Published in Dalton Transactions 39, issue 371, issue 7, 1673-1688, 2010
which should be used for any reference to this work

Arene ruthenium complexes as anticancer agents†

Georg Süss-Fink

Institut de Chimie, Université de Neuchâtel, Case postale 158, CH-2009 Neuchâtel, Switzerland. E-mail: georg.suess-fink@unine.ch; Fax: +41 32 718 2511; Tel: +41 32 718 2405

† Dedicated to Professor Pierre Dixneuf, one of the pioneers of arene ruthenium chemistry and a personal friend, on the occasion of his retirement.

Neutral or cationic arene ruthenium complexes providing both hydrophilic as well as hydrophobic properties due to the robustness of the ruthenium–arene unit hold a high potential for the development of metal-based anticancer drugs. Mononuclear arene ruthenium complexes containing *P*- or *N*-donor ligands or *N*,*N*-, *N*,*O*- or *O*,*O*-chelating ligands, dinuclear arene ruthenium systems with adjustable organic linkers, trinuclear arene ruthenium clusters containing an oxo cap, tetranuclear arene ruthenium porphyrin derivatives that are photoactive, as well as hexanuclear ruthenium cages that are either empty or filled with other molecules have been shown to be active against a variety of cancer cells.

Introduction

Cancer is the second leading cause of death in economically developed countries and the third leading cause of death in developing countries.[1] Statistically, one in eight Europeans will develop cancer during his or her lifetime.[2] Although the estimated incidence rates show rising trends for both sexes, the age-standardized cancer mortality rate has been falling continuously among women since 1970 and among men since 1985.[3] The increase of the survival rates is due to better cancer treatment, in particular thanks to the introduction of efficient anticancer drugs which largely contributed to this improvement.

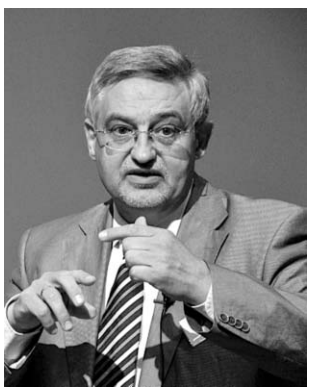
Metal-based cancer chemotherapy

Platinum-based drugs have been in clinical use for cancer treatment for more than 30 years.[4] The landmark discovery of the antitumoural properties of *cis*-diamminedichloroplatinum(II) (cisplatin) by Rosenberg in 1965 heralded a new area of anticancer research based on metallopharmaceuticals.[5] To date, cisplatin and its analogues (Fig. 1) are some of the most effective chemotherapeutic agents in clinical use.[6] The square-planar Pt(II) drugs are activated by slow hydrolysis of the anionic ligands,[7] the corresponding cationic aqua complexes thus formed act by binding to DNA.[8] The current understanding of the structure–activity relationships and the state of the art in targeted chemotherapy with platinum-based drugs has been reviewed recently by Reedijk.[9] The evolution of platinum-based anticancer agents is a beautiful example of how it was possible to turn a serendipitous discovery into pharmaceuticals. However, platinum-based drugs are not without problems: Their high toxicity and incidence of drug resistance remain the main challenges in their clinical application.[10,11]

In the search for anticancer agents containing metals other than platinum (thus overturning the platinum paradigm), ruthenium compounds turned out to be the most promising ones.[12] The ligand exchange kinetics of metal complexes in aqueous solution, which seem to be crucial for the anticancer activity and which vary for the different metal cations by as much as fourteen orders of magnitude (rates from 10^{-6} to 10^{+8} s^{-1}), are very similar for platinum(II) and ruthenium(II) complexes; in both cases the ligand exchange processes are quite slow and may take hours (rates 10^{-3} to 10^{-2} s^{-1}).[13] Ruthenium has therefore been considered to be an attractive alternative to platinum, in particular since many ruthenium compounds are not very toxic and some ruthenium compounds have been shown to be quite selective for cancer cells.[14,15] This is believed to be due to the ability of ruthenium to mimic iron in binding to biomolecules. As cancer cells overexpress transferrin receptors to satisfy their increased demand for iron, ruthenium-based drugs (containing the iron homologue ruthenium) may be delivered more efficiently to cancer cells.[16,17]

