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Classical and Non-Classical Ruthenium-Based Anticancer Drugs: Towards Targeted Chemotherapy

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Ruthenium-based anticancer chemotherapies are making significant advances in clinical trials. Until recently, the focus has been on coordination complexes, and mechanisms such as "activation by reduction" and "transferrin-targeted delivery" have been proposed to account for the excellent cytotoxicity and low general toxicity of these complexes. More recently organoruthenium compounds, which to some extent appear not to follow the established rules, have started to be investigated. Despite such differences, similar activities between certain coordination and organometallic compounds suggest similar modes of action are present. DNA, the classic target, is believed to be the dominant mechanism for cytotoxicity with certain ruthenium drugs, while with others, non-classical targets are thought to be more important. In this article we describe these features and show how both ruthenium coordination complexes and organoruthenium compounds represent an ideal scaffold for further drug design and optimisation.

Introduction

The landmark discovery of the antitumoural properties of *cis*-diamminedichloroplatinum(II) (*cisplatin*), by Rosenberg in 1965, heralded a new era of anticancer research based on metallopharmaceuticals.[1] To date, cisplatin and its analogues are some of the most effective chemotherapeutic agents in clinical use (see Figure 1), often as the first line of treatment in testicular and ovarian cancers.[2,3] However, they are not without their problems. In particular, their high toxicity and incidence of drug resistance, acquired or intrinsic, remain the main challenges in their clinical application.[4] These limitations have provided the motivation for alternative chemotherapeutic strategies. For example, *satraplatin* is a platinum(IV) prodrug, that could be orally administered, and is reduced by intracellular biomolecules to yield cytotoxic platinum(II) moieties once inside the cell.[5] Platinum(IV) compounds with functionalised ligands have also been harnessed to defeat glutathione-S-transferase-mediated drug resistance, to target estrogen receptor-positive breast cancer and as a pro-drug for photo-chemotherapy (see Figure 1).[6] There are also a number of platinum(II) compounds that target aspects of cancer cells to enhance uptake and improve selectivity.[7] At the same time, the search for effective anticancer compounds based on other metal centres has intensified. In particular, anticancer drugs based on ruthenium have gained significant prominence.

Ruthenium is an attractive alternative to platinum: besides the rich synthetic chemistry, ruthenium has a range of oxidation states (Ru^{II}, Ru^{III} and Ru^{IV}) accessible under physiological conditions, which is unique among the platinum-group metals.[8] This feature is significant since the activities of most metal-based anticancer drugs are dependent on their oxidation states. In addition, ruthenium compounds are known to be less toxic than their platinum counterparts.[8–10] This is believed to be due to the ability of ruthenium to mimic iron in the binding to biological molecules, such as albumin and transferrin, although plati-

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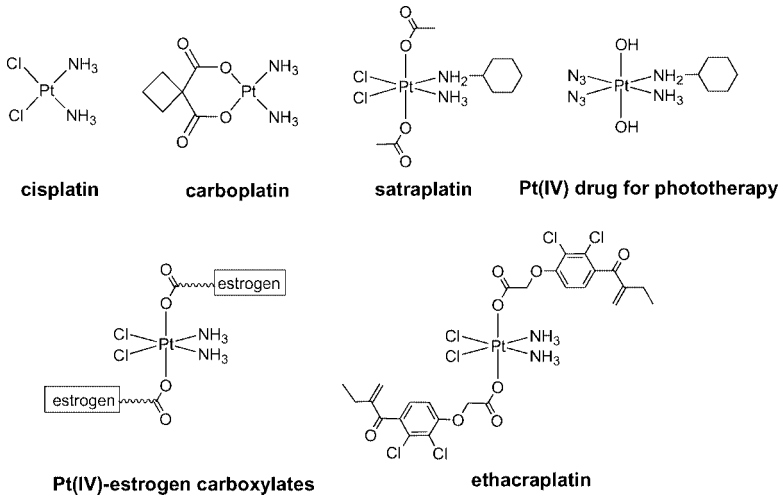


Figure 1. Clinical and preclinical platinum-based anticancer drugs.

num drugs can also bind to these proteins. Since rapidly dividing cells, such as cancer cells, have a greater demand for iron, transferrin receptors are over-expressed, thereby allowing ruthenium-based drugs to be more effectively delivered to cancer cells.[9,10] In addition, the "activation by reduction" mechanism could also account for the lower general toxicity of some ruthenium compounds.[9] Two ruthenium-based anticancer drugs, namely NAMI-A and KP1019 (see Figure 2), have successfully completed phase 1 clinical trials and are scheduled to enter phase 2 trials in the near future.[11–13]

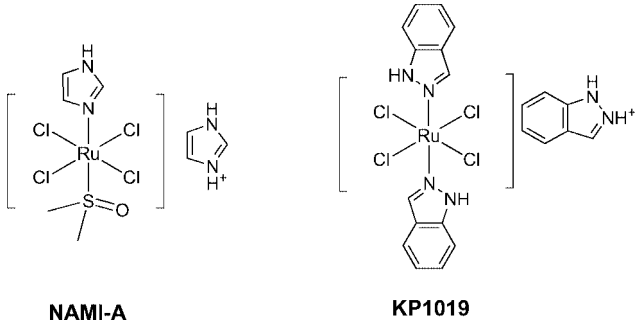


Figure 2. Ruthenium-based anticancer drugs under clinical evaluation.

In the past two decades, a new approach to treating cancer, known as targeted therapy, has started to take root.[14] The new strategy involves targeting cellular signalling pathways of cancer cells, yielding highly effective cancer treatments with much reduced severe side effects.[14,15] A few of them, e.g. Imatinib mesylate and Erlotinib hydrochloride (see Figure 3), have demonstrated such potential that their approval processes were fast-tracked by the US Food and Drug Administration. Eventually, targeted therapies could become mainstream as the first line therapeutic options, replacing existing classical anticancer drugs such as 5-fluorouracil and cisplatin. It is therefore worthwhile to consider if the existing approaches being used to develop ruthenium-based anticancer drugs are adequate.

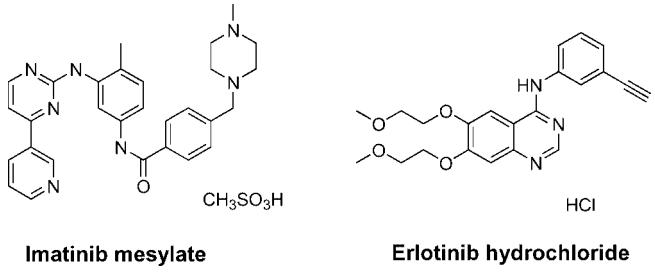


Figure 3. Drugs for targeted therapy in clinical use.

Classical versus Targeted Chemotherapy

Classical (or conventional) chemotherapy, which originated in the 1950s, refers to drugs interfering with replication and mitotic processes of tumour cells.[14] Historically, these were the main "target" of chemotherapy, since cancer biology was not well established at that time and little was known about the causes of cancer and its mechanism, so the general therapeutic strategy was largely premised on the fact that cancer cells replicate their DNA more frequently than normal cells and hence are more susceptible to DNA damage. Examples of cytotoxic substances include alkylating agents, mitosis inhibitors and topoisomerase inhibitors. In recent years, with the advent of molecular oncology, the study of cancer at the molecular level has been made possible.[14] The discovery of receptors and growth factors, such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), cyclin-dependent kinases (CDK) that are upregulated in cancer cells, provides new "targets" for cancer therapy.[15,16] Whereas the strategy of classical chemotherapy relies on damaging cancerous cells more than normal cells, targeted therapies are far more specific and their toxicity profile more manageable. Important classes of target therapeutic agents include monoclonal antibodies that interfere with the activities of EGFR, VEGF or proteasome, small molecule inhibitors of the tyrosine ki-

nase receptor (which includes EGFR), as well as immunotherapeutic agents that trigger an immune response against CD20 antigens.[15,17]

The main drawback of classical chemotherapy strategies is that by blocking the metabolism of rapidly dividing malignant cells, it inadvertently inflicts damage on healthy cells that divide frequently, such as hair follicles, bone marrow and cells lining of the gastrointestinal tract. Consequently, there are associated side effects, such as hair loss, anaemia and neutropenia, that are often severe and limit the treatment options.[18] In contrast, targeted therapies focus on specific cellular signalling pathways on which the cancer cells depend for growth, proliferation, metastasis and angiogenesis, and are therefore much more selective.[15] It is of no doubt that the centre of efforts in anticancer research has shifted towards the latter approach, and while targeted therapies are still in their infancy, they offer considerable potential.[14,16]

Despite the potential of targeted therapies, classical chemotherapeutic drugs, like cisplatin, remain the most effective and widely available drugs in use. This is because classical chemotherapeutic approaches target the most fundamental aspect of cancer cells, their rapidly dividing nature, whereas targeted approaches are contingent on discovering unique and specific features of particular cancer cells. As it stands, most targeted therapeutic drugs are only effective in fairly specific types of cancer, e.g. Imatinib mesylate for chronic myelogenous leukaemia, Erlotinib for advanced non-small cell lung cancer, etc. This limits their applicability and most common cancers cannot be treated with "targeted" chemotherapeutic agents, although this is expected to change in the future.

