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Ruthenium(II) Arene Compounds as Versatile Anticancer Agents

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Abstract: Ruthenium-based compounds are an attractive alternative to clinically used platinum drugs due to several features including a wide range of accessible oxidation states, varied synthetic chemistry and typically lower general toxicities. One series of ruthenium(II)-arene-pta, RAPTA (pta = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1] decane) compounds has been found to show particularly high selectivity towards cancer cells in both *in vitro* and *in vivo* studies.

Keywords: Anticancer · Bioorganometallic · Chemotherapy · Ruthenium

Introduction

Ruthenium-based compounds are gaining increasing interest as a potential alternative to platinum-based chemotherapeutic agents, having been shown to display cytotoxicities close to those of cisplatin and to be effective against cancers which cannot be treated by cisplatin.[1] Moreover, certain ruthenium compounds have been found to display a higher selectivity towards cancerous cells than platinum drugs, leading to reduced side effects. This selectivity has been attributed, at least in part, to their ability to mimic iron;[2,3] rapidly dividing cells such as cancer cells have an elevated need for iron which is delivered to the cells *via* the plasma protein transferrin. Consequently transferrin receptors are over-expressed on the surface of cancer cells, providing an effective means for ruthenium complexes to accumulate in the diseased cells.[3–5] Two ruthenium(III)-based drugs, KP1019,[6] and NAMI-A,[7] have currently completed phase I clinical trials. Both complexes behave quite differently *in vivo* to cisplatin; NAMI-A has been shown to be a strong inhibitor of metastasis while having little effect on the primary tumour,[8] KP1019 effectively reduces colorectal tumours where cisplatin shows limited activity.[9] In both cases, the metal centre is proposed to bind to serum proteins[10] and to be reduced by the cancer cell environment to give an active Ru(II)complex.[11]

More recently, investigation has begun into the anticancer activity of organometallic Ru(II) arene compounds.[12] Different concepts have been explored, including mono- and bifunctional compounds such as RAPTA complexes,[13] targeted approaches and kinase inhibitors,[14,15] and multinuclear ruthenium arene compounds.[16–18] Monofunctional $[Ru((\eta^6\text{-arene})Ru(en)Cl]^+$ type complexes (where en = ethylenediammine or its derivatives) were found to have *in vitro* cytotoxicites similar to that of cisplatin. DNA has been identified as a probable target for the complexes, with preferential binding to guanine bases.[19]

RAPTA complexes (Fig. 1, $[Ru(\eta^6\text{-arene})(pta)Cl_2]$) though structurally similar, exhibit a very different cytotoxicity profile to the $[Ru(\eta^6\text{-arene})Ru(en)X]^+$ series. While IC_{50} values determined in a range of cancer cell lines are high, the complexes generally show no cytotoxicity towards the healthy cell model HBL-100. Moreover, *in vivo* studies on RAPTA-C ($[Ru(\eta^6\text{-p-cymene})(pta)Cl_2]$), RAPTA-B ($[Ru(\eta^6\text{-benzene})(pta)Cl_2]$) and RAPTA-T ($[Ru(\eta^6\text{-toluene})(pta)Cl_2]$), revealed excellent inhibition of metastases growth in addition to high selectivity and extremely low general toxicity, while little effect on the primary tumour was observed.[20,21] Further *in vivo* studies on RAPTA-C showed that it was also active on Ehrlich ascites carcinoma.[22] Interestingly, the behaviour of RAPTA complexes both *in vitro* and *in vivo* is very similar to that of the drug NAMI-A, despite the latter species being an inorganic Ru(III) species with a far more complicated hydrolytic decomposition.[23] The high selectivity and promising anti-metastases activity of RAPTA-C and RAPTA-B has prompted the study of a large number of RAPTA analogues with a range of properties.

Design and Activity of RAPTA Complexes

RAPTA complexes are easily synthesised in two steps (Scheme 1), involving the synthesis of the Ru(II)-arene chloro-bridged dimer from hydrated ruthenium trichloride and the appropriate diene,[24] followed by addition of two equivalents of pta.

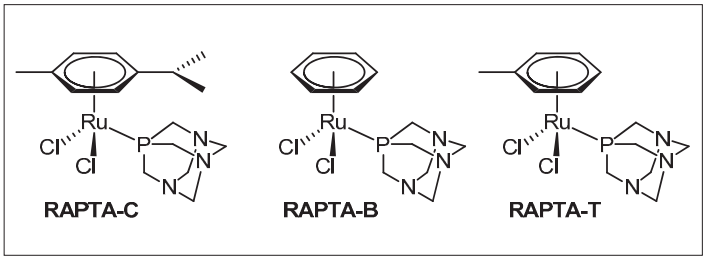

Fig. 1. First series of RAPTA compounds that show promising antimetastasic activity.

