International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.6, No.1, pp 828-837, Jan-March 2014

Ruthenium Complexes: - Potential candidate for Anti-Tumour Activity

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Abstract : Cancer is a class of diseases characterized by uncontrolled growth of abnormal cells, either by direct growth into adjacent tissue or by migration of cells to distant sites. The major goal in anticancer drug discovery process is to discover and develop innovative therapies that exhibit a real improvement in effectiveness and/or tolerability. A transition metal complex is a species consisting of a transition metal coordinate (bonded to) one or more ligands. Transition metal ions usually form complexes with a well defined number of ligands. Metal with many "d"electrons will have lower co-ordination number. Regardless of the achievements of cisplatin, the drug is efficient in only a limited range of cancers. Also cisplatin has severe side effects: often causing severe nausea and vomiting, bone marrow suppression and kidney toxicity. Ruthenium complexes have potential antitumor activity with less adverse effects. Some ruthenium complexes have been shown to be effective against cancers not readily treated by cisplatin. Ruthenium has the ability to mimic iron in binding to specific molecules such as albumin and transferring. Therefore the delivery of the ruthenium drugs can be more effective.
Keywords- Cancer, innovative therapies, transition metal, ruthenium complex.

Introduction

Cancer

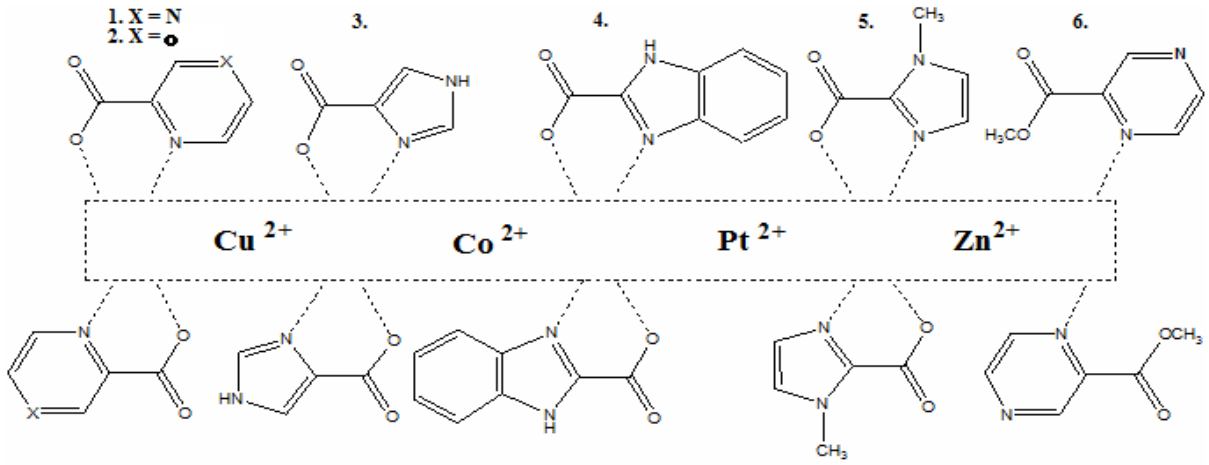
Cancer is a class of diseases characterized by uncontrolled growth of abnormal cells anywhere in the body and the ability of these cells to invade other locations in the body, either by direct growth into adjacent tissue or by migration of cells to distant sites. In Cancer, abnormalities in the genetic material of the affected cells have been observed. Damage to DNA causes unregulated growth, resulting in mutations to genes that encode proteins controlling cell division. It can thus form an encapsulated benign tumor, which leads to invasion and destruction of adjacent tissues. On the other hand, non-encapsulated malignant tumors grow rapidly, and can spread to various regions of the body and metastasize. 90% of death related to cancer is due to metastasis, which is a secondary growth though originating from the original primary tumour. Tumorigenesis is thus an accumulation of successive mutations in proto-oncogenes and suppressor genes deregulating the cell cycle. The key event to tumorigenesis is translocations or deletions and changes that affect the chromatin structure such as methylation of DNA or acetylation of histones. Cancer is still one of the more difficult

diseases to treat and was responsible for ca. 13% (7.6 million) of all deaths worldwide in 2008 [1]. An increase in cancer death rates to 12 million is estimated by 2030.