Ruthenium compounds with anticancer properties

The first ruthenium compounds to be studied for cancer activity were chloro-ammine complexes: Durig *et al.* had observed



Georg Süss-Fink (*photograph courtesy of Johan Eriksson*)

Georg Süss-Fink graduated from the Technische Universität München in 1974, where he remained for his Doctorate (1977) with Professor Max Herberhold. After a postdoctoral year (1977–78) with Professor (now Lord) Jack Lewis (University of Cambridge) and a visiting professorship (1983–84) with Professor Pierre Dixneuf (Université de Rennes), he received his Habilitation (1984) from the University of Bayreuth. Following two years as Associate Professor (1986–88) at the Technische Hochschule Aachen, he joined the Université de Neuchâtel as Professor of Chemistry in 1988. His research focuses on ruthenium chemistry, from organometallics and catalysis to nanomaterials and biomedical applications.

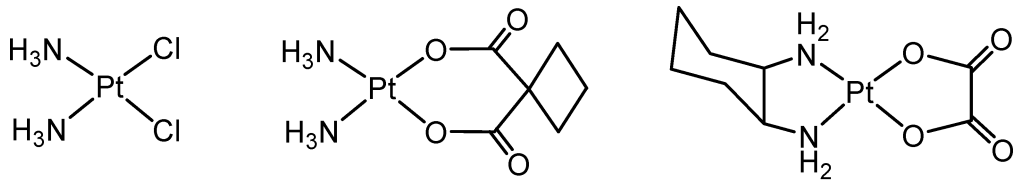


Fig. 1 Platinum(II) complexes approved worldwide for clinical use in cancer treatment.

in 1976 that the ruthenium(III) complex *fac*-$Ru(NH_3)_3Cl_3$ induces filamentous growth of *E. coli* cells, at the same concentration as the required concentration of cisplatin for the same effect.[18] In 1980, this complex as well as the related ruthenium(II) complex *cis*-$Ru(NH_3)_4Cl_2$ were evaluated for their anticancer properties by Clarke.[19] However, although active, these compounds were not soluble enough for pharmaceutical use.[20] In the following years, a large number of Ru(II) and Ru(III) compounds were studied for their cytotoxic properties, in particular polypyridyl complexes such as *cis*-Ru(*N*,*N*-bipy)$_2$Cl$_2$ and *mer*-Ru(*N*,*N*,*N*-terpy)Cl_3 (bipy = 2,2′-bipyridine, terpy = 2,2′:6′2″-terpyridine),[21,22] aminocarboxylato complexes such as Ru(*N*,*N*,*O*,*O*-pdta)Cl_2 or [Ru(*N*,*N*,*O*,*O*,*O*-edta)Cl]$^-$ (pdta = 1,2-propylenediaminetetraacetato, edta = ethylenediaminetetraacetato) as potassium salt,[23,24] dimethylsulfoxide complexes such as *cis*- and *trans*-Ru(*S*-dmso)$_4Cl_2$ (dmso = dimethylsulfoxide),[25-27] and arylazopyrdine complexes such as Ru(*N*,*N*,*N*,*N*-azpy)$_2Cl_2$ (azpy = 2-phenylazopyridine).[28,29] Since Ru(II) coordinates more rapidly to biomolecules,[20] it has been suggested that Ru(III) is reduced *in vitro* to Ru(II).[30] This is possible, because cells contain reducing agents such as glutathione, and tumour cells are often hypoxic (poor in O_2) and more acidic than normal tissue. This activation mechanism proposed by Clarke has become known as the "activation by reduction" hypothesis.[31]