In recent years, ruthenium-based drug research appears to be moving away from classical approaches into the realm of "non-classical" ruthenium-based drugs. It is useful to take stock of the developments so far, drawing on the lessons learnt with respect to drug design and development. In this review, we explore the major classes of classical and non-classical ruthenium-based drugs and highlight the principal and emerging development strategies.

Classical Ruthenium Anticancer Drugs

Polypyridyl-Ru-Complexes

Polypyridyl-Ru systems have been exploited extensively as molecular DNA probes, given their photoluminescence properties and the ability of polypyridyl ligands to intercalate DNA. The large, rigid, multidentate polypyridyl ligands confer shape and chirality to the ruthenium complexes that could be exploited to achieve customised DNA-binding properties. This has provided the motivation to develop polypyridyl-Ru complexes as DNA-targeting anticancer agents, and a large number of these complexes have been screened for anticancer activity. Typical polypyridyl ring ligands include 2,2′-bipyridine (bpy), 1,10-phenanthroline (phen) and 2,2′:6′2′′-terpyridine (terpy) as they are commercially available and readily form stable complexes with ruthenium (see Figure 4). Some of the earliest polypyridyl-Ru complexes studied for potential anticancer properties include *cis*-[Ru(bpy)$_2$Cl$_2$] (both Δ and Λ enantiomers) and *mer*-[Ru(terpy)Cl$_3$]. In vitro, *mer*-[Ru(terpy)Cl$_3$] was significantly more cytotoxic (L1210, HeLa) than both enantiomers of *cis*-[Ru(bpy)$_2$Cl$_2$], which matches the in vivo data (BALB/c × C$_{57}$BL/6 female mice).[19] This trend correlates to the ability of *mer*-[Ru(terpy)Cl$_3$] to form DNA-interstrand crosslinks, whereas the inactive *cis*-[Ru(bpy)$_2$Cl$_2$] appears to exhibit no such interactions.[19,20] There have also been numerous examples of polypyridyl-Ru complexes comprising one or more [Ru(bpy)$_2$L]$^{2+}$ or [Ru(phen)$_2$L]$^{2+}$ units [where L = derivatised quinolines, 2,6-(2′-benzyimidazolyl)pyridine/chalcone, aryldiazo-β-diketonate, 4-substituted thiosemicarbazides, 4-substituted thiopicolinanalides, 2-phenylazoimidazole, etc.] in an attempt to improve the DNA-intercalating ability of the complex.[21] However, it is not clear if there has been any serious attempt to advance these compounds into clinical trials. A series of DNA-binding ruthenium(II) complexes with tetradentate cyclam rings and varying DNA-intercalating quinonediimide ligands were studied in vitro (KB-3–1, KB-V1). It was noted that cytotoxicity was linked to the ability of the quinonediimide ligands to intercalate, although the IC$_{50}$ values were considered too high to be of interest.[22] Reedijk et al. reported an example of a NO-containing polypyridyl-Ru complex that readily liberates NO upon irradiation under a mercury lamp and that could potentially be applied in phototherapy.[23] The complex *cis*-(Cl,Cl)-[Ru^{II}(terpy)(NO)Cl$_2$]Cl exhibited good cytotoxicity towards A2780 human ovarian carcinoma cell lines, significantly higher than that of *mer*-[Ru(terpy)Cl$_3$], cisplatin or carboplatin, although their activity under irradiation was not reported. Harding et al. reported the synthesis of ruthenium analogues of streptonigrin, a DNA-targeting antitumour antibiotic.[24] On the basis of structural activity studies, the investigators identified the key structures responsible for their activity and synthesised quinolinide and bipyridine ligands to mimic the active sites. Subsequent dicarbonyl(dichloro)ruthenium(II) complexes [Ru(CO)$_2$Cl$_2$L] (where L = quinolinide and bipyridine mimics of streptonigrin) were prepared, although it appears that no further in vitro studies were undertaken. More recently,

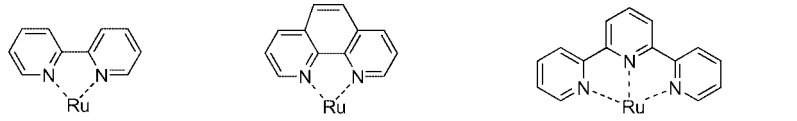


Figure 4. Polypyridyl ligands used in ruthenium anticancer complexes.

a heteronuclear (Pt, Ru) complex, comprising a Ru(terpy) moiety, with a highly flexible bridging chain was developed (see Figure 5).[25] The Pt end was designed to bind directly to DNA, leaving the Ru(terpy) end to intercalate, thereby providing additional anchor support. Ultimately, this type of hybrid complex could provide the basis for customised DNA-targeting agents that could form long-range DNA adducts.

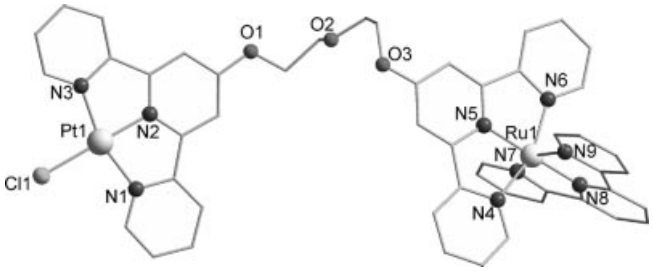


Figure 5. Ball and stick representation of a heteronuclear (Pt, Ru) complex that potentially coordinates *and* intercalates DNA.

Ru-Polyaminocarboxylate Complexes

The use of polyaminocarboxylate (pac) ligands in metallopharmaceutical applications is of growing interest not only because of their ability to bind strongly with metal centres but also because their amino and carboxylate binding entities are akin to those in biological systems.[26] One of the earlier pac-Ru complexes evaluated for anticancer activity is the highly water-soluble dichloro(1,2-propylenediaminetetraacetate)ruthenium(III), Ru(pdta)Cl_2 (see Figure 6), which demonstrated antitumoural activity in vivo (EAT, L1210, P388, MX-1, M5076) with low systemic toxicity.[27] The presence of the two chloride ligands in the *cis*-conformation was found to be an important feature of its biological activity; under physiological conditions, the chlorides rapidly hydrolyse.[28] Ru(pdta)Cl_2 was found to alter the DNA conformation in pHV14 DNA and inhibit DNA lysis by restriction enzymes.[29] The structurally similar K[Ru^{III}(eddp)Cl_2] complex (where eddp = ethylenediamine-*N*,*N*-di-3-propionate) also displayed cytotoxicity in vitro (HeLa, BT-20, HT-29) and was found to induce DNA cleavage.[30] A related compound, K_2[Ru^{III}(dmgly)Cl_4] (where dmgly = *N*,*N*′-dimethylglycine), was reported to be cytotoxic towards the murine C6 astrocytoma cell line but not towards primary rat astrocytes, further demonstrating the selective toxicity of ruthenium complexes towards cancer cells.[31] Another class of pac-Ru complexes that has been extensively studied, K[Ru^{III}(pac)Cl], contains ethylenediaminetetraacetatic acid and its derivatives as the pac ligands (see Figure 6).[26,32] Like Ru(pdta)Cl_2, these complexes also rapidly hydrolyse to yield Ru(pac)(H_2O) species at low pH. In vitro cytotoxicity against tumour cell lines (MCF-7, NCI-H460, SF-268) showed K[Ru^{III}(pdta)Cl] and K[Ru^{III}(edta)Cl] to be more efficacious inhibitors of cell growth, presumably because of their higher reactivity towards nucleotides.[26,32,33] In addition, several of these complexes were found to be effective NO scavengers and protease inhibitors, thus they could be used to treat various diseases or serve as antiviral agents.[34]

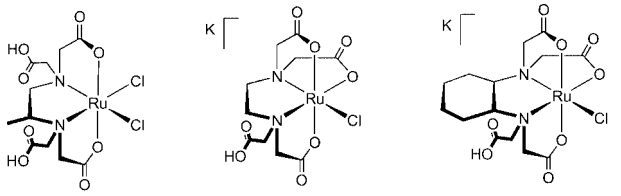


Figure 6. Examples of Ru-pac complexes investigated for anticancer activity (cdta = cyclohexane-1,2-*trans*-diamine-*N*,*N*,*N*′,*N*′-tetraacetate).

