)-benzene complex exhibited higher activity against the tested cancer cells with low IC_{50} values. In addition, the interaction between synthesized Ru(II)-arene complexes and DNA/HSA was explored by absorption and emission spectroscopy methods. The complexes bind via intercalation and exhibit good binding affinity toward DNA [86].

1.1.2.1.4 The Z Leaving Group

The leaving group (Z), which is typically a halide ion, plays an important role in the activation of Ru(II)arene complexes given that with its release from the metal centre, a vacant site for coordinating potential biomolecules is readily made available. Initial studies showed that the substitution of the chloride leaving group by other halides, such as iodide, seemed to have only a small effect on the cytotoxicity [57].The hydrolysis of Ru–Z bonds is also affected by pH and the concentration of Z in the medium. The anticancer efficacy of arene Ru(II) complexes can also affected by the water solubility and volume of the chelating ligand and leaving group [51]. The evidences suggest that a combination of fast hydrolysis rates and high reactivity usually leads to non-cytotoxic complexes, similarly very slow hydrolysis rates and chemical inertness leads to low *in vitro* cytotoxicity [87]. An exception to this observation was found when the leaving group is not a halide but a thiophenolate [56]. This

compound was found active, displaying an IC_{50} value of 23 μM against A2780 human ovarian cancer cells, despite its inertness towards hydrolysis. Pettinari *et. al.* determined the anticancer efficacies of arene Ru(II) complexes (*Fig. 1.16*), with a 4-(biphenyl-4-carbonyl)-3-methyl-1-phenyl-5-pyrazolonate ligand and different monodentate ligands (Cl, CH_3OH, pta) [88].The nature of the monodentate ligands was critical in terms of the DNA binding affinities of the Ru(II) complexes, with a rank order of pta analogues > CH_3OH analogues > chloride analogues.

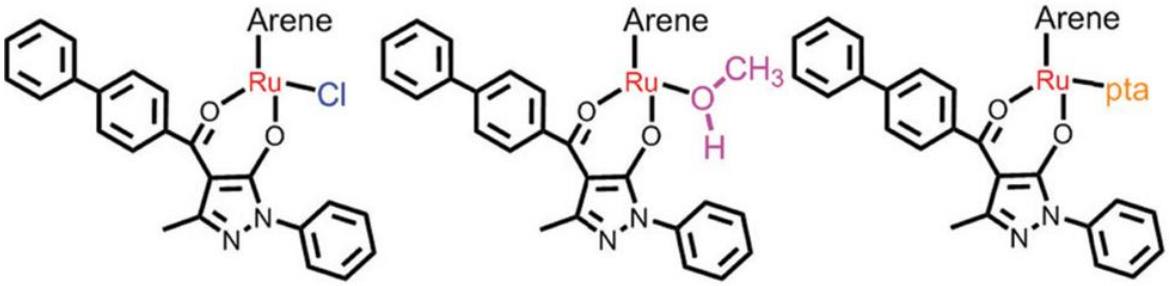

Fig. 1.16: *Ru(II) arene complexes with different monodentate ligands*

Two water soluble half-sandwich Ru(II)–arene complexes of the type [Ru(η^6 -arene)(metformin)Cl]Cl (η^6 –arene = p-cymene; benzene) have been isolated and characterized. Stronger DNA binding affinity of the p-cymene complex was attributed to its hydrophobic interaction with DNA through the methyl and isopropyl groups of arene ligand. DNA docking studies revealed that p-cymene complex binds to DNA in the major groove, which is stabilized by hydrogen bonding interactions. The p-cymene complex also exhibited higher affinity to bind to BSA than benzene complex in the hydrophobic region. The complex displayed more cytotoxic activity than its analogue against human breast, lung and ovarian cancer cells and was found to be non-toxic to normal kidney cells [89]. Quadruplex nucleic acids with guanine rich sequences have vital roles in the biology of cancers and have emerged as promising targets for small molecules. Hager etal. synthesized four novel Ru(II) arene complexes with 1,3-dioxoindan-2-carboxamides ligands bearing pendant naphthyl groups designed to bind quadruplexes by stacking interactions and coordination. Substitution of the chlorido leaving ligand with pyridine has been observed to improve the hydrolytic stability of such complexes, influence their interaction with quadruplexes and cytotoxicity against ovarian cancer cells [90]. Ruthenium (II) based complexes with phosphine ligands (*Fig. 1.17*) have been reported recently. Some of them displayed strong antiproliferative properties on several types of cancer including colon, breast, and lung. Notably, two of Ru(II) complexes exhibited IC_{50} around 2 μM, which is exceptional for these kinds of complexes. It

was evident that there is a dramatic influence of the nature of the arene moiety coordinated to the ruthenium center on the cytotoxicity of the complexes [91].