The first real breakthrough in the area of classical ruthenium complexes was the introduction of imidazole and indazole ligands by Keppler: the isoelectronic ruthenium(III) compounds [imiH]*trans*-[Ru(*N*-imi)$_2Cl_4$] and [indH]*trans*-[Ru(*N*-ind)$_2Cl_4$] (imi = imidazole, ind = indazole) were active against a number of tumour models, in particular against platinum-resistant colorectal autochthonous tumours.[32] Alessio and Sava reported the imidazole-dimethylsulfoxide ruthenium(III) complex *trans*-[Ru(*N*-imi)(*S*-dmso)Cl_4]$^-$ to be specifically active against solid metastasizing tumours in mice.[33] After extensive preclinical tests, the compounds [indH]*trans*-[Ru(*N*-ind)$_2Cl_4$] (KP1019) and [imiH]*trans*-[Ru(*N*-imi)(*S*-dmso)Cl_4] (NAMI-A) (see Fig. 2) went into clinical trials.[4] In accordance with the "activation by reduction" hypothesis,[31] NAMI-A*R*, obtained by reduction of NAMI-A with ascorbic acid prior to administration, was found to be even more efficient than NAMI-A itself against metastasis growth.[34]

Arene ruthenium complexes with antitumoural and antimetastatic properties

Organometallic compounds are generally considered to be toxic and unstable, undergoing decomposition when exposed to air and water. While this statement is true for many organometallics, generalisations of this type are misleading: there are an increasing number of stable, water-soluble organometallic complexes, and aqueous-phase organometallic chemistry is a thriving subject for industrial catalysis,[35,36] as well as for biologically related topics,[37] thus overcoming old teaching paradigms.[38] The term "bioorganometallic chemistry" was introduced in 1985 by Jaouen,[39] stimulating also the search for organometallic anticancer drugs.

The field of antitumoural and antimetastatic arene ruthenium complexes was pioneered by Dyson and by Sadler,[40,41] after the notion of using arene ruthenium compounds as anticancer agents had first been introduced by Tocher *et al.* in 1992, who had observed a cytotoxicity enhancement by coordinating the

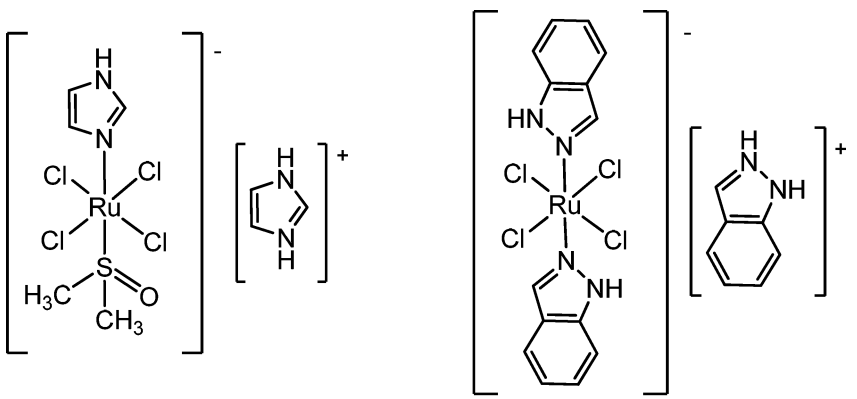


Fig. 2 Ruthenium(III) complexes in clinical trials for cancer treatment.