Treatment on cancer is mostly based on chemotherapy, radiotherapy, hormone and surgery. However, a major disadvantage associated with chemotherapy is their severe toxicity and lack of tolerability in some patients. The non-selective biodistribution throughout the body is the cause of their toxicity. Therefore, the major goal in anticancer drug discovery process is to develop innovative therapies that exhibit a real improvement in effectiveness and/or tolerability.

Transition metal complex

A transition metal complex is a species consisting of a transition metal coordinated (bonded) to one or more ligands (neutral or anionic non-metal species). Coordinate compounds are easily formed by transition metals with free *d* orbital's that can accept the donor electron pairs. Transition metal ions usually form complexes with a well-defined number of ligands. Oxidation state often dictates particular coordination geometry. The coordination is through the heterocyclic nitrogen and carboxylate oxygen donor atom.



The critical factors in the structure of metal ion complex depend on the type of ligand and the oxidation state of metal, which regulate the biological activity of the metal-based drugs. If the oxidation number is high, the metal can accept more electrons from ligands and vice versa. Metal with many d electrons will have lower co-ordination number. The success of the clinical applications of the platinum complex has stimulated considerable interest in searching for new metal complexes as modern therapeutic, diagnostic and radiopharmaceutical agents. The importance of transition metal complexes can be revealed in catalysis, material synthesis, biological systems and photochemistry. They may be attached to the metal through a single atom (monodentate) or bound to the metal through two or more atoms (bidentate or polydentate). Polydentate ligands are also called chelating agents [2].

History

In the year 1965 Rosenberg *et.al* serendipitously discovered the anticancer properties of cisplatin, cis-$[PtCl_2(NH_3)_2]$. Cisplatin approved by North American Food and Drug Administration in the USA (FDA), is still one of the world's best selling anticancer drugs and is serving over the past 30 years. It is used for the cure of over 90% of cases of testicular cancer and it plays an important role in some cancer treatments such as ovarian, head and neck cancer, cervical cancer, bladder cancer, melanoma, and lymphomas. However, for some tumours cisplatin may produce acquired immunity during the treatment thus limiting its use. The adverse effect associated with cisplatin includes nausea, vomiting, bone marrow suppression and kidney toxicity. Current research on metal complexes is directed partly towards the development of complexes that kill the types of cancer cells that have an inherently same response to current chemotherapy, and partly to drugs whose main aim is to diminish the severe side effects as in cisplatin.[3-7]

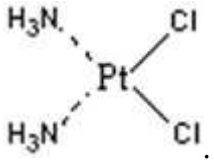


Fig. Cisplastin

The effectiveness of cisplatin is limited due to its severe toxicity, resistance to the drug, and unwanted side effects[8–12]. Moreover, this has led to the search for complexes of other transition metals with interesting biological properties[13–24], wider ranges of activity, and lower systemic toxicities.

Ruthenium complexes are increasingly gaining interest as potential alternatives to platinum-based chemotherapeutic agents. Cancers not readily treated by cisplatin [25], some ruthenium based complexes have been shown to be effective. Among all ruthenium-based anticancer agents, ruthenium-DMSO complexes are believed to have greater potential because of their selectivity for solid tumor metastases and lower host toxicity[26].

Several ruthenium complexes have been found to display a significantly higher degree of selectivity towards cancerous cells than the leading commercially available platinum derived drugs, which results in reduced damage to healthy tissues.[27]

Development of a new anticancer agent

The development of a new anticancer agent is a multi-stage process and includes steps such as synthesis, characterization, and proof of biological activity, pre-clinical and clinical screenings [28]. Testing for the biological activity requires the measurement of the biological effect in *in vitro* (in the cancer cell lines) and *in vivo* (in animals) screens.

There are three methods to develop new tumor-inhibiting complexes[29]

1. through the synthesis of derivatives of cisplatin,

2. Synthesizing tumor-inhibiting non-platinum complexes,

3. Synthesizing the platinum complexes linked to carrier systems that have the ability to accumulate the drug in organs and tissues.

The first method seems to be less promising because it will lead to drugs that will be quite similar to cisplatin. However, to reduce the toxic side effects in comparison to the parent compound, attempts can be made, as made in the development of carboplatin, or change the tumor selectivity, as in the case of oxaliplatin. The second method aims at preparing compounds with a central heavy metal ion other than platinum. Owing to different chemical properties of the metal range of activity thus can be changed. However, this is one of the most difficult and risky approach of scientific research compared to the first strategy but the opportunities for a breakthrough are greater. In the third method, the platinum complex is linked to carrier molecules, which is known as drug targeting. The aim of this approach is to synthesize a platinum drug that possesses high selectivity towards malignant cells. It can be done for tumors containing biochemical targets different in structure or quantity from the normal tissues.