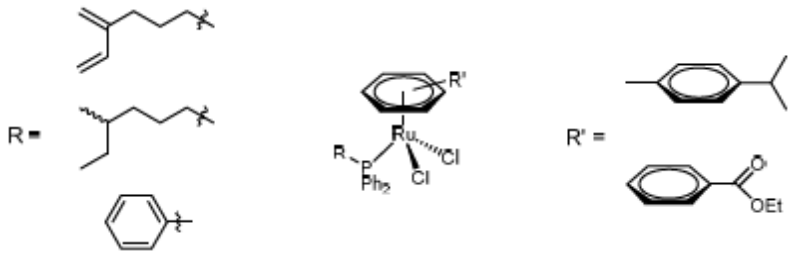

Fig. 1.17: *Structures of the Ru(II)-based complexes investigated*

Synthesis and antiproliferative activity of a new fluorescent 4-amino-1, 8-naphthalimide Tröger's base-Ru(II)-curcumin organometallic conjugate was reported (*Fig. 1.18*) [92]. The merging of two anticancer active structures (Ru-Cur and TBNap) within a single conjugate increased the anticancer potency of TB-Ru-Cur and its cationic nature facilitated the cellular uptake as expected. TB-Ru-Cur showed a fast cellular localisation and higher antiproliferative activity than the precursors.

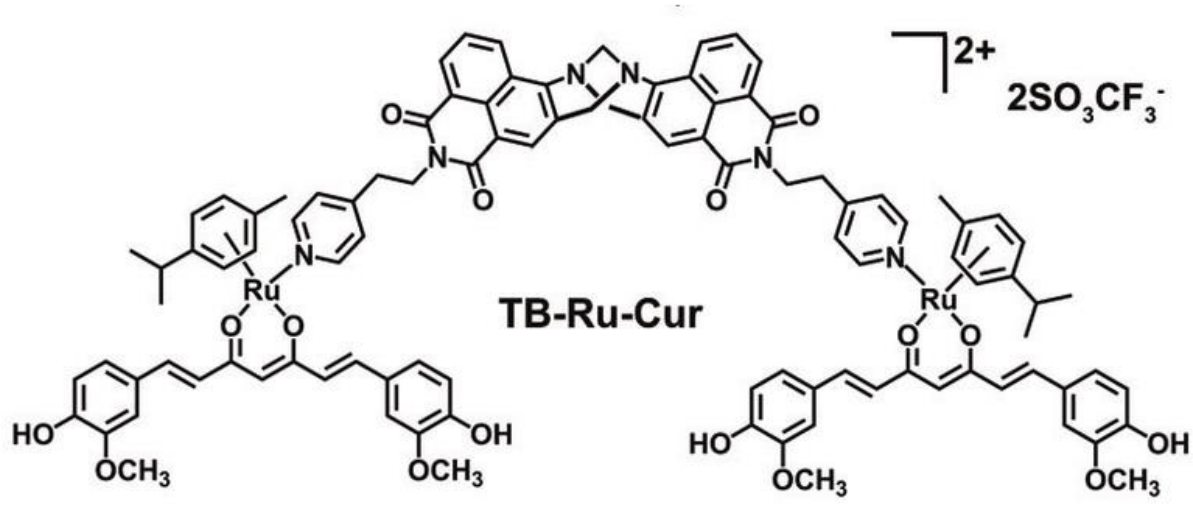

Fig. 1.18*: Structure of TB-Ru-Cur*

1.2 Summary:

It can be summarized from the vast literature that ruthenium drugs have promising anticancer activity in the *in vitro* and *in vivo* models compared to platinum (II) compounds, due to their low systemic toxicity. In general, the activity of ruthenium compounds are mainly determined by the ligand combination and coordination geometry between ruthenium and its

ligands, mostly with regard to their reactivity, hydrophobicity, binding, cellular uptake and intracellular distribution. In this regard, several Ru (II) compounds have high selectivity and targeting ability, which helps in improving their efficiency towards the cancer cells and further minimized their toxicity in normal cells. These complexes function in a different way to classical chemotherapies.