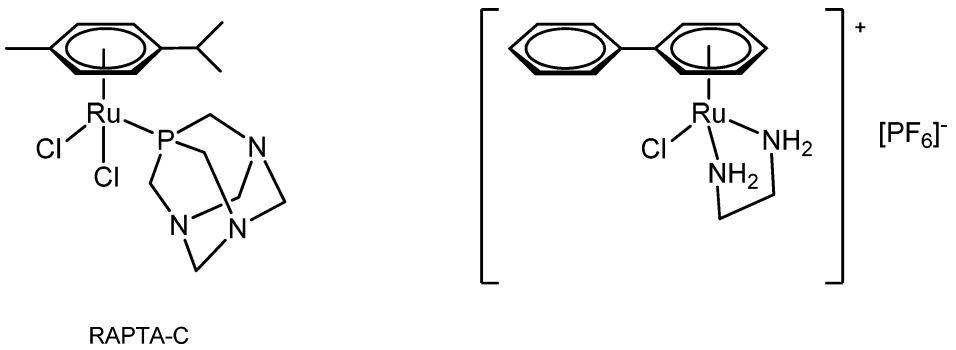


Fig. 3 Prototype anticancer arene ruthenium complexes reported by Dyson and by Sadler.

anticancer agent metronidazole [1-β-(hydroxyethyl)-2-methyl-5-nitro-imidazole] to a benzene ruthenium dichloro fragment.[42] Initially, the prototype arene ruthenium(II) complexes evaluated for anticancer properties in 2001 were (η^6-*p*-$MeC_6H_4Pr^i$)Ru(*P*-pta)Cl_2 (pta = 1,3,5-triaza-7-phospha-tricyclo-[3.3.1.1]decane), termed RAPTA-C, from Dyson's laboratory,[43] and [(η^6-C_6H_5Ph)Ru(*N*,*N*-en)Cl]$^+$ (en = 1,2-ethylenediamine) as hexafluorophosphate salt as well as some analogues from Sadler's laboratory (Fig. 3).[44] Although RAPTA-C exhibits only a low activity *in vitro*, it is very active *in vivo*, where it inhibits lung metastases in CBA mice; like NAMI-A, RAPTA-C is also an antimetastatic agent.[40] The underlying design of this type of anticancer agents and the understanding of their mode of action are summarized in some excellent review articles.[6,40,41,45,46]

Recent developments with arene ruthenium complexes

Apart from a supposed low general toxicity and a supposed high selectivity of ruthenium compounds for cancer cells,[14,15] the main reasons for the flourishing design of arene-ruthenium-based anticancer drugs are the amphiphilic properties of the arene ruthenium unit, provided by the hydrophobic arene ligand counterbalanced by the hydrophilic metal centre, and the synthetic diversity of the arene ligand, which is an excellent scaffold for the coupling of organic segments for targeted chemotherapy.[40]

Another important feature is the hydrolysis of Ru–X bonds to give ruthenium-aqua species (aquation), while the arene–ruthenium bond is robust. The corresponding aqua complex will exist over a range of pH, but for pH > pK_a the hydroxo complex formed by deprotonation will be predominant. As hydroxide is a less labile ligand than water, it will not so easily be displaced by biomolecule targets. Aquation of the chloro complexes might be suppressed extracellularly by high chloride concentrations (0.1 M) but becomes possible after the complex enters the cell due to lower Cl^- concentrations (4–25 mM) found intracellularly.[41]

Mononuclear arene ruthenium complexes containing *P*- or *N*-donor ligands

The influence of structural variations on the anticancer activity of RAPTA-C was studied in detail by Dyson (Fig. 4): Variation of the arene ligand in the prototype complex (η^6-*p*-$MeC_6H_4Pr^i$)Ru(*P*-pta)Cl_2 (**1**) revealed that both the *para*-cymene derivative **1** (RAPTA-C) and the benzene derivative (η^6-C_6H_6)Ru(*P*-pta)Cl_2 (**2**) (RAPTA-B) inhibit metastasis growth in addition to possessing low general toxicity.[47] The toluene and hexamethylbenzene derivatives (η^6-C_6H_5Me)Ru(*P*-pta)Cl_2 (**3**) (RAPTA-T) and (η^6-C_6Me_6)Ru(*P*-pta)Cl_2 (**4**) (RAPTA-H) are slightly more cytotoxic.[6] Variation of the anionic ligands led to the bromo, iodo and isothiocyanato analogues **5–7**, all these complexes (Fig. 4) are cytotoxic and show antimicrobial but no antiviral activity.[48] Replacement of the two chloro ligands in **1** by bridging anionic ligands gave the oxalato complex (η^6-*p*-$MeC_6H_4Pr^i$)Ru(*P*-pta)(*O*,*O*-C_2O_4) (**8**),[49] and the diketonato complexes [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*P*-pta)(*O*,*O*-R_2acac)]$^+$ (**9–11**),[50] which resist hydrolysis without this phenomenon having a great impact on the cytotoxicity.