Dimethyl Sulfoxide-Ru Complexes

As early as 1988, *cis*- and *trans*-Ru(DMSO)$_4X_2$ (where X = Br, Cl) were screened for antitumour activity (C75B1/BD2F1 female mice with lung carcinoma) and *trans*-Ru(DMSO)$_4Cl_2$ was 20-fold more active against metastasis than its *cis* counterpart (see Figure 7).[35,36] In leukaemic mice, the complexes were able to prolong the lifespan of the host without affecting the number of tumour cells.[35,37] No further development of *cis*- and *trans*-Ru(DMSO)$_4Cl_2$ was reported, presumably in favour of NAMI-A which was found to be a strong antimetastatic agent by the same investigators. However, analogues of *cis*- and *trans*-Ru(DMSO)$_4Cl_2$ containing chelating DMSO ligands such as bis(methylsulfinyl)ethane, bis(ethylsulfinyl)ethane (BESE), bis(propylsulfinyl)ethane and bis(methylsulfinyl)propane have since been reported and studied in vitro (CHO).[38] The compounds were found to be non-cytotoxic under aerobic and hypoxic conditions, despite accumulating significantly within the cellular DNA. *cis*-Ru(DMSO)$_4Cl_2$, in particular, has been used as a synthon for numerous compounds such as *cis*,*cis*,*trans*-RuL_2(DMSO)$_2Cl_2$ (where L corresponds to cytotoxic nitrofurylsemicarbazone ligands), although the conjugated products did not offer improved cytotoxicity (MCF-7, TK-10, HT-29).[39] Similarly, complexes with derivatised DMSO, *cis*,*cis*,*trans*-RuL_2("DMSO")$_2Cl_2$ and *cis*-Ru("DMSO")$_2$(mal)$_2$ type compounds (where L = metroidazole and mal = maltol or ethylmaltol), show no advantageous improvement in cytotoxicity (MDA-MB-435S).[40] *cis*-Ru(DMSO)$_4Cl_2$ has been used to prepare heterodinuclear Pt–Ru compounds crosslinked by 1,4-diaminobutane, i.e. [{*cis*,*fac*-RuCl_2-(DMSO)$_3$}($NH_2(CH_2)_4NH_2$){*cis*-Pt(NH_3)Cl_2}], with a view to develop a compound that could crosslink DNA–DNA or DNA–protein structures (see Figure 7).[41] On the basis of DNA-binding studies, it was demonstrated that the heterodinuclear compound could form specific DNA lesions that could cross-link proteins to DNA, but its sensitivity to light and rapid hydrolysis prevented further evaluation.[25,42] More recently, both *cis*- and *trans*-Ru(DMSO)$_4Cl_2$ were investigated for their phototoxicity under UVA illumination.[43] Both complexes exhibited photodependent cytotoxicity against human and murine mela-

noma cell lines (SK-MEL 188, S91) – the *trans* isomer was more photocytotoxic. In addition, their reactivity towards oligonucleotides was found to be greatly accelerated through a photochemical pathway when irradiated by UVA.

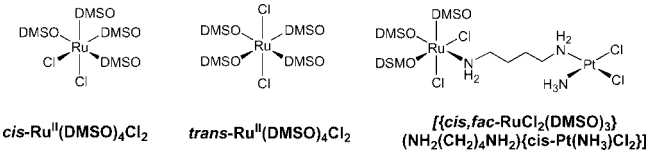


Figure 7. Examples of DMSO–ruthenium compounds evaluated for antitumour activity.

Ruthenium Arylazopyridine Complexes

Arylazopyridine ruthenium(II) complexes, $Ru(azpy)_2Cl_2$ (where azpy = 2-phenylazopyridine), represent a class of well-characterised anticancer compounds with a strong structural dependency. Although these complexes can exist in up to five different isomeric forms, arising from the lack of a twofold symmetry axis, only three have been reported, namely the α, β and γ isomers, and their structures unequivocally established by X-ray crystallography (see Figure 8).[44,45] The structural characteristics have a significant impact on the efficacy of the compounds as cytotoxic agents. Reedijk et al. reported that the high cytotoxicities of the α and γ isomers in vitro (A498, EVSA-T, H226, IGROV, MCF-7, WIDR, M19) were comparable to those of cisplatin and 5-fluorouracil and is about 10-fold higher than that of the corresponding β isomer. This result is surprising since the α and β isomers are structurally similar and differ only in the orientation of the azpy ligand.[45,46] The addition of methyl groups to either the pyridine or phenyl moiety, as in $Ru(tazpy)_2Cl_2$ and $Ru(mazpy)_2Cl_2$ (tazpy = *o*-tolylazopyridine and mazpy = 4-methyl-2-phenylazopyridine), did not alter the trend, validating the structure–activity relationship of the isomers on cytotoxicity.[46] DFT calculations suggest that the ability of the $Ru(mazpy)_2Cl_2$ isomers to intercalate to DNA decreases from γ > α > β isoforms on the basis of the geometric and electronic factors, which correlates with the observed cytotoxicity.[47] The γ isomer has the most preferential geometric arrangement of the mazpy ligand for DNA intercalation, as well as the lowest LUMO energy level and smallest HOMO–LUMO energy gap. Accordingly, it is the most reactive towards DNA. A mixed ligand analogue, *cis*-Ru(bpy)(azpy)Cl_2, which is structurally similar to α-$Ru(azpy)_2Cl_2$, was found to be 2–10 fold more cytotoxic in vitro (A498, EVSA-T, H226, IGROV, MCF-7, WIDR, M19) than *cis*-$Ru(bpy)_2Cl_2$ but much less cytotoxic (>50-fold) than either α- or β-$Ru(azpy)_2Cl_2$.[48] The replacement of the chloride ligands with a 2,2′-bipyridine group, as in $[Ru(bpy)_n(azpy)_{3-n}](PF_6)_2$, also did not offer any significant advantage over the parent α-$Ru(azpy)_2Cl_2$.[49] Some water-soluble derivatives of the α isoform, where the chloride ligands are replaced by bridging carboxylate ligands, e.g. oxalate, malonate or 1,1-cyclobutanedicarboxylate, overcome one of the main limitation of these complexes.[50] Although the compounds are 5–10 fold less cytotoxic than α-$Ru(azpy)_2Cl_2$ (A2780, A2780cisR) and slightly less cytotoxic than cisplatin, its cytotoxicity is comparable to that of carboplatin (see Figure 9). Another water-soluble derivative $[NEt_4]_2[Ru(sazpy)_2Cl_2]$ (where sazpy = 2-phenylazopyridine-5-sulfonate), which contains a sulfonate functionality on the azpy group,[51] was >100-fold less cytotoxic (A2780, A2780cisR) than α-$Ru(azpy)_2Cl_2$. More recently, dinuclear analogues with bridging azpy ligands, comprising two azpy units joined at the *para* position of the phenyl rings by a bridging methylene group, have been reported.[52] The supramolecular complexes each contain two $Ru(azpy)_2Cl_2$ moieties arranged in either the α or γ isoforms. Three isomers have been isolated that contain either α/α-, α/γ- or γ/γ- "$Ru(azpy)_2Cl_2$" units and their structures have been confirmed by X-ray crystallography (see Figure 9). The α/γ and γ/γ isomers were tested in vitro on cancer and non-tumourgenic cell lines (T47D, HBL-100) with the γ/γ isoform exhibiting the highest cytotoxicity – >30-fold higher than that of cisplatin.

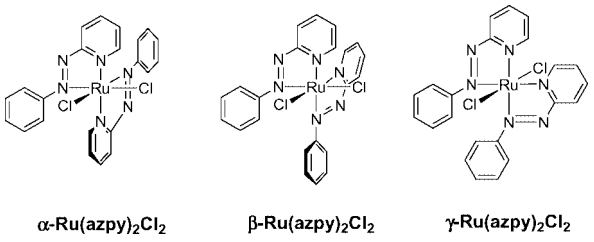


Figure 8. Different isomeric forms of $Ru(azpy)_2Cl_2$ complexes.

Organometallic Arene–Ruthenium-Complexes

Organometallic ruthenium complexes bearing η^6-arene ligands have been investigated extensively as potential anticancer drug candidates. One of the earlier examples is (η^6-benzene)Ru(DMSO)Cl_2 (see Figure 10), which has been shown to strongly inhibit topoisomerase II activity by cleavage complex formation.[53] The authors suggested that the ruthenium complex interacts with DNA and forms cross-links with topoisomerase II. The complex exhibited antiproliferative activity in vitro (Crit-2), but it is inconclusive if there is a direct link to its ability to inhibit topoisomerase II activity. Arene–ruthenium complexes containing BESE, which is essentially a bidentate "DMSO" ligand, have been tested in vitro (MDA-MB-435s), but their cytotoxicities were more than 5-fold higher than that of cisplatin, and no further investigation was reported.[54] In contrast, monofunctional Ru^{II} complexes of the type $[(\eta^6\text{-arene})Ru(en)X]^+$ (where en = ethylenediammine or its derivatives and X = halide) exhibit high cytotoxicity in vitro (A2780, A2780cisR, A2780adr, HT29, Panc-1, NX02), comparable to that of cisplatin, that is dependent on the arene ligand (see Figure 10).[55] Large arene ligands, such as biphenyl and tetrahydroanthracene, improved the cytotoxicity of the drug. Replacement of the en ligand with bpy or tmeda (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine) resulted in complexes with poor cytotoxicity.[56] Extensive oligonucleotide studies have been carried out and $[(\eta^6\text{-cymene})Ru(en)X]^+$ have been found to preferentially bind to guanine bases to