1.3 Aim and outline of this thesis:

The second generation (post-platinum) well know transition metal chemotherapeutics that possess unique properties granting them, at least in preclinical studies, more selective entry into tumor cells with fewer toxic effects to normal cells. A large number of laboratory data demonstrates that several ruthenium (II) and (III) complexes display antitumor activity on variety of cancer cell lines and animal models. Taking insights from the clinical progress of NAMI-A, KP1019 and RAPTA type ruthenium complexes, a group of related complexes have been presented here along with studies of their biological activities.

The subject is first introduced earlier in **Chapter 1** with an overview of the established ruthenium anticancer agents with a special mention of those, under clinical trials. Special attention is given to the mechanisms of action of these compounds, as well as the structure-activity relationships that are known to date.

Chapter 2 encompasses synthesis and detailed characterization of four different ligands series which have been chosen with special focus on their biological relevance. These ligands were to be used in the preparation of binuclear ruthenium (II) arene complexes.

Piano stool type organometallic binuclear ruthenium(II) arene complexes with a general structural formula **[(η^6-p-cym) (L)Ru(μ-im /azpy)Ru(L)(η^6-p-cym)]Cl_n** (*p-cym* = *p*-cymene $MeC_6H_4Pr^i$; *L* = N,O-, S,N- and O,O- donor ligands discussed in chapter 2; im= imidazole; azpy = 4,4’ azopyridine) were synthesized and well characterized in **Chapter 3** with an aim to check for their bioactivities.

In **Chapter 4** the binding interactions of the synthesized binuclear ruthenium (II) arene complexes with two important biomolecules; DNA and Serum albumin; were looked into using spectroscopic techniques. The cytotoxicities of the binuclear ruthenium complexes against human cervical cell line HeLa were evaluated.

Finally **Chapter 5** offers a summary and a cumulative discussion of the results presented in this thesis.

1.4 References:

[1] Valastyan, S.; Weinberg, R.A. *Cell*, **2011**, 147, 275.

[2] Thun, M. J.; DeLancey, J. O.; Center, M. M.; Jemal, A.; Ward, E. M. *Carcinogenesis* **2010**, 31, 100.

[3] (a) Galanski, M. *Recent Pat. Anti-Can Drug Discov*., **2006**, 1, 285. (b) Shaili, E. *Sci. Prog,*. **2014**, 97, 20.

[4] (a) McQuitty, R. J. *Sci. Prog.*, **2014**, 97, 1−19. (b) Hurley, L. H. *Nat. Rev. Cancer* **2002**, 2, 188. (c) Hartinger, C. G.; Dyson, P. J. *Chem. Soc. Rev.*, **2009**, 38, 391.

[5] Ohmichi, M.; Hayakawa, J.; Tasaka, K.; Kurachi, H.; Murata, Y.; *Trends Pharmacol. Sci*., **2005**, 26, 113.

[6] Bergamo, A.; Gaiddon, C.; Schellens, J. H. M.; Beijnen, J.H.; Sava, G. *J. Inorg. Biochem*., **2012**,106, 90.

[7] (a) Poynton, F. E.; Bright, S. A.; Blasco, S.; Williams, D. C.; Kelly, J. M. ; Gunnlaugsson, T. *Chem. Soc. Rev.*, **2017**, 46, 7706. (b) Notaro, A.; Gasser, G. *Chem. Soc. Rev.*, **2017**, 46, 7317.

[8] Thota, S. *Mini-Rev. Med. Chem.*, **2016**, 16, 771.

[9] Biersack, B. *Mini-Rev. Med. Chem*., **2016**, 16, 804.

[10] Su, W.; Tang, Z.; Li, P. *Mini-Rev. Med. Chem*., **2016**, 16, 787.

[11] Abid, M.; Shamsi, F.; Azam, A. *Mini-Rev. Med. Chem*., **2016**, 16, 772.

[12] Allardyce, C. S.; Dyson, P. J. *Platinum Met. Rev*., **2001**, 45, 62.