On the other hand, the water-soluble phosphine ligand pta seems to play a significant part in determining the selectivity for cancer cells. When pta was replaced by the *N*-methylated pta-Me$^+$, the selectivity was lost, complex [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*P*-pta-Me)Cl_2]$^+$ (**12**) being equally cytotoxic in both cancerous and non-tumourigenic cell lines.[47] However, replacement of pta by ptn (3,7-dimethyl-7-phospha-1,3,5-triazabicyclo[3.3.1]nonane), a *P*,*N*-four-electron donor, to give [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*P*,*N*-ptn)Cl]$^+$ (**13**) (Fig. 4) had little influence on the cytotoxicity with respect to that of **1**.[51] In contrast to the expectations, the introduction of hydrogen-bonding substituents in the arene ring, such as in [(η^6-$C_6H_5CH_2CH_2NH_3$)Ru(*P*-pta)Cl_2]$^+$ (**14**), does not enhance the cytotoxicity but actually has the reverse effect.[52]

The question of whether the aromatic fragment in the ruthenium complexes of the RAPTA type (piano-stool geometry) is essential for the anticancer activity was addressed by Alessio, who synthesized the 1,4,7-trithiacyclononane ([9]aneS_3) complex ([9]aneS_3)Ru(*P*-pta)Cl_2 (**15**), which is isoelectronic to **1** without being organometallic (Fig. 5), and derivatives thereof. The results clearly suggest that the aromatic ligand is not an essential feature for the cytotoxicity and that it can be effectively replaced by another face-capping six-electron ligand with low sterical demand and hydrophobic properties.[53,54]

Replacing the arene ligand with another π-ligand, the five-electron donor cyclopentadienyl system showed a remarkable cytotoxicity dependence on the substituents at the ring: While the complex (η^5-C_5H_5)Ru(*P*-pta)$_2$Cl (**16**) is inactive on human ovarian cancer cell lines, the water-soluble but lipophilic complexes (η^5-$C_5HCH_2Bu^tBu^t{}_2OR$)Ru(*P*-pta)$_2$Cl (**17**: R = Me, **18**: R = Et) are more active than the RAPTA compounds, the cytotoxicity being comparable to that of cisplatin; this has been explained by an increased uptake due to the high lipophilicity.[55] Complex **19** containing a 3-hydroxy-2-pyridone ligand (Fig. 5), which exists in