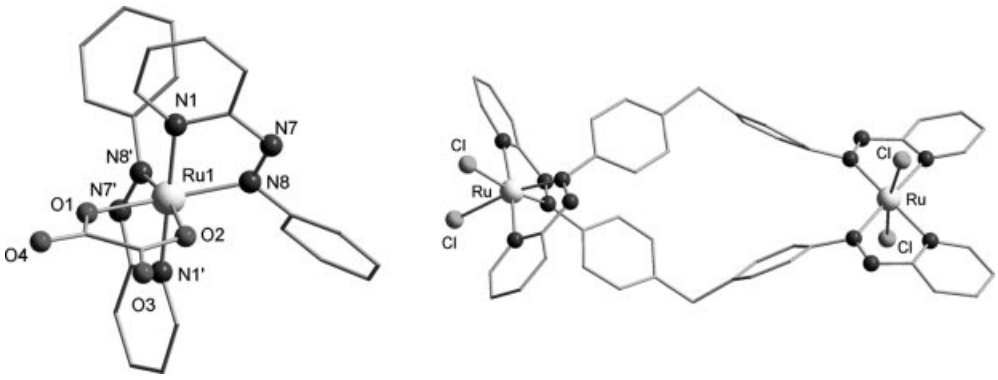


Figure 9. Ball and stick representation of derivatives of α-Ru(azpy)$_2$Cl$_2$. Unlabelled spheres represent N atoms.

form monofunctional DNA adducts.[57] This was supported by NMR spectroscopic studies that suggest that the binding of the [(η^6-arene)Ru(en)X]$^+$ moiety to guanine is enhanced through H-bonding interactions between the en ligand and the exocylic oxygen atom (i.e. C^6–O) on the guanine nucleotide.[58] In contrast, binding to adenine is weakened because of steric interactions between the en ligand and the exocyclic amino groups (i.e. C^6–NH$_2$),[58] whereas (η^6-cymene)-Ru(acac)Cl (where acac = acetylacetonate) [see Figure 10] binds to both adenine and guanine bases – the authors noted that these results could lead to compounds designed to target specific DNA nucleotides.[59] With large arene rings systems, e.g. dihydroanthracene and tetrahydroanthracene, binding of [(η^6-arene)Ru(en)X]$^+$ complexes to nucleotide bases was promoted by hydrophobic arene–purine base π–π stacking interactions, which could explain the enhanced cytotoxicity of those derivatives.[60] There was also evidence of dynamic chiral recognition of ethylguanine by the diastereomers of [(η^6-biphenyl)Ru(Et-en)Cl]$^+$, realizing the concept of induced-fit recognition of DNA by chiral derivatives of ruthenium complexes.[61]

Non-Classical Ruthenium Anticancer Drugs

Antimetastatic NAMI-A Type Complexes

Imidazolium *trans*-[tetrachloro(DMSO)(imidazole)ruthenate(III)] H$_2$im[*trans*-RuCl$_4$-(DMSO)Him], more commonly known as NAMI-A (see Figure 11), was the first ruthenium-based anticancer drug to enter clinical trials – as

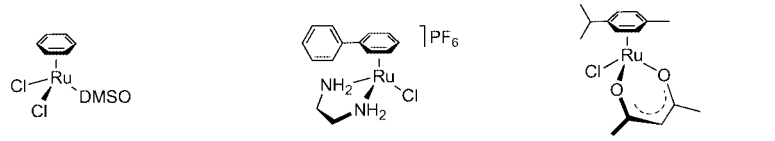


Figure 10. Examples of organometallic (η^6-arene) ruthenium anticancer drugs.

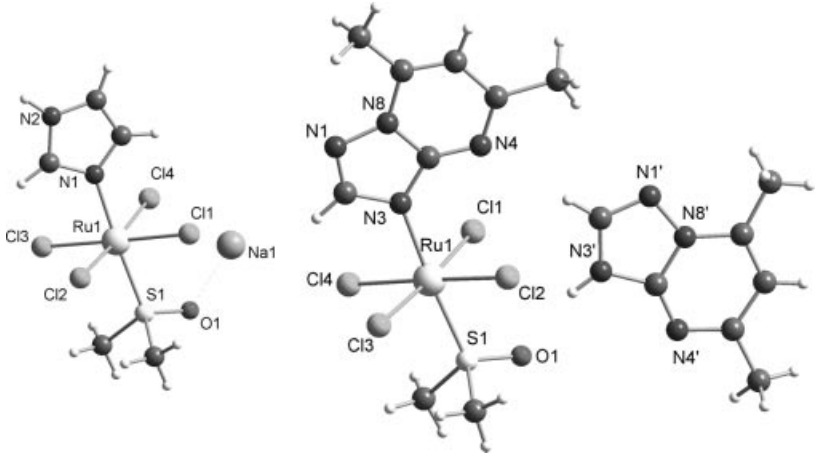


Figure 11. Ball and stick representation of NAMI-A precursor, Na[*trans*-RuCl$_4$–(DMSO)Him] (left) and Hdmtp[*trans*-RuCl$_4$-(DMSO)dmtp] (right).

a drug candidate against non-small cell lung cancer (NSCLC).[62] Compared to other transition-metal-based drugs such as cisplatin, NAMI-A is unique. In vitro, it is virtually devoid of cytotoxicity, while in vivo, it inhibits lung metastases formation and reduces metastases weight without affecting the primary tumour,[63] in contrast to platinum-based drugs which typically exhibit strong growth inhibition on primary tumours. Indeed, both in vitro and in vivo data appear to exclude DNA as the primary target, in line with the observation that the binding of NAMI-A to DNA is much weaker than that of platinum complexes.[63–65] Instead, strong binding to serum proteins is observed and the drug could potentially exploit receptor-mediated delivery by transferrin for selective delivery to cancer cells.[66] There is strong evidence linking the antimetastatic behaviour to the lack of cytotoxicity in vitro, cell-cycle changes corresponding to cell arrest in the premitotic G_2-M phase and inhibition of Matrigel invasion, which could constitute an in vitro screening strategy for ruthenium complexes with similar properties.[67] Phase 1 clinical trials showed that the drug was well-tolerated in patients, reversible blister formation is the dose-limiting toxicity.[12] Several NAMI-A analogues have been studied for potential antimetastatic activity as a means to ascertain the structure–activity relationships in this class of compounds. NAMI-A derivatives [*mer*-$RuCl_3$(DMSO)(acv)(H_2O)] and [*mer*-$RuCl_3$(DMSO)(acv)-(MeOH)] and [*trans*-$RuCl_4$(DMSO) guaH] (where acv = acyclovir and gua = guanine) exhibited low cytotoxicity in vitro (TS/A), similar to NAMI-A.[68] Analogues with the dmtp ligand (where dmtp = 5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyridmidine) were also investigated and found to be slightly cytotoxic in vitro (TS/A, KB, B16-F10).[69] In particular, Hdmtp[*trans*-$RuCl_4$(DMSO) dmtp] exhibited a similar in vivo profile to NAMI-A (CBA mice with MCa carcinoma), strongly inhibiting lung metastases formation without significantly affecting the primary tumours (see Figure 11).[69] This trend has also been replicated in NAMI-A analogues with pyrazine, pyrazole and bidentate *N*-heterocyclic ligands, e.g. 4,4′-bipyridine, 1,2-bis(4,4′-pyridyl)ethane, suggesting that the imidazole fragment is not an essential feature for the antimetastatic property of NAMI-A.[70]

Exploiting the Transferrin Delivery Mechanism

Indazolium *trans*-[tetrachlorobis(1*H*-indazole)ruthenate-(III)] H_2in[*trans*-$RuCl_4(Hin)_2$], KP1019 (see Figure 12), the only other ruthenium drug presently undergoing clinical evaluation, contains two indazole ligands in the *trans* conformation (whereas in NAMI-A the imidazole ligand is *trans* to a DMSO moiety). Unlike NAMI-A, KP1019 is significantly cytotoxic in vitro against colorectal cell lines (SW480, HT29) by induction of apoptosis.[71] There is also evidence of P-glycoprotein (Pgp) mediated drug resistance to KP1019, although other multidrug resistance (MDR) associated proteins (MRP1, BCRP and LRP) did not affect its activity significantly.[72] The drug was evaluated in vivo against autochthonous colorectal tumours in rats and was found to be highly effective in reducing tumour growth (superior to 5-fluorouracil, the most effective drug in clinical use against colorectal cancer).[11] In contrast, cisplatin was inactive in the in vivo model. The activity of KP1019 is attributed, at least in part, to transferrin-mediated drug transport, with KP1019 binding strongly to transferrin in the iron-binding pockets.[73] Indeed, its imidazolium analogue, which binds more weakly to transferrin, is taken up less effectively by transferrin and is also less cytotoxic in vitro.[71] Another possible mechanism in play is that the drug is activated by reduction, from Ru^{III} to Ru^{II}, selectively in hypoxic tumour tissue by endogenous bioreductants such as glutathione. It appears that KP1019 induces apoptosis in colorectal cell lines predominantly by the intrinsic mitochondria pathway and that DNA could be a target, although studies have shown that the DNA lesions formed by KP1019 is different to that by cisplatin.[11,74] In phase 1 clinical trials, the drug was well-tolerated, and five out of six patients treated achieved disease stabilisation.[11,13] A large number of KP1019 analogues, comprising different types and numbers of *N*-heterocyclic rings, have been reported.[75,76] Notably, it was found that increasing the number of indazole ligands, as in *trans*-$Ru^{II}Cl_2(Hin)_4$ and [*trans*-$Ru^{II}Cl_2(Hin)_4$]Cl, improved the in vitro cytotoxicity significantly (CH1, SW480).[76] This was correlated with the increased cellular uptake and a higher reduction potential of the homologues, in line with the "activation by reduction" hypothesis.