[13] Alessio, E. *Eur. J. Inorg. Chem*., **2017**, 2017, 1549.

[14] M J Clarke, M.J.; F Zhu, F.; Frasca, D.R.; *Chem. Rev*. **1999**, 99, 2511.

[15] Thota, S.; Rodrigues, D. A.; Crans, D. C.; Barreiro, E. J. *J. Med. Chem.*, **2018**, 61, 5805.

[16] Clarke, M. J. *Coord. Chem. Rev.*, **2003**, 236, 209.

[17] Clarke, M. J.; Jansen, B.; Marx, K. A.; Kruger, R. *Inorg. Chem. Acta.*, **1986**, 124, 13.

[18] Clarke, M. J.; Zhu, F. C.; Frasca, D. R. *Chem. Rev.*, **1999**, 99, 2511.

[19] Clarke, M. J.; Bitler, S.; Rennert, D.; Buchbinder, M.; Kelman, A. D. *J. Inorg. Biochem.*, **1980**, 12, 79.

[20] Bratsos, I.; Bergamo, A.; Sava, G.; Gianferrara, T.; Zangrando, E.; Alessio, E. *J. Inorg. Biochem.*, **2008**, 102, 606.

[21] Alessio, E.; Mestroni, G.; Nardin, G.; Attia, W. M.; Calligaris, M.; Sava, G.; Zorzet, S. *Inorg. Chem.*, **1988**, 27, 4099.

[22] Coluccia, M.; Sava, G.; Loseto, F.; Nassi, A.; Boccarelli, A.; Giordano, D.; Alessio, E.; Mestroni, G. *Eur. J. Cancer*, **1993**, 29, 1873.

[23] Mestroni, G.; Alessio, E.; Sava, G.; Pacor, S.; Coluccia, M.; Boccarelli, A. *Met-Based. Drugs*, **1994**, 1, 41.

[24] Bergamo, A.; Messori, L.; Piccioli, F.; Cocchietto, M.; Sava, G. *Invest. New Drugs* **2003**, 21, 401.

[25] Alessio, E.; Mestroni, G.; Bergamo, A.; Sava, G. *Curr. Top. Med. Chem.*, **2004**, 4, 1525.

[26] Cocchietto, M.; Sava, G. *Pharmacol. Toxicol.*, **2000**, 87, 193.

[27] Bergamo, A.; Gava, B.; Alessio, E.; Mestroni, G.; Serli, B.; Cocchietto, M.; Zorzet, S.; Sava, G. *Int. J. Oncol.*, **2002**, 21, 1331.

[28] Messori, L.; Orioli, P.; Vullo, D.; Alessio, E.; Iengo, E. *Eur. J. Biochem.*, **2000**, 267, 1206.

[29] Barca, A.; Pani, B.; Tamaro, M.; Russo, E. *Mutat. Res.-Fundam. Mol. Mech. Mutagen.*, **1999**, 423, 171.

[30] Sanna, B.; Debidda, M.; Pintus, G.; Tadolini, B.; Posadino, A. M.; Bennardini, F.; Sava, G.; Ventura, C. *Arch. Biochem. Biophys.*, **2002**, 403, 209.

[31] Rademaker-Lakhai, J. M.; Bongard, D. V. D.; Pluim, D.; Beijnen, J.H.; Schellens, J. H. *Clin. Cancer Res.*, **2004**, 10, 3717.

[32] Alessio, E. *Eur. J. Inorg. Chem.*, **2017**, 1549.

[33] Uivarosi, V.; Badea, M.; Olar, R.; Marinescu, D.; Nicolescu, T. O.; Nitulescu, G. M. *J. Therm. Anal. Calorim.*, **2011**, 105, 645.

[34] Kapitza, S.; Pongratz, M.; Jakupec, M. A.; Heffeter, P.; Berger, W.; Lackinger, L.; Keppler, B. K.; Marian, B. *J. Cancer Res. Clin. Oncol.,* **2005**, 131, 101.

[35] Groessl, M.; Reisner, E.; Hartinger, C.G.; Eichinger, R.; Semenova, O.; Timerbaev, A. R.; Jakupec, M. A.; Arion, V.B.; Keppler, B. K. *J. Med. Chem.*, **2007**, 50, 2185.