The major problem in the development of anticancer drugs is the large leap from the preclinical *in vitro* and *in vivo* studies to clinical trials. The cause for this is the great difference between the experimental animal models and the individual patient tumors, making the therapeutic situation of the cancer patients much more complex.[30]

Theoretical advantages in using ions of transition metals other than platinum include:

A. Having an additional co-ordination site in octahedral and altered shape of the complex

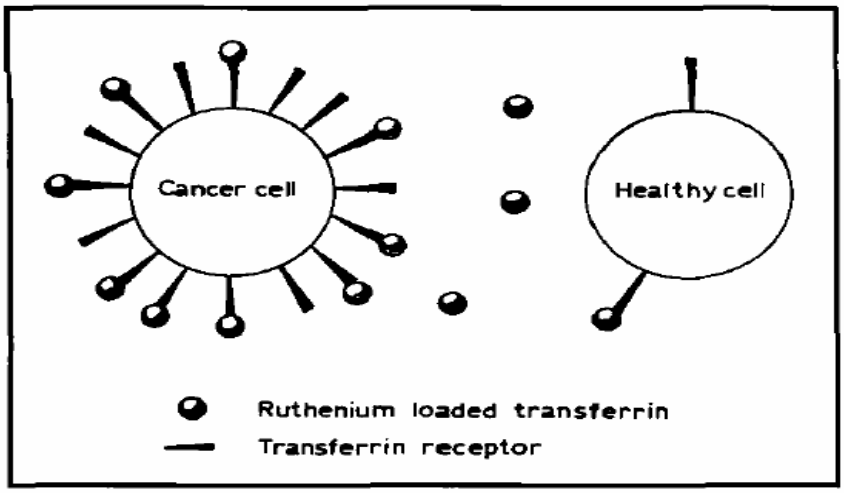
B. Alterations in ligand affinity and substitution kinetics,

C. Changes in oxidation state,

D. Photodynamic approaches to therapy.

Ruthenium complexes

An interesting alternative to platinum is the development of anticancer drug ruthenium having three oxidation states Ru(II), Ru(III) and Ru(IV), which are all accessible under physiological conditions. One of the earliest reports about the antitumor activity of ruthenium complexes dates back to 1970 and 1980s[31,32]. An advantage of ruthenium compounds is their less toxicity compared to platinum counterparts[33]. As like platinum, ruthenium has the ability to mimic iron in binding to specific molecules such as albumin and transferrin. As in cancer cell there is always an increase in the demand for iron, the transferrin receptors are thus over expressed and therefore the delivery of the ruthenium drugs can be enhanced[34].



Three most important advantages of using ruthenium complexes as anticancer agents are

1) The well-developed coordination chemistry,

2) Rates of ligand exchange reactions comparable to that of platinum, and

3) The octahedral coordination geometry[35].

[HIm]- Trans-[RuCl4(DMSO)(Im)] (NAMI-A) (see Fig. 1) is the first representative of ruthenium (III) complexes undergoing clinical trial. This complex developed by Sava et al. in Trieste, possesses outstanding antimetastatic properties [36]. Secondly, the complex [HInd] trans-[RuCl4(Ind)2] (KP1019, FFC14a) (see Fig. 1) was chosen among several ruthenium(III) compounds prepared and evaluated for their potential anticancer activity and is currently undergoing clinical trial. Indeed, KP1019, developed by Keppler et al., shows very encouraging antitumor properties[37].