Ruthenium-Based Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMs), such as tamoxifen (see Figure 13), are a class of drug that have been successfully used to treat hormone-dependent (ER-positive) breast cancer, i.e. which express the estrogen receptor ERα. At the same time, there is no satisfactory therapeutic treatment for hormone-independent (ER-negative) breast tumours that contain another estrogen receptor ERβ, which accounts for about one-third of breast cancer cases. Jaouen et al. have developed a series of hydroxytamoxifen derivatives comprising ferrocene (hydroxyferrocifen) (see Figure 13), and other potentially cytotoxic organometallic fragments of rhodium, manganese, titanium and rhenium have been reported, representing a strategy to defeat breast tumours which contain both ERα and ERβ receptors.[77] Hydroxytamoxifen analogues containing organometallic ruthenocenes and their biological evaluation in vitro (MCF7, MDA-MB231) have also been reported.[78] Although the hydroxytamoxifen-Ru complexes (see Figure 13) were found to bind strongly to both ERα and ERβ, unlike hydroxyferrocifen, hydroxytamoxifen-Ru exhibits no cytotoxic effect towards ER-negative breast cancer cell lines, possibly because of the increased stability of the ruthenium fragment compared to the iron analogue with respect to oxidation, and thus would not offer any therapeutic advantage over existing treatment options. Nonetheless, the authors noted that the hydroxytamoxifen-Ru complexes could be suitable for radioimaging of tumour cells using either of two γ-emitting Ru isotopes, ^{97}Ru and ^{103}Ru.[78]

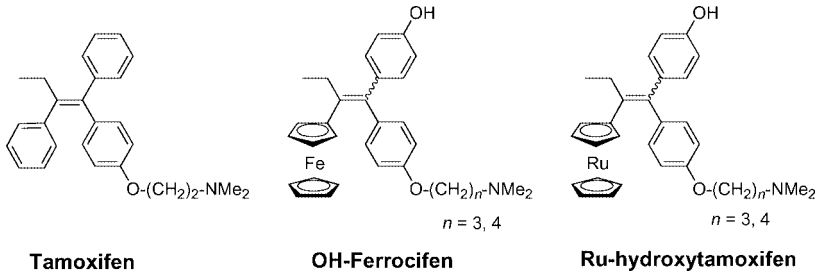


Figure 13. Derivatives of tamoxifen.

Ruthenium Ketoconazole Complexes

Ketoconazole (KTZ) and clotrimazole (CTZ) are azole compounds that were originally developed as antifungal agents. They also exhibit anticancer properties and ketoconazole in particular is being used in the clinic as a second-line agent for hormone-refractory prostate cancer. Ketoconazole-ruthenium $Ru(KTZ)_2Cl_2$ and clotrimazole-ruthenium $Ru(CTZ)_2Cl_2$ complexes were originally developed to treat tropical diseases,[79] but more recently, they have been evaluated for anticancer activity. In vitro, $Ru(KTZ)_2Cl_2$ and $Ru(CTZ)_2Cl_2$ were found to be more effective inhibitors of cell growth proliferation (C8161) than the parent ligands KTZ and CTZ.[80] The effects of $Ru(KTZ)_2Cl_2$ on various cell signalling pathways were investigated. In particular, it was observed that A431 spheroids, known to overexpress EGF-R and to be resistant to either $Ru(KTZ)_2Cl_2$ or C225 *anti*-hEGF-R monoclonal antibody (MAb), were susceptible towards a combination treatment of $Ru(KTZ)_2Cl_2$ and the C225 antibody. C225 is an experimental monoclonal antibody presently under clinical evaluation as a targeted therapeutic agent against EGFR-expressing metastatic colorectal cancer. The authors suggested that $Ru(KTZ)_2Cl_2$ could potentially be used to enhance other targeted therapeutic treatment methods.[80]

Ruthenium-Based Protein Kinase Inhibitors

The three-dimensional structure of many biomolecules is complicated and their syntheses are often difficult given the need to maintain specific conformity and spatial orientation. With a view to develop synthetic compounds with superior biological activity, Meggers et al. reported an organometallic ruthenium complex that mimics the shape of a known protein kinase inhibitor, staurosporine (see Figure 14).[81,82] The strategy exploits the relative ease of synthesising ruthenium moieties of a specific spatial conformation to mimic aspects of an inhibitor not easily accessible using purely organic scaffolds.[81,83,84] The authors further demonstrated the superior binding of the ruthenium mimic to Pim-1, a protein kinase, and reported co-crystallisation of the protein with the ruthenium complex.[81] This provides a basis for the development of further transition-metal-based enzyme inhibitors and could lead to new types of organometallic-targeted therapeutic agents.

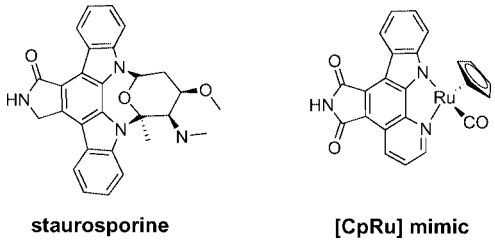


Figure 14. Staurosporine and an organometallic ruthenium mimic.

Arene PTA Ruthenium(II) (RAPTA) Complexes

The RAPTA compounds comprise a class of organometallic ruthenium(II) complexes with a monodentate 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (pta) ligand and a η^6-arene ligand. The compounds are readily synthesised in two steps: the first step involves the reaction of C_6-dienes,

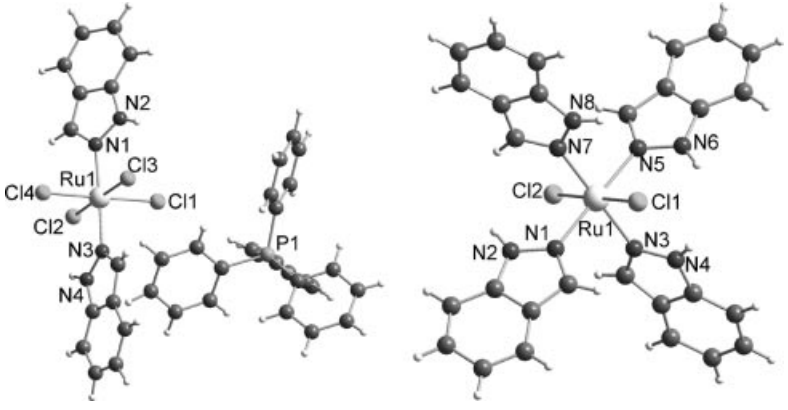
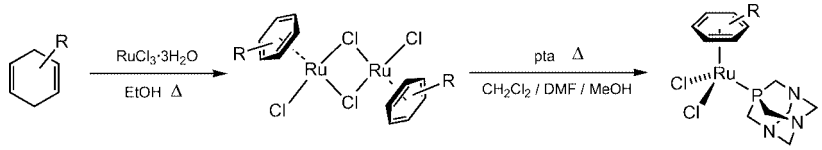


Figure 12. Ball and stick representation of $PPh_4[trans\text{-}RuCl_4(Hin)_2]$ (left) and $trans\text{-}Ru^{II}Cl_2(Hin)_4$ (right).



Scheme 1. General synthesis of RAPTA complexes.