[36] Vargiu, A. V.; Robertazzi, A.; Magistrato, A.; Ruggerone, P.; Carloni, P. *J. Phys. Chem. B,* **2008**, 112, 4401.

[37] Hartinger, C. G.; Zorbas-Seifried, S.; Jakupec, M. A.; Kynast, B.; Zorbas, H.; Keppler, B. K. *J. Inorg. Biochem.,* **2006**, 100, 891.

[38] Jakupec, M. A.; Arion, V. B.; Kapitza, S.; Reisner, E.; Eichinger, A.; Pongratz, M.; Marian, B.; Graf von Keyserlingk, N.; Keppler, B. K. *Int. J. Clin. Pharmacol. Ther.*, **2005**, 43, 595.

[39] Dickson, N.R.; Jones, S.F.; Burris, H.A.; Ramanathan, R.K.; Weiss, G.J.; Infante, J.R.; Bendell, J.C.; McCulloch, W.; Von Hoff, D.D. *J. Clin. Oncol.,* 2011, 29, 2607.

[40] Trondl, R.; Heffeter, P.; Kowol, C.R.; Jakupec, M.A.; Berger, W.; Keppler, B.K. *Chem. Sci.*, **2014**, 5, 2925.

[41] Bytzek, A. K.; Koellensperger, G.; Keppler, B. k.; Hartinger, C. G. *J. Inorg. Biochem.,* **2016**, 160, 250.

[42] Dwyer, F. P.; Gyarfas, E. C.; Rogers, W. P.; Koch, J. H. *Nature,* **1952**, 170, 190.

[43] Li, G.; Sun, L.; Ji, L.; Chao, H. *Dalton Trans.*, **2016**, 45, 13261.

[44] Kilah, N.L.; Meggers, E. *Aust. J. Chem.*, **2012**, 65, 1325.

[45] Tan, C.; Wu, S.; Lai, S.; Wang, M.; Chen, Y.; Zhou, L.; Zhu, Y.; Lian, W.; Peng, W.; Ji, L. *Dalton Trans.*, **2011**, 40, 8611.

[46] Chen, Y.; Guan, R.; Zhang, C.; Huang, J.; Ji, L.; Chao, H. *Coord. Chem. Rev.,* **2016**, 310, 16.

[47] Smithen, D. A.; Yin, H.; Beh, M. H.; Hetu, M.; Cameron, T. S.; McFarland, S. A. *Inorg. Chem.,* **2017**, 56, 4121.

[48] Clarke, M. VCH Verlagsgesellschaft, Weinheim, and VCH Publishers, New York, **1993**, 129.

[49] Murray, B. S.; Babak, M. V.; Hartinger, C. G.; Dyson, P. *J. Coord. Chem. Rev.,* **2016**, 306 (part 1), 86.

[50] Dougan, S. J.; Sadler, P. J. *Chimia.*, **2007**, 61, 704.

[51] Suss-Fink, G. *Dalton Trans.*, **2010**, 39, 1673.

[52] Chen, F.W.H.; Parsons, S.; Oswald, D.H. I.; Davidson, J.E.; Sadler P.J. *Chem. Eur. J.*, **2003**, 9, 5810.

[53] Yan, Y. K.; Melchart, M.; Habtemariam, A.; Sadler, P. J. *Chem. Commun.,* **2005**, 4764.

[54] Liu; H. K.; Wang. F.; Parkinson, J.A.; Bella, J.; Sadler P.J. *Chem. Eur. J.*, **2006**, *12*, 6151.

[55] Bugarcic, T.; Nováková, O.; Halámiková, A.; Zerzánková, L.; Vrána, O.; Kašpárková, J.; Habtemariam, A.; Parsons, S.; Sadler, P. J.; Brabec**,** V. *J. Med.Chem*., **2008**, 51, 5310.

[56] Wang F. Y.; Habtemariam, A.; van der Geer, E. P. L.; Fernandez, R.; Melchart, M.; Deeth, R, J.; Aird, R.; Guichard, S.; Fabbiani, F. P. A.; Lozano-Casal, P.; Oswald, I. D. H.; Jodrell, D. I.; Parsons, S.; Sadler, P. *J. Proc. Natl. Acad. Sci.*, **2005**, 102, 18269.