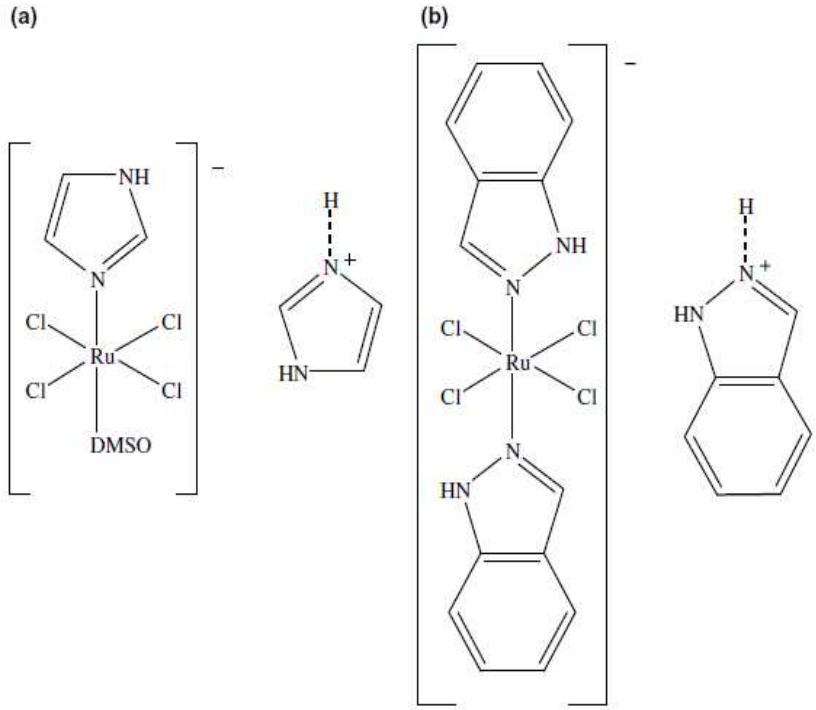
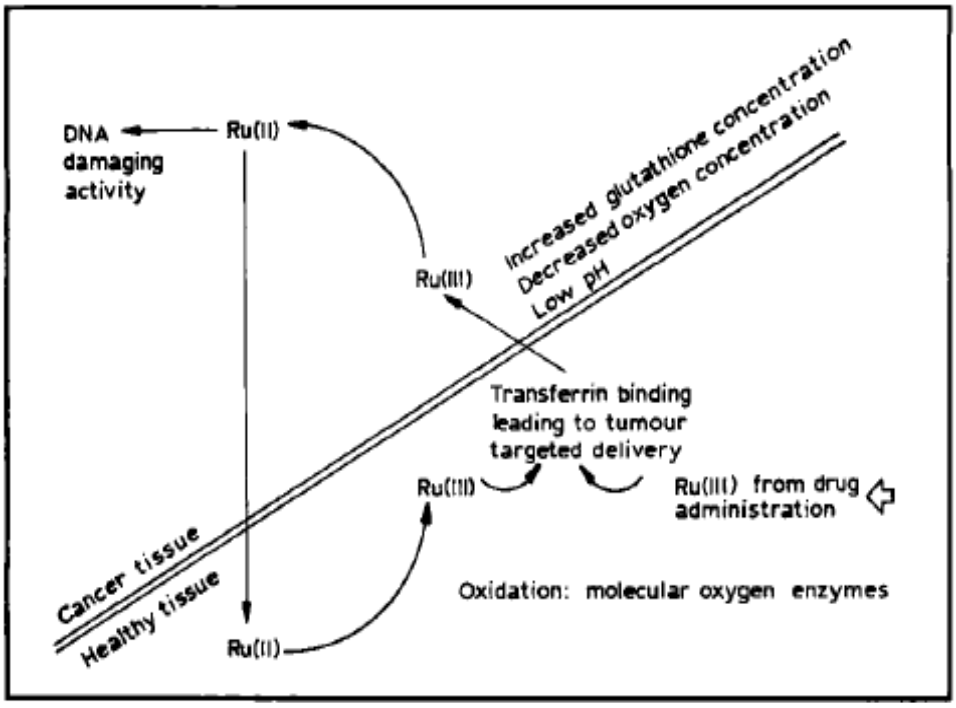


Fig. 1. Schematic drawing of (a) HIm *trans*-[$RuCl_4$(DMSO)(Im)] (NAMI-A) and (b) HInd *trans*-[$RuCl_4(Ind)_2$] (KP1019, FFC14a).

Solid tumors have lower oxygen level than normal cells and this result in conversion of ruthenium (III) complex to ruthenium (II) by reduction. And it is observed that compound activated by reduction result in lower toxicities[38].



Proper oxygen conditions are required in the solid tumors for chemotherapy to be effective. Moreover, reduction is facilated by the presence of reducing species such as glutathione and low extracellular pH. Thus, an appropriate redox potential is important for ruthenium (III) complexes than their cytotoxic properties alone[39].

By coordination geometry ruthenium complex are distinctly different from cisplatin and in principle, they are capable of interacting with DNA.