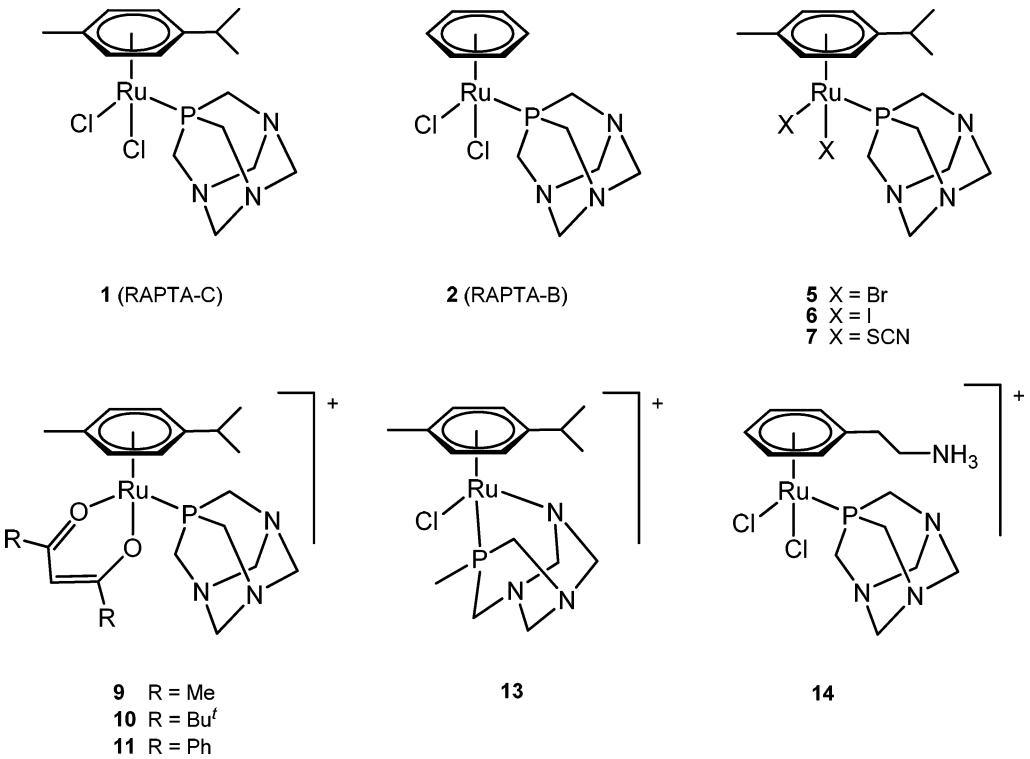


Fig. 4 Structural variations of the ligands in the lead compound RAPTA-C.

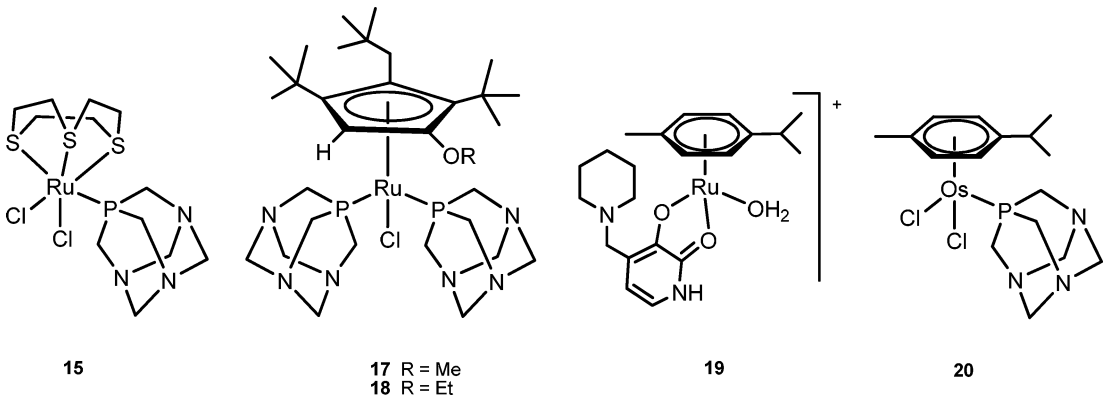
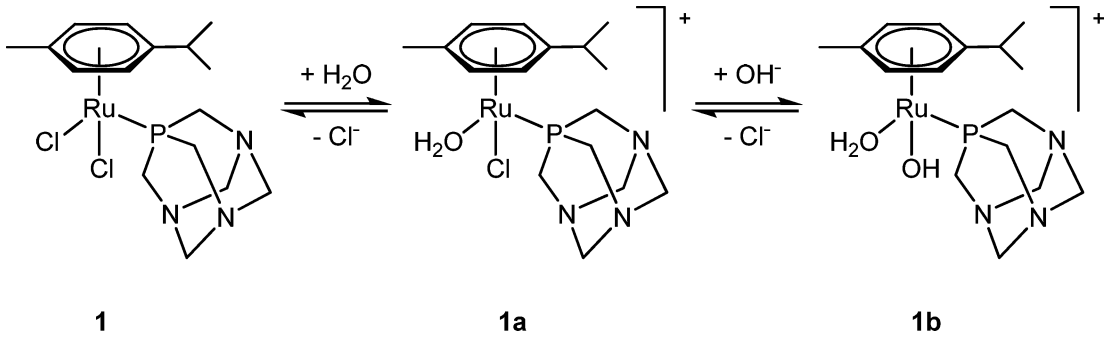