[57] Aird, R. E.; Cummings, J.; Ritchie, A. A.; Muir, M.; Morris, R. E.; Chen, H.; Sadler, P. J.; Jodrell, D. I. *Br. J. Cancer*, **2002**, 86, 1652.

[58] Novakova, O.; Malina, J.; Suchankova, T.; Kasparkova, J.; Bugarcic, T.; Sadler, P.J.; Brabec, V. *Chem. Eur. J*., **2010**, 16, 5744.

[59]Sipka, S.G.; Stepanenko, I. N.; Lazic, J.M.; Bartel, C.; Jakupec, M.A.; Arion, V.B.; Keppler, B.K. *Dalton Trans.*, **2009**, 3334.

[60] Allardyce, C.S.; Dyson, P.J.; Ellis, D.J.; Heath, S.L. *Chem. Commun*., **2001**, 1396.

[61]Bratsos, I.; Gianferrara, T.; Alessio, E.; Hartinger, C.G.; Jakupec, M.A.; Keppler, B.K. Bioinorg. *Med. Chem*., ed. E. Alessio, Wiley-VCH, Weinheim, **2011**, 151.

[62] Zeng, L.; Gupta, P.; Chen, Y.; Wang, E.; Ji, L.; Chao, H.; Chen, Z.S. *Chem. Soc. Rev*., **2017**, 46, 5771.

[63]Adhireksan, Z.; Palermo, G.; Riedel, T.; Ma, Z.; Muhammad, R.; Rothlisberger, U.; Dyson, P.J.; Davey, C.A. *Nat. Commun*., **2017**, 8, 14860.

[64]Pettinari, R.; Marchetti, F.; Condello, F.; Pettinari,C.; Lupidi, G.; Scopelliti, R.; Mukhopadhyay, S.; Riedel, T.; Dyson, P.J. *Organomet*., **2014**, 33, 3709.

[65] Casini, A.; Gabbiani, C.; Sorrentino, F.; Rigobello, M. P.; Bindoli, A.; Geldbach, T. J.; Marrone, A.; Re, N.; Hartinger, C.G.; Dyson, P.J. *J. Med. Chem*., **2008**, 51, 6773.

[66] Morris, R.E.; Aird, R.E.; Murdoch, P. D. S.; Chen, H.; Cummings, J.; Hughes, N.D.; Parsons, S.; Parkin, A.; Boyd. G.; Jodrell, D.I. *J. Med. Chem*., **2001**, 44, 3616.

[67] Habtemariam, A.; Melchart, M.; Ferna´ndez, R.; Parsons,S.; Oswald, I.D.; Parkin, A.; Fabbiani, F.P.; Davidson, J.E.; Dawson, A.; Aird, R.E. *J. Med. Chem.*, **2006**, *49*, 6858.

[68] Chen, H.; Parkinson, J.A.; Morris, R.E.; Sadler, P.J. ,*J. Am. Chem. Soc.*, **2003**,125, 173.

[69] Liu, H. K.; Berners-Price, S. J.; Wang, F. Y.; Parkinson, J. A.; Xu, J.; Bella, J.; Sadler, P. J. *Angew. Chem.-Int. Ed*., **2006**, 45, 8153.

[70] Reedijk, J. *Chem. Commun.*, **1996**, 801.

[71] Smalley, K.S.M.; Contractor, R.;Haass, N.K.; Kulp, A.N.; Gokcumen, G.E.A.; Williams, D.S.; Bregman, H.; Flaherty, K.T.; Soengas, M.S.; Meggers, E.;Herlyn, M. *Cancer Research,* **2007**, 67, 209.

[72]Meggers, E.; Gokcumen, G.E.A.; Gründler, K.; Frias, C.; Prokop, *A.; Dalton Trans*, **2009**, 48,10882.

[73] Meggers, E.;Gokcumen, G.E.A.; Bregman, H.; Maksimoska, J.; Mulcahy, S.P.; Pagano, N.;Williams, D.S. *Synlett*, **2007**,8,1177.

[74] Debreczeni , J.E.; Bullock, A.N.; Atilla, G.E.; Williams, D.S.; Bregman, H.; Knapp, S.; Meggers, E. *Angew. Chem. Int. Ed.*, **2006**, 45, 1580.