typically derived using Birch reduction of the desired arene, with hydrated $Ru^{III}Cl_3$ to yield dimeric $[(\eta^6\text{-arene})Ru^{II}Cl_2]_2$.[85] In the second step, 2 equiv. pta are added to $[(\eta^6\text{-arene})RuCl_2]_2$ in organic solvents to yield the desired RAPTA complex (see Scheme 1). The reaction is thermodynamically favoured and proceeds in high yield. Alternatively, ligand exchange using the arene-labile complex $[(\eta^6\text{-}PhCO_2Et)RuCl_2]_2$ can be carried out for arene ligands that cannot be readily reduced by Birch reduction, such as benzocrown ethers, but yields are lower.[86] In comparison to typical phosphane ligands, pta is relatively compact and sterically undemanding, with a cone angle of 103° (c.f. PPh_3 143° and PMe_3 134°).[87] As such, RAPTA compounds are generally air-stable complexes with good thermodynamic stability. The pta ligand can also be readily derivatised to form acetylated species, e.g. 3,7-diacetyl-1,3,7-triaza-5-phosphabicyclo[3.3.1]nonane (pta-Ac) or methylated species (pta-Me^+), further expanding the scope of possible RAPTA compounds.[87] Importantly, the presence of the phosphorus atom makes the characterisation of RAPTA compounds by ^{31}P NMR spectroscopy fairly straightforward. RAPTA is also unusual in comparison with other phosphane ruthenium complexes in that it is not only soluble in polar organic solvents, but also in water. The prototype $[(\eta^6\text{-cymene})Ru^{II}(pta)Cl_2]$, RAPTA-C, has been central to biological evaluation and is the reference compound from which other RAPTA compounds are developed (see Figure 15). Two key approaches have thus been adopted. The first is centred on evolving RAPTA into an effective drug that is highly selective towards tumour cells with a low systemic toxicity. In vitro evaluation and oligonucleotide-binding studies supplemented by computational studies were undertaken, with a view that DNA is a possible target, as with most transition-metal-based anticancer drugs. The second approach is aimed at discovering "non-classical" chemotherapeutic applications for RAPTA complexes and to study drug interactions at the proteome level. Early on, specific protein binding interactions were detected with RAPTA, which could be the basis for new therapeutic targets beyond DNA. Furthermore, the remarkably similar in vivo activity of RAPTA-T and NAMI-A suggests that they could respond to similar targets.[10] The development of complementary proteomic techniques as well as rational drug design for RAPTA complexes has been carried out. On the basis of these two approaches, more than 20 RAPTA complexes of the general formula (arene)Ru^{II}(pta)X_2, where X = Cl, Br, I, SCN or bridging carboxylate ligands, have so far been synthesised and studied (see Scheme 2). In addition, RAPTA analogues containing other organometallic fragments, namely [CpRu], [Cp*Ru], [Cp*Rh] and [CyOs] have also been studied.[88]

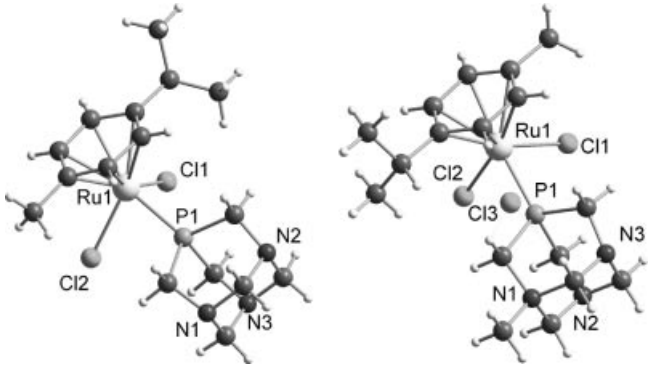
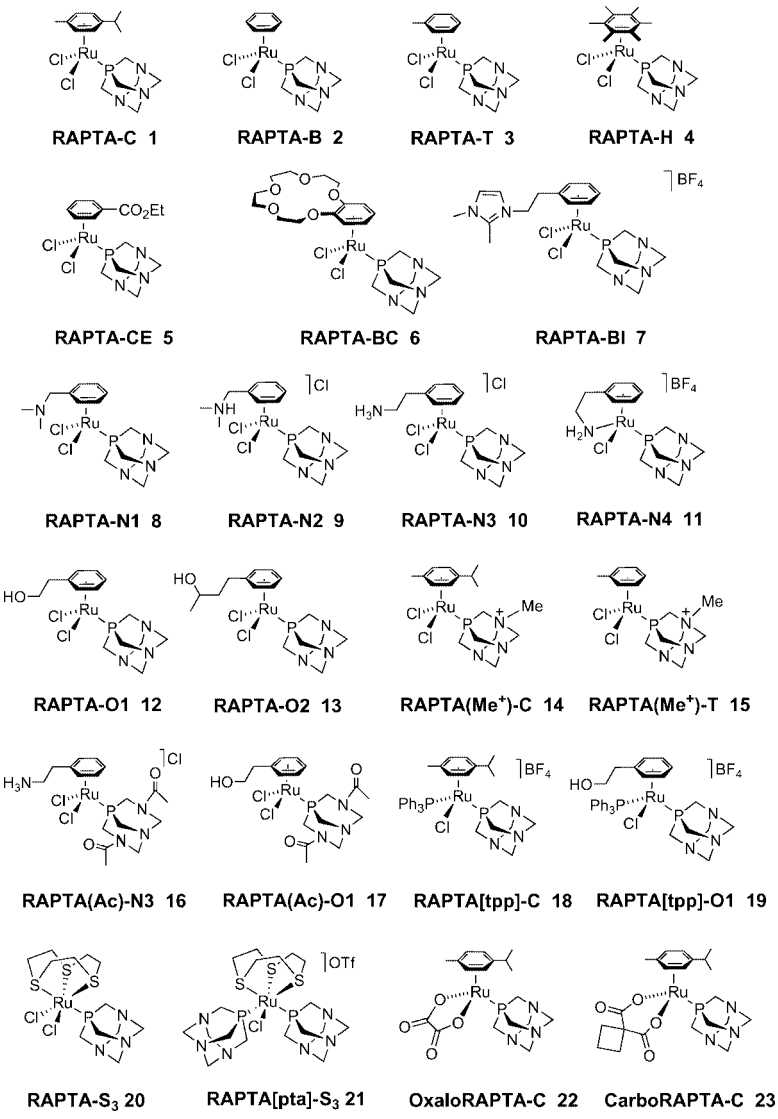


Figure 15. Ball and stick representation of RAPTA-C **1** (left), RAPTA(Me^+)-C **14** (right).

Evolution of RAPTA Complexes: Hybrid Classical / Non-Classical Compounds

The motivation to study RAPTA complexes as potential anticancer drugs came from the observation that RAPTA-C **1** induces pH-dependent DNA damage against *E Coli* pBR322 DNA plasmids, with a significant retardation of the migration of the supercoiled DNA at pH <7.0 because of unwinding of the DNA as a result of drug interactions.[89] This provided a means to target cancer cells, since they generally exhibit lower pH as a result of metabolic changes, partly because of accelerated cell division. Binding studies using calf thymus DNA further suggested that RAPTA-C stabilises the DNA upon binding (see Figure 16), with a significant increase in the melting temperature of the DNA-RAPTA-C adduct.[90] This suggests that the reaction between DNA and RAPTA-C could be thermodynamically driven and thus highly favourable. However, the RAPTA complexes do not show selective binding to DNA in vitro, proteins and RNA appear to be the main intracellular targets.[91]

The reactivity of RAPTA complexes towards single-strand oligonucleotides, studied by ESI-MS, shows that RAPTA-C **1** and its methylated analogue RAPTA(Me^+)-C **14** bind to the 14-mer oligomer (5′-ATACATGGTACATA-3′) across a range of pH values and concentrations, irrevocably with the loss of the chloride and arene ligands, while the pta ligand remains intact.[92] Although the purine bases could, in principle, offer heterocyclic aromatic rings for an arene-type π-bonding mode with the ruthenium centre, DFT calculations revealed that such binding would be unlikely, and it is more probable that the interaction is based on multiple coordinative N-donor bonds, such binding only possible with RNAs.[92]



Scheme 2. Family of RAPTA complexes.

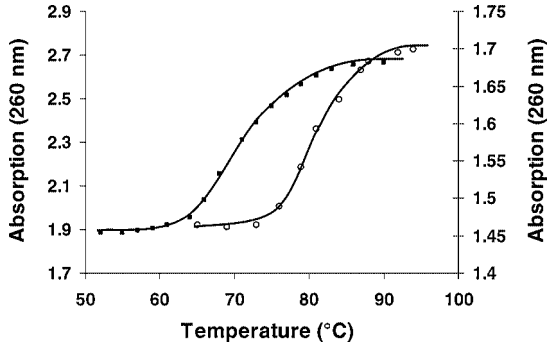


Figure 16. DNA melting curves of calf thymus DNA alone (■) and calf thymus DNA with RAPTA-C (1:1 ratio) (○), buffer: 10 mM phosphate buffer and 50 mM $NaClO_4$.

The cytotoxicity of the RAPTA complexes is generally very low, at least in both TS/A murine adenocarinoma and non-tumourgenic HBL-100 human mammary cell lines in comparison to cisplatin (see Table 1).[90] However, the lead RAPTA complexes **1**–**3** exhibit selective cytotoxicity towards the TS/A cancer cell lines relative to HBL-100, which provided early indication that the complexes are benign towards healthy cells. This is important since high systemic toxicity is associated with the drastic side-effects of cisplatin and related drugs and limits the amount of drug that can be administered. Similarly, NAMI-A is non-cytotoxic to both TS/A and HBL-100 cell lines up to 1 mM concentration. The pta fragment appears to play a significant part in determining this selectivity. When pta was replaced with pta-Me^+, as in **14** and **15** (see Figure 15), selectivity was lost and the compounds were equally toxic in both the cancerous and non-tumourgenic cell lines.[90] This coincides with the hypothesis that RAPTA compounds derived their cytotoxic activity through the protonation of its pta ligand under hypoxic pH conditions,[89] although such a process is deemed unlikely given the low pK_a of the coordinated pta moiety.