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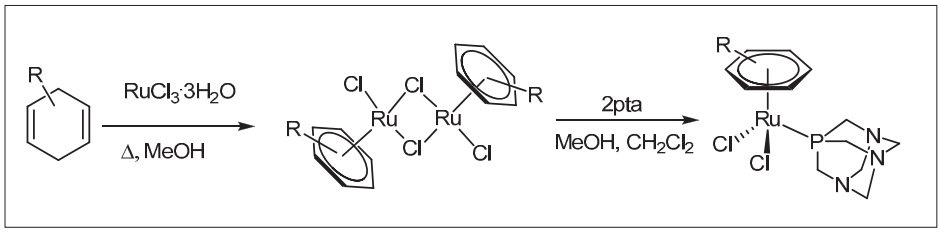


Scheme 1. General synthesis of RAPTA compounds.

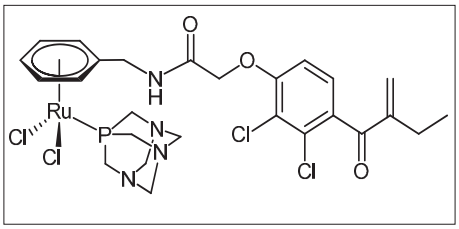


Fig. 3. RAPTA-EA with ethacrynic acid tethered to the arene ligand.

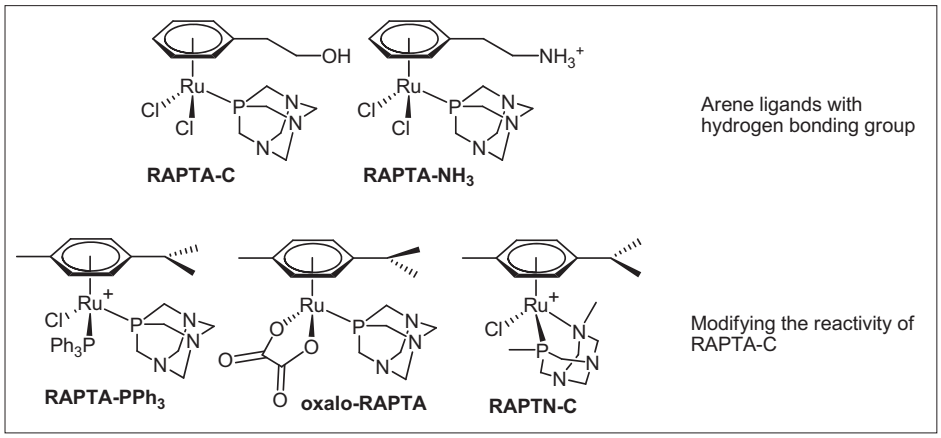


Fig. 2. Examples of modified RAPTA compounds.

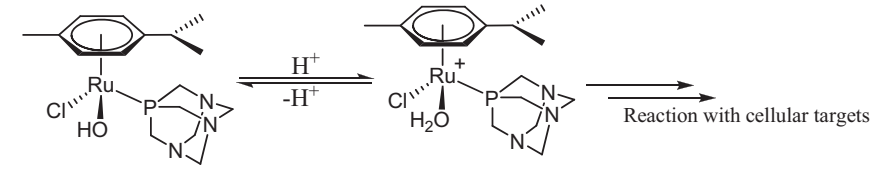
The pseudo octahedral complex comprises an η^6-arene ligand, a phosphine ligand, 7-phospha-1,3,5-triazatricyclo [3.3.1.1]decane, and two chloride ligands. The arene increases the lipophilicity of the compound facilitating uptake into the cell, while the hydrophilic pta increases solubility in aqueous medium, potentially allowing oral delivery. In aqueous solution, hydrolysis occurs rapidly through loss of a chloride ligand with the major species present being $[Ru(\eta^6\text{-arene})(pta)Cl(H_2O)]^+$.[25] This process is suppressed at elevated concentrations of NaCl such as that found in the blood stream (100 mM), suggesting that *in vivo* the drug is activated by hydrolysis on entering the cell where the NaCl concentration is much lower (4 mM), in a similar manner to cisplatin.[26] This hypothesis is supported by analysis of adducts to cellular targets such as oligomers or model proteins where chloride ions are always lost.[27,28] Interestingly, the rate of hydrolysis does not seem to dictate activity; replacement of the two chloride ligands by an oxalato (oxalo-RAPTA, Fig. 2) significantly decreases the rate of hydrolysis,[29] however reactivity towards a single stranded oligomer and *in vitro* activity remain essentially unchanged.