Fig. 5 RAPTA analogues showing similar or enhanced cytotoxicities.

aqueous solution as a mononuclear complex cation (pH < 7) or as a neutral trinuclear complex, in which the deprotonated pyridone nitrogen atom replaces the aqua ligand of a neighbour ruthenium atom (pH > 8.5), is slightly less active and also less selective than **1** towards human ovarian cancer cells; it was not possible to ascertain whether or not a pH-dependent process involving a structural change takes place.[56] Finally, arene osmium and pentamethylcyclopentadienyl rhodium RAPTA analogues such as **20** (Fig. 5) have been shown to have very similar cytotoxicity profiles to those of **1**, suggesting that the anticancer potential of neighbouring elements to ruthenium should not be overlooked.[57]

Since these findings were somewhat controversial, and as the modes of action of RAPTA-type anticancer compounds were largely unexplored,[16] some mechanistic and modelling studies have been undertaken. The aquation of RAPTA-C (**1**) in water was studied by UV-Vis and NMR spectrometry: during the hydrolysis process in 1 μM solution, the percentage of **1** decreases to 18%, the cationic aqua-chloro complex **1a** is predominant at 70%, while the cationic aqua-hydroxo complex **1b** is present at 12%, the equilibrium being reached after 20 minutes (Scheme 1).[58] Aquation seems to be the activating step in the cytotoxicity of **1**, hydrolysis is suppressed in the blood plasma where the chloride concentration is about 100 mM, but occurs once the compound penetrates the cell cytoplasma where chloride concentrations are much lower. Then the labile aqua ligand is prone to substitution by biomolecules.[40] The hydrolysis-resistant complexes **8–11**, which are also cytotoxic, are presumably activated by another mechanism which may involve ring slippage of the arene.[49,50]

It is generally believed that the target of arene-ruthenium-based anticancer drugs within the cancer cell is DNA or RNA, but serum proteins may also be targets.[40] Early on, it had been observed that RAPTA-C (**1**) induces pH-dependent DNA damage.[43] Binding



Scheme 1 Hydrolysis of **1** (RAPTA-C) in pure water at 1 or 2 micromolar concentration.

studies using calf thymus DNA further suggested that **1** causes denaturation by double-helix stabilisation upon binding.[47] Indeed, **1** showed pH-dependent reactivity towards the DNA model compound 2′-deoxiguanosione-5′-monophosphate (dGuaRP) with an up to tenfold higher amount of ruthenium in the dGuaRP-bound form at pH = 6 (typical for cancer cells) as compared to pH = 7.4 (typical for healthy cells),[59] and **1** was found to react with purine bases as DNA model compounds, in particular with guanine.[60] However, in the reaction with the single-strand 14-mer oligonucleotide d(ATACATGGTACATA) it was not possible to identify preferential binding sites; the binding occurs with loss of the chloro ligand and – surprisingly – also the arene ligand, while the pta ligand remains coordinated.[61] On the basis of DFT calculations, it was suggested that the interaction with the arene-free ruthenium fragment is based on multiple N-donor bonds, possible only in RNA, therefore RNA and serum proteins appear to be the main intracellular targets.[6,61] Sadler isolated the arene ruthenium enzyme complex (η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*-lysozyme)Cl_2 from the reaction of [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(H_2O)Cl_2 with the single-chain protein lysozyme (containing 129 amino acid residues); this enzyme complex could be structurally characterized and showed the ruthenium to be coordinated to the N3 atom of the histidine-15 unit of lysozyme.[62]