Table 1. IC_{50} values of RAPTA complexes on TS/A and HBL100 cell lines[a] and ruthenium uptake in TS/A cells.[b]

Complex		Aromatic	pta	Anion	IC_{50} (TS/A) [μM]	IC_{50} (HBL-100) [μM]	Selectivity Index	Intracellular RAPTA [μg/10^6 cells]	Intracellular RAPTA [$\times10^{-4}$ M]
		Fragment	Fragment						
RAPTA-C	**1**	cymene	pta	–	507	>1000	>2.0	0.12±0.02	2.55±0.06
RAPTA-B	**2**	benzene	pta	–	231	>1000	>4.3	0.13±0.02	3.26±0.04
RAPTA-T	**3**	toluene	pta	–	74	>1000	>13.5	0.16±0.02	2.9±0.3
RAPTA-H	**4**	hexamethylbenzene	pta	–	199[d]	>300[d]	>1.5	0.15±0.01	3.16±0.27
RAPTA-CE	**5**	ethyl benzoate	pta	–	103[d]	>300[d]	>2.9	3.40±0.41	54.67±6.66
RAPTA-BC	**6**	benzo-15-crown-5	pta	–	159[d]	>300[d]	>1.9	0.28±0.02	4.63±0.35
RAPTA-BI	**7**	2,3-dimethyl-1-(2-phenylethyl)-1*H*-imidazolium·BF_4	pta	BF_4^-	66[d]	>300[d]	>4.5	0.19±0.04	3.03±0.67
RAPTA-N1	**8**	*N*,*N*′-dimethylbenzylamine	pta	–	458	813	1.8	0.04±0.004	0.95±0.09
RAPTA-N2	**9**	*N*,*N*′-dimethylbenzylamine·HCl	pta	Cl^-	449	603	1.3	0.10±0.02	2.02±0.38
RAPTA-N3	**10**	benzylamine·HCl	pta	Cl^-	820	666	0.8	0.05±0.005	1.05±0.09
RAPTA-N4	**11**	benzylamine·HCl	pta	BF_4^-	>1000	612	<0.6	0.06±0.01	1.14±0.21
RAPTA-O1	**12**	2-phenylethanol	pta	–	570	778	1.4	0.06±0.01	1.33±0.20
RAPTA-O2	**13**	1-phenylbutan-3-ol	pta	–	505	891	1.8	0.07±0.01	1.46±0.30
RAPTA(Me^+)-C	**14**	cymene	pta-Me^+	Cl^-	>300	246	<0.8	–	–
RAPTA(Me^+)-T	**15**	toluene	pta-Me^+	Cl^-	110	77	0.7	0.33±0.08	5.9±1.4
RAPTA(Ac)-N3	**16**	benzylamine·HCl	pta-Ac	Cl^-	>1000	>1000	1.0	0.05±0.01	0.81±0.19
RAPTA(Ac)-O1	**17**	2-phenylethanol	pta-Ac	–	538	715	1.3	0.07±0.006	1.39±0.12
RAPTA[tpp]-C	**18**	cymene	pta, PPh_3	BF_4^-	>100[c]	37[c]	<0.4	–	–
RAPTA[tpp]-O1	**19**	2-phenylethanol	pta, PPh_3	BF_4^-	124	82	0.7	–	–
RAPTA-S_3	**20**	[9]$anes_3$	pta	–	650	738	1.1	–	–
RAPTA[pta]-S_3	**21**	[9]$anes_3$	2× pta	OTf^-	388	>1000	>2.6	–	–

[a] 72 h exposure at a maximum concentration of 1000 μM, unless otherwise stated. Cell growth inhibition was determined using the MTT assay. [b] 24 h exposure at concentration of 100 μM. Ru levels determined using GF-AAS. [c] Compounds were tested in medium containing 1.0% DMSO, at a maximum concentration of 100 μM. [d] Compounds were tested up to a maximum concentration of 300 μM.

The aromatic fragment is significant, to a certain degree, in influencing the drug uptake and in vitro activity. The presence of the ester group significantly improved the drug uptake in RAPTA-CE **5**, with a corresponding improvement in cytotoxicity against TS/A cells. Upon entering the cells, endogenous esterases could cleave the ester bond, thereby retaining the charged species within the cell walls.[90] Addition of functional groups such as benzocrown and an imidazolium moiety appeared to improve cytotoxicity towards TS/A, but not to a significant degree to warrant further studies.[90] RAPTA complexes with H-bonding groups attached to the arene ring (complexes **8**–**13**, **16**, **17** in Scheme 2) were developed and found to be generally more reactive than lead compounds **1**–**3** with respect to oligonucleotide binding.[93] However, in vitro, they are not only less cytotoxic towards TS/A cells but also less selective between the cell lines. This correlated with drug uptake studies on TS/A cells which showed that the uptake of these complexes decreased, presumably because of the incorporation of the hydrophilic H-bonding substituents. The addition of the hydrophobic triphenylphosphane (tpp) ligand in RAPTA complexes **18** and **19** reversed the trend. The complexes were found to be cytotoxic towards both cell lines, hence poorly selective, presumably because of increased drug uptake (see Figure 17).[94] The replacement of the aromatic fragment with a [9]aneS_3 ligand, RAPTA-S_3 **20**, results in only a slight decrease in selectivity and cytotoxicity, alluding to the fact that the aromatic fragment may not be an essential feature for in vitro activity and could be effectively replaced by another face-capping ligand with low steric demand (see Figure 17).[95] Interestingly, RAPTA[pta]-S_3 **21**, with [9]aneS_3 and two pta ligands, shows good selectivity and cytotoxicity towards TS/A cells, comparable to that of **1** and **2**. RAPTA analogues are presently being evaluated.

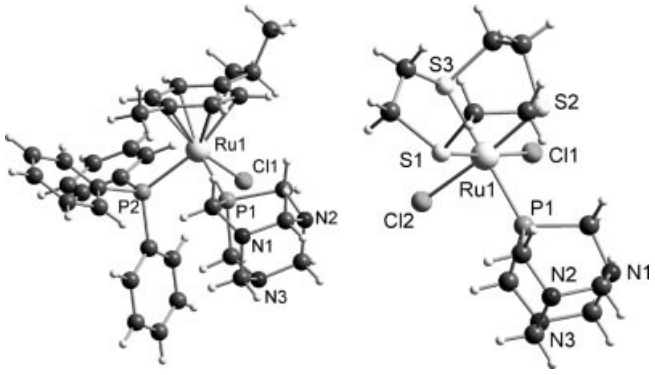


Figure 17. Ball and stick representation of RAPTA[tpp]-C **18** (left) and RAPTA-S_3 **20** (right); the BF_4 anion on **18** was omitted for clarity.

One of the potential issues with regard to eventual clinical application is the behaviour of drugs in water. Second-generation cisplatin drugs with oxalate and 1,1-cyclobutane-dicarboxylate ligands have been developed to improve the stability and solubility of the platinum complexes in water.[2] RAPTA complexes are prone to hydrolysis and would have to be administered in saline to suppress the cleavage of the chloride ligands. With a view to develop drugs that could resist hydrolysis in aqueous media, RAPTA complexes **22** and **23**, bearing chelating carboxylate ligands instead of the two chloride ligands, have been

developed (see Figure 18).[96] They were found to be kinetically more stable than **1**, and essentially, retained the carboxylate ligand in aqueous solution. Preliminary investigations show that these derivatives exhibit the same order of cytotoxicity in vitro (A549, T47D, MCF7, HT29) as **1** and exhibit similar oligonucleotide binding characteristics, hence proving to be a possible replacement for RAPTA-C in clinical studies.[96]

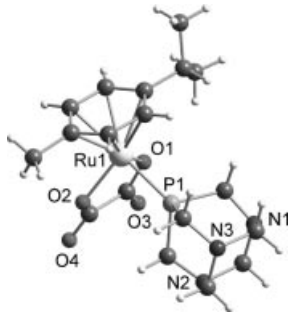
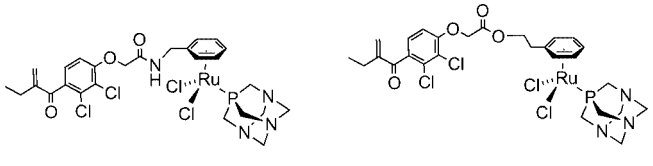


Figure 18. Ball and stick representation of OxaloRAPTA-C **22**.

As a strategy to enhance drug efficacy, the next generation of RAPTA complexes should be more "targeted". Such complexes could have multiple modes of activity, functionalised to achieve specific, desirable outcomes. As proof of concept, RAPTA complexes designed to inhibit Glutathione-S-Transferases (GST), a cytosolic detoxification enzyme associated with drug resistance, have been developed (see Figure 19.).[97] GST catalyses the conjugation of intracellular xenobiotics with glutathione, which is then expelled from cells by the GS-export pump. RAPTA complexes conjugated through the arene ring to ethacrynic acid, a known inhibitor of GST enzymes, were found to be effective GST inhibitors with significantly increased cytotoxic activity, i.e. showing comparable cytotoxicity to that of cisplatin in cell lines known to contain elevated levels of GST (A549, HT29, T47D). Other potential enzyme targets are presently being investigated.