The half sandwich structure can be easily functionalised to modulate the properties of the complexes. A wide range of arene ligands are accessible from either commercially available dienes, those prepared by Birch reduction or subsequent functionalisation of the coordinated η^6-arene. It was proposed that introducing hydrogen bonding functionalities to the arene ligand (Fig. 2) would increase cytotoxicity through stronger binding to DNA[30] as has previously been observed for titanocene-type drugs.[31] The functionalised arenes do promote reactivity towards a 14-mer 5'-ATACATGGTACATA-3', but *in vitro* proved to be less active, attributed to reduced uptake into the cell, yet significantly more toxic towards a healthy cell model. The poor correlation between DNA binding and cytotoxicity implies that DNA is not the main target for RAPTA compounds. A similar effect was observed when a chloride ligand is replaced by a triphenylphosphine group to give a monocationic compound (Fig. 2). While the hydrophobic PPh_3 improves uptake into the cell and subsequently cytotoxicity, and reactivity towards DNA, the monochloride compounds proved to be far more toxic towards the non-tumorogenic cell model than their bischloride analogues. Interestingly, they also showed reduced affinity for the model proteins.[28]

This trend is reversed in the Ru-ptn series where the pta ligand is replaced by a chelating analogue ptn (3,7-dimethyl-7-phospha-1,3,5-triazabicyclo[3.3.1]nonane).[32] The chelate complexes are strongly resistant to hydrolysis, with hydrolysis requiring up to several days, and they show little reactivity towards DNA. However their *in vitro* activities are comparable to or better than those of their pta analogues and their affinity for model protein ubiquitin slightly higher. While it is clear that RAPTA complexes can bind to DNA, it appears increasingly unlikely that this is the primary target, and interactions with proteins seem to play a greater role. Recently, enzyme inhibition tests on cathepsin B, an enzyme associated with tumour metastases, with a number of RAPTA showed them to be very good inhibitors, with a correlation between cytotoxcity and enzyme inhibition observed, suggesting a possible pathway in the inhibition of metastases tumour growth.[33]

The facile functionalisation of the RAPTA structure has been further exploited to incorporate molecules of biological importance. It is recently been shown that RAPTA-C binds strongly to glutathione, and when the drug is bound to a protein such as ubiquitin, glutathione can cleave the ruthenium–ubiquitin bonds.[34] In a strategy previously used with considerable success in a Pt(IV) complex,[35] ethacrynic acid, a strong inhibitor of the enzyme glutathione-s-transferase (GST)[36] associated to drug resistance, was tethered to a RAPTA complex to incorporate the dual antiproliferative and antiresistance mechanisms in a single molecule (Fig. 3). RAPTA-EA proved to be a strong inhibitor of GST in addition to showing very promising *in vitro* activity.[37]

Increasingly, molecular modelling and DFT calculations are being used in conjunction with experimental techniques to rationalise physical and chemical properties of putative drug candidates. Simulated binding of RAPTA-C to DNA show that the molecule binds preferentially to the N(7) site of guanine, which has also been observed experimentally,[27] and that the complex can kink the structure of the DNA strand by binding two neighbouring guanine bases.[30] Docking experiments with the enzyme cathepsin B revealed that sterically hindered RAPTA had a much poorer fit to the active site, leading to a less stable adduct which is consistent with the lower enzyme inhibition values.[33] Calculations may even be used to direct the synthesis of new potential drugs: a study into hydrolysis products of lead RAPTA compounds ($[Ru(\eta^6\text{-arene})(pta)Cl(H_2O)]^+$) revealed the aqua ligand to have a pK_a value of approximately 9. It was envisaged that a complex with lower pK_a, around 7, could



Scheme 2. pH-Controlled selectivity targeting the acidic tumour environment.

be deprotonated at cellular pH to give a less active hydroxy complex, ([Ru(η^6-arene)(pta)Cl(H_2O)]$^+$, Scheme 2) while remaining protonated in the more acidic environment of a tumour.[25] Addition of fluorous substituents to the arene ring was proposed as means of lowering the pK_a and the synthesis and evaluation of such compounds is currently on-going.

Conclusions

Recent years have seen significant advances in the design of ruthenium-based drugs, with two compounds already in clinical trials. The RAPTA series show a cytotoxicity profile very different to platinum compounds and do not seem to primarily target DNA. *In vivo* studies are promising, with RAPTA compounds showing good selectivity towards tumour cells and RAPTA-EA able to overcome drug resistance, two major problems associated with cisplatin. Design of new potential drugs is facilitated by molecular modelling and improved understanding of potential targets and pharmacokinetics.

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