An ESI-MS characterisation of protein adducts showed reaction of **1** with horse heart cytochrome-c and with hen egg white lysozyme revealed stable metalloadducts containing [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*P*-pta)] and [(η^6-*p*-$MeC_6H_4Pr^i$)Ru] fragments, preferentially bound to surface histidines.[63] Recently, it was found that RAPTA-C (**1**) as well as its derivates and analogues inhibit two specific enzymes that are believed to be targets in cancer chemotherapy: RAPTA compounds are potent inhibitors of cathepsin B and weak inhibitors of thioredoxin reductase.[64] It has also been shown that **1** induces apoptosis and slows down cell division in cancer cells.[65]

The RAPTA moiety was tethered to human serum albumin (HSA), known to accumulate in tumours by modification of HAS with hydrazine groups and by subsequent hydrazone formation with the RAPTA-aldehyde derivative **21** (Fig. 6), causing a twentyfold increase of the cytoxicity with respect to **1**.[66] On the other hand, replacement of pta by 3,5,6-bicyclophosphite-α-D-glucofuranoside ligands leads to RAPTA analogues such as **22**, which is more cytotoxic than RAPTA compounds such as **1**, due to its increased lipophilicity.[67]

Beyond the conventional approach of drug discovery, relying largely on screening for biological activity, deriving structure–activity relationships and testing for improved drug efficiency, targeted drug design becomes more and more important: the next generation RAPTA derivatives and analogues were designed to have multiple modes of activity, functionalized to achieve specific outcomes.[6]

The glutathione transferase (GST) P1-1, a cytosolic detoxification enzyme, is often found in solid tumours, and its overexpression after exposure to anticancer agents has been reported.[68] Ethacrynic acid is an effective GST inhibitor, particularly with GST P1-1, where it binds competitively to the H-site.[69] As proof of concept, the RAPTA derivatives **23** and **24** (Fig. 7), in which the

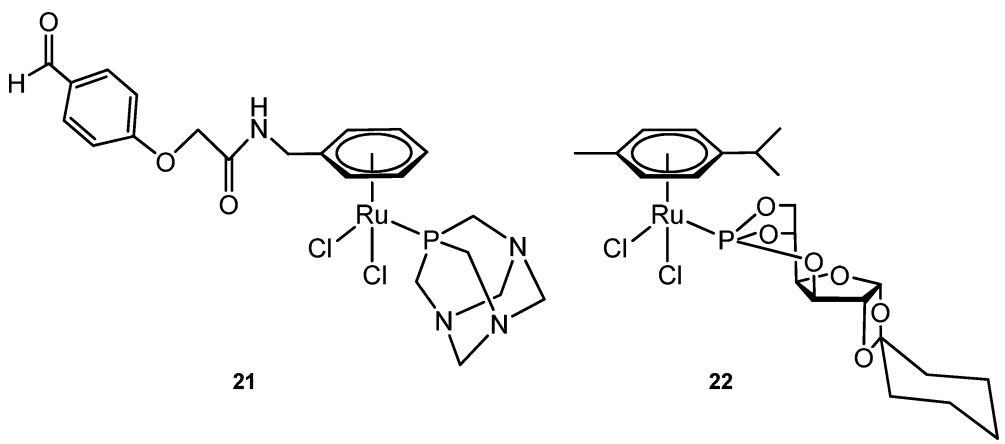