24 **25**

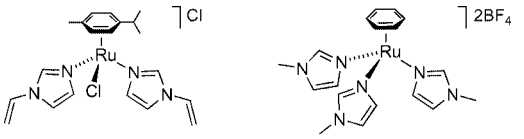
Figure 19. RAPTA complexes designed to defeat GST-mediated drug resistance.

Beyond the Classical Approach: RAPTA as Antimetastatic Agents

Despite the differences in oxidation state and ligand sphere, the striking semblance of RAPTA complexes to NAMI-A in vitro, primarily their low toxicity towards non-tumourgenic HBL-100, prompted the investigation of the lead RAPTA complexes **1**, **2** and **3** for potential antimetastatic activity.[90] In vivo experiments on MCa mammary carcinoma in CBA mice were used to evaluate the effects of the drugs i.p. on primary tumour growth and lung metastases formation. Firstly, it was found the lead RAPTA complexes were well tolerated by the mice at very high doses, consistent with in vitro data. Secondly, a low sustained treatment regime of RAPTA-C at 4× 100 mg/kg/day resulted in significant reduction in both lung metastases weight and number in treated mice whilst leaving the primarily tumour unaffected. In fact, under this regime 40% of the mice were completely free from metastases at the end of the treatment. This phenomenon was replicated in RAPTA-B and RAPTA-T under various treatment regime conditions and at 3× 80 mg/kg/day, RAPTA-T was able to reduce lung metastases in treated mice by 75%.[91] This result is comparable to that of NAMI-A which typically at 6× 35 mg/kg/day, reduces lung metastases by 71–90% and metastases number by 40–60%. Accordingly, there is no corresponding activity on the primary tumour. While the RAPTA complexes displayed marginally less antimetastatic activity than NAMI-A, it is much less toxic to mice and can potentially be administered at much higher dosage.

It is therefore apparent that the antimetastatic behaviour is not unique to NAMI-A, but applicable to other classes of ruthenium complexes. In the case of the well-studied NAMI-A, it is apparent that DNA is not the target, and more likely, activity is a consequence of drug–protein interactions. Therefore, a proteomic-based analytical approach based on 2-D PAGE and laser-ablation inductively-coupled mass spectrometry (ICP-MS) is being developed to identify the specific proteins interacting with ruthenium-based drugs.[98,99] The analytical technique utilises well-established methods in protein separation and exploits the high sensitivity of ICP-MS instruments in detecting non-endogenous transition-metal isotopes (in the order of parts per billion). While the technique is still under development, it has been used effectively to identify drug–protein interactions in human blood plasma, and was used to identify the main protein target of cisplatin in bacteria using 1D PAGE.[99,100]

At the same time, with a view to rationally develop other ruthenium-based antimetastatic agents, a new class of imidazole (η^6-arene)ruthenium complexes of the general formula [(η^6-arene)$Ru^{II}Cl_2$(imid)], [(η^6-arene)Ru^{II}Cl$(imid)_2$]X or [(η^6-arene)$Ru^{II}(imid)_3]X_2$ (where imid = imidazolium ligand, X = Cl, BF_4, BPh_4, 15 examples) have been prepared, combining the unique structural aspects of NAMI-A and the "piano-stool" arene ruthenium(II) complexes.[101] The derived complexes exhibit similar cytotoxicity with RAPTA complexes in vitro (TS/A, HBL-100) and several of the complexes exhibit selectivity towards cancer cells. Specifically, [(η^6-cymene)Ru^{II}Cl(vinylimid)$_2$]Cl **26** and [(η^6-benzene)$Ru^{II}(mimid)_3](BF_4)_2$ **27** have been identified for further in vivo experiments (see Figure 20).[101] Further derivatisation of the imidazolium moiety, to yield antimetastatic ruthenium complexes conjugated to ethacrynic acid and other inhibitors of multidrug resistance-associated enzymes, is also being carried out.[97]



26 **27**

Figure 20. New class of RAPTA-NAMI antimetastatic drug candidates.

Outlook - Towards Targeted Chemotherapy

Drug discovery and evaluation strategies for ruthenium-based anticancer complexes rely largely on the classical approach of screening for biological activity using specific in vitro or in vivo models, identifying potential drug candidates, deriving structure–activity relationships and thereafter cycles of structural modifications and testing for improved drug efficacy. These tests are "result-oriented", especially those based on the effect of the drug on cell viability, and reveal immediately the inhibitory effect of the drug on cell growth proliferation. However, even if potential drugs are identified, to establish directly the drug mechanism is not trivial, especially where there is no target definition in the drug design, and investigators often rely on peripheral experiments in order to rationalise drug behaviour. It is therefore fortunate that ruthenium, and indeed other transition metals not endogenous to biological systems, can be detected using ICP-MS to high levels of sensitivity. Analytical methods based on ICP-MS could therefore directly establish the fate of ruthenium drugs once administered. Keppler et al. employed capillary electrophoresis and size exclusion chromatography techniques, coupled to ICP-MS, to determine drug–protein binding of ruthenium drugs in human blood plasma, and much is now known about metal-drug plasma-protein interactions.[102] We have demonstrated the efficacy of proteomic methods in protein separation on polyacrylamide gels, in conjunction with laser-ablation ICP-MS, to establish drug–protein interactions in the human plasma and from cell samples.[98,100] The ultimate goal is to create a map of drug–protein interactions in the proteome of a cancer cell line after treatment with ruthenium drugs. This is important not only in establishing the mechanism of drug activity, but also as a way of identifying areas in the proteome on which ruthenium drugs could target, and hence towards a more systematic way of developing non-classical ruthenium drugs.

It is debatable whether the inhibition of cell growth proliferation or cytotoxicity should continue to be the predominant "measure of success" for ruthenium-based anticancer drugs. Many ruthenium drugs are known for their low systemic toxicity as compared to established platinum-based drugs. If cytotoxicity is the sole determinant, it is unlikely that ruthenium drugs would offer significant therapeutic advantages. Instead, attributes like cancer-cell selectivity or low systemic toxicity need to be incorporated into drug evaluation, which would focus preclinical drug development towards highly selective ruthenium drugs of low toxicity. Furthermore, existing cytotoxicity assays ignore other aspects of ruthenium drugs that would have greater therapeutic scope. Indeed, NAMI-A failed the in vitro evaluation using the NCI's protocol against a 60-panel cell lines and would have been omitted for further evaluation if the conventional method of drug screening were strictly followed.[98] Sava et al. demonstrated the antimetastatic potential of ruthenium-based drugs, and we have subsequently shown that this attribute is not confined to NAMI-A, while Keppler et al. has exploited known ruthenium–transferrin interactions for drug delivery.[11,63,64] Clearly, more suitable models for testing ruthenium drugs are needed but it is imperative that these testing methods exploit the unique biological profile of ruthenium.[10]

A relatively uncharted area that holds much potential for the application of ruthenium-based drugs is combination therapy. An example is the ketoconazole ruthenium complex which is found to potentiate A431 cells overexpressing EGF-R to C225 MAb treatment, although the mechanism is not fully understood.[80] More recently, in vivo experiments (CBA mice with MCa carcinoma) showed that the combination treatment of NAMI-A and cisplatin led to a synergistic enhancement in the inhibition of metastases formation and growth of primary tumour in mice, as compared to the treatment of NAMI-A or cisplatin alone. At the same time, the increase in toxicity was additive, which was anticipated since both drugs are based on transition-metal centres and discharged mainly by renal excretion.[98] Such a strategy would be extremely attractive but would be dependent on finding other unique applications of ruthenium drugs, such as the antimetastatic behaviour of NAMI-A, which is beyond the scope of existing therapies.

Beyond developing better ways to augment existing drug discovery strategies, Meggers et al. have demonstrated the viability of organometallic ruthenium-based protein-kinase inhibitors, in a strategy that employs chiral ruthenium moieties surrounded by stable inert ligands as a spatial replacement for the carbohydrate moiety which is difficult to synthesise. In this way, the ruthenium centre plays a relatively minor role in determining biological activity and serves more as a connector for other ligands which provides the shape and conformation.[81,83] The sheer range of possible ligands of different shapes and sizes gives a high degree of synthetic flexibility, but it would not be unreasonable to replace the ruthenium by other transition metals and those which occur in living systems may prove preferable. The possibility of ruthenium clusters extending the scope of this approach could also be worth considering, especially since clusters have already been shown to have pharmacological potential.[103] Another approach would be to modify existing receptor and inhibitor molecules, e.g. in the approaches taken by Jaouen et al. in the design of SERMs and by our group in the development of multifunctional RAPTA complexes.[78,97] Nonetheless, developing ruthenium complexes for target therapies would invariably involve structural biology in the design phase of the receptor/inhibitor molecule. Designing the receptor/inhibitor molecule around the ruthenium scaffold in the beginning, rather

than in retrospect, could lead to viable ruthenium drugs with well-defined and predictable modes of activity.

It is clear that with two ruthenium compounds proceeding through clinical trials, combined with the limited number of platinum drugs that have entered the clinic since the discovery of cisplatin and putative drugs based on other transition metals being withdrawn from clinical trials, ruthenium systems hold great potential. With the new strategies outlined in this review, it is likely that we will witness increased research efforts in this domain.

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