[75]Bregman, H.; Meggers, E.; *Org. Lett.*, **2006**, 8, 5465.

[76] Savić, A.; Gligorijević , N.; Aranđelović , S.; Dojčinović , B.; Kaczmarek, A.M.; Radulović , S.; Deun , R.V.; Hecke, K.V.; *J. Inorg. Biochem*., **2020**, 202, 110869.

[77]Lara, S.B.; Salassa, L.; Habtemariam A.; Sadler, P.J. *Chem. Commun.*, **2009**, 6622.

[78]Lara, S.B.; Salassa, L.; Habtemariam, A.; Novakova, O.; Pizarro, A.M.; Clarkson, G.J.; Liskova, B.; Brabec V.; Sadler, P.J. *Organomet*., **2012**, 31, 3466.

[79]Wang, T.; Zhou, Q.; Zhang, Y.; Zheng, Y.; Wang, W.; Hou, Y.; Jiang, G.; Cheng, X.; Wang, X.; *RSC Adv*., **2016**, 6, 45652.

[80]Chelopo, M.P.; Pawar, S.A.; Sokhela, M.K.; Govender, T.; Kruger H.G.; Maguire, G.E.; *Eur. J. Med. Chem*., **2013**, 66, 407.

[81] Motswainyana M.W.; Ajibade, P.A.; *Adv. Chem*., **2015**, 2015, 859730.

[82] Sandland, J.; Savoie, H.; Boyle, R.W.; Murray, B.S.; *Inorg. Chem.*, **2020**, 59, 7884.

[83]Frik, A.M.M.; Elie, B.T.; Gonzalo, O.; de Mingo, D.R.; Sanau, M.; Sa´nchez-Delgado, R.; Sadhukha, T.; Prabha, S.; Ramos, J.W.; Marzo, I.; Contel, M.; *J. Med.Chem*., **2014**, 57, 9995.

[84]Yellol, G.S.; Donaire, A.; Yellol, J.G.; Vasylyeva, V.; Janiak C.; Ruiz, J.; *Chem. Commun*., **2013**, 49, 11533.

[85] Yellol, J.; Pe´rez, S.A.; Buceta, A.; Yellol, G.;Donaire, A.; Szumlas, P.; Bednarski, P.J.; Makhloufi, G.; Janiak C.; Espinosa, A.; *J. Med. Chem*., **2015**, 58, 7310.

[86]Muralisankar, M.; Dheepika, R.; Haribabu, J.; Balachandran, C.; Aoki, S.; Bhuvanesh, N.S.P.; Nagarajan, S. *ACS Omega*, **2019**, 4, 11712.

[87] Peacock, A.F.A.; Peter J. Sadler, P.J.*Chem. Asian J.*, **2008**, *3*, 1890.

[88] Pettinari, R.; Pettinari, C.; Marchetti, F.; Skelton, B.W.; White, A. H.; Bonfili, L.; Cuccioloni, M.; Mozzicafreddo, M.; Cecarini, V.; Angeletti, V.M.; Nabissi M.; Eleuteri, A.M. *J. Med. Chem*., **2014**, 57, 4532.

[89] Gopalakrishnan, D.; Ganeshpandian, M.; Loganathan, R.; Bhuvanesh, N.S.P.; Sabina, X.J.; Karthikeyan, J. *RSC Adv*., **2017**, 7, 37706.

[90]Hager, L.A.; Mokesch, S.; Kieler, C.; Castro, S.A.D.; Baier, D.; Roller, A.; Kandioller, W.; Keppler, B.K.; Berger, W.; Salassa, L.; Terenzi, A.; *Dalton Trans*., **2019**, 48, 12040.

[91] Chotard, F.; Dondaine, L.; Balan, C.; Bettaieb, A.; Paul, C.; Gendre P.L.; Bodio E.; *New J. Chem*., **2018**,**42**, 8105.

[92] Shanmugaraju, S.; Poulsen, B.L.C.; Arisa, B.T.; Deivasigamani Umadevi, D.; Dalton, L. H.; Hawes, C. S.; Adrián, E.S.; Savyasachi, J. A.; Watson, G. W.; Williams, D.C.; Gunnlaugsson, T. *Chem. Commun*., **2018**, 54, 4120.