Ruthenium is similar to iron in certain aspects of its coordination chemistry and redox activity and it might target iron dependent mechanisms. It fits into the active centre of transfferin. The cytotoxic potency correlates well with the redox potential. Activation of caspase 3 causes apoptosis and is detectable 2-6 hour after exposure, an enzyme involved in apoptosis. Another complex, KP1339, activates caspase 7.

Synthesis of transition metal complexes

The reactions were generally done at room temperature either with water as a solvent, ethanol or acetone and by placing the reaction mixture in refrigerator. The ratios of ligand to metal were 2:1 in almost all complexes.

E.g: - Synthesis of ruthenium-pyrazole complexes

A) Synthesis of mer-[$RuCl_3$ (DMSO-S)$_2$(DMSO-O)(pyz)]

mer-[$RuCl_3$(DMSO-S)$_2$ (DMSO-O)] (0.1 g, 0.23 mmol) and pyrazole (0.017 g, 0.25 mmol) were dissolved in dichloromethane (10 mL) and stirred at room temperature for 20 h. The solution was evaporated to 3 mL and 10 drops of hexane were added. The flask was kept in a refrigerator. The resulting solution yields red crystals (yield 0.184 mmol, 80%). Anal. Calcd. for $C_7H_{16}C_{l3}N_2O_2RuS_2 \cdot CH_2C_{l2}$ (516.68) C,18.6; H, 3.5; N, 5.4. Found: C, 18.87; H, 3.82; N, 5.11. Selected IR absorption bands in KBr (cm-1): νSO1103, (S-DMSO), 909 (O-DMSO), νC=C (pyz) 1627, νC=N (pyz) 1409. UV–vis (H_2O): 386 nm (ε=27.11 M-1 cm-1); 525 nm (ε=8.34 M-1 cm-1).

B) Synthesis of mer-[$RuCl_3$(bpy)(dmpyz]

The aim was to synthesize mer-[$RuCl_2$(bpy)(DMSO-S) (dmpyz)]CF_3SO_3 by refluxing mer-[$RuCl_3$(DMSO-S)(bpy)] with equimolar amounts of silver triflate and an excess amount of 3,5-dimethylpyrazole. However, the X-ray structure revealed that the isolated product is mer-[$RuCl_3$ (bpy)(dmpyz)] 3, not what we expected. Compound 3 was also synthesized by the direct reaction of mer- [$RuCl_3$(DMSO-S)(bpy)] with an excess amount of 3,5-dimethylpyrazole. mer-[$RuCl_3$(DMSO-S)(bpy)] (0.1 g, 0.23 mmol), 3,5-dimethylpyrazole (0.167 g, 1.74mmol) and acetone (35 mL)were placed in a flask,which was heated to reflux for 24 h. The volume of solution was reduced to 3 mL and 10 drops of hexane were added. On cooling at 4°C the resulting solution yields orange crystalline solid (yield 0.156 mmol, 68%). Calcd. for $C_{15}H_{16}Cl_3N_4Ru$ (459.74) C, 39.2; H, 3.5; N, 12.2. Found: C, 38.8; H, 3.41; N, 11.81. Selected IR absorption bands in KBr (cm-1): νC=C, 1573 (m), νC=N, 1448 (s) (pyz), νC-N (bpy) 1607 (m), UV–vis (H_2O): 435 nm (ε=47.89 M-1 cm-1); 375 nm (ε=8.29M-1 cm-1). Unfortunately, our attempt to synthesize the similar complex with pyrazole ligand was unsuccessful.

C) Synthesis of mer-[$RuCl_3$(DMSO-S)(dmpyz)$_2$]

A solution of mer-[$RuCl_3$(DMSO-S)$_2$(DMSO-O)] (0.2 g, 0.45 mmol) and 3,5-dimethylpyrazole (0.334 g, 3.48 mmol) in dichloromethane (15 mL) was heated to reflux for 9 h. The brown solution was evaporated to 3 mL and then 1 mL of hexane was added. On cooling at 4°C a reddish-orange solid was separated (yield 0.262 mmol, 58%). Product was purified by column chromatography with ethyl acetate and hexane (40/60) as eluent. Anal. Calcd. $C_{12}H_{20}Cl_3N_4ORuS \cdot H_2O$ (495.83): C, 29.06; H, 4.95, N, 11.35. Found: C, 29.18.; H, 5.24; N, 10.95. Selected IR absorption bands in KBr (cm-1): νSO1086 (S-DMSO), νC=C (pyz) 1632, νC=N (pyz) 1398. UV–vis (H_2O): 362 nm (ε=12.20 M-1 cm-1) [40].

Characterisation of transition metal complexes

The complexes were characterized using available analytical methods, such as-

1) IR and UV-Vis spectroscopy

2) HPLC chromatography was performed for all complexes.

3) For some complexes X-ray crystallography could be performed and also for these complexes LC-MS studies were done.

4) The LC/MS studies were performed in search of the molecular weights of the complexes

The purpose of the study was to check the stability of the complex in aqueous solution and to determine whether its stability is affected by the presence of thiols such as glutathione, which are found in relatively high concentrations in cancer cells. The investigation of their potential stability under biological conditions was undertaken in the stability studies at 37 °C over 24 h.

Characterisation and analysis

The ruthenium compound has been characterised by various analytical methods (NMR, mass spectroscopy, elemental analysis). Elemental analysis (%) for $RuCl_2C_{26}H_{24}N_4O_2\_2CH_3COCH_3$ (acetone from crystallisation): Expt. (Calc.) C, 53.50 (53.93); N, 7.84 (7.86); H, 5.24 (5.09) %. ESI-MS measurements of a freshly prepared solution of crystalline compound in methanol–water (80:20 v/v) showed major peaks at m/z = 560.88 and 524.91 with a ruthenium isotopic pattern, corresponding to the following species, [1-Cl^-] and [1-$2Cl^- + H^+$], respectively[40].

The hydrolysis rate was determined, capillary electrophoresis studies showed in a competitive reaction between KP1019 and the four 50-nucleotide monophosphates primarily formation of GMP and AMP adducts, and CD as well as ESI-MS investigations gave insight into the stoichiometry of specific binding between KP1019 and transferrin[41-43].

The primary tumors were sensitive to ruthenium complex and it caused apoptosis via the mitochondrial dependent pathway and probably acts as a pro-drug. The complex can react with transferrin in the blood and is then released from transferrin at acidic pH of the cellular endosomes.

NAMI-A is unique compared to cisplatin. In contrast to cisplatin the compound in in-vitro shows almost no cytotoxic activity while in vivo it reduces the metastatic potential without affecting the primary tumor[44-46]. On the other hand, KP1019 showed cell growth inhibitory effects in the colorectal cancer cell lines SW480 and HT29. Activity is superior to 5- fluorouracil in experimental therapy of colorectal carcinoma in rat [47, 48]. This compound was also tested in human cancer cell lines such as LCLC-103H, A-427, 5637, MCF-7, RT-4 and DAN-G. However, in these cell lines the agent was found inactive.

Application

- Ruthenium complexes have less adverse affects and potential antitumor activity.
- Cancers not readily treated with cisplatin, some ruthenium complexes have found to be effective.
- Three main properties that make ruthenium compounds well suited to medicinal applications are:

(i) Rate of ligand exchange.

(ii) The range of accessible oxidation states and

(iii) The ability of ruthenium to mimic iron in binding to certain biological molecules.

Conclusion

Organmetallic complexes provide versatile platforms for drug design. These complexes offer the possibility of novel mechanisms of action compared to purely organic drugs and have the potential for combating drug resistance as well as treating currently intractable conditions**.** Low toxicity makes the ruthenium complex important in clinical use. This is due to the ability of ruthenium to mimic the binding of iron to bimolecular, exploiting the mechanisms that the body has evolved for nontoxic transport of iron. Oxidation and reduction reaction in our body is catalysed by the redox potential between the different accessible oxidation states occupied by the ruthenium, depending on the physiological environment. It is also true for other disease condition as in cancer, the biochemical changes that accompany disease alter the physiological environment, enabling ruthenium compounds to be selectively activated in diseased tissues. These two features makes the

ruthenium drugs exhibit low toxicity compared to other platinum metal compounds and thus makes drug promising in the clinical.

Future ruthenium drugs

- Highly promising anticancer activity in cells, animals and humans have been showed by ruthenium compounds. To date, two compounds are being evaluated in phase II clinical trials.
- However, a major limitation in ruthenium drug discovery is their unknown mode of action. This leads to possible strategies for future ruthenium drug design.
- More research could be directed at eliciting the mode of action of existing ruthenium compounds. Then, armed with detailed information about how ruthenium behaves in biological media – what it prefers to bind to, how it gets into cells etc – chemists can begin to design ruthenium drugs which have a high affinity for cancer targets, with far less severe side effects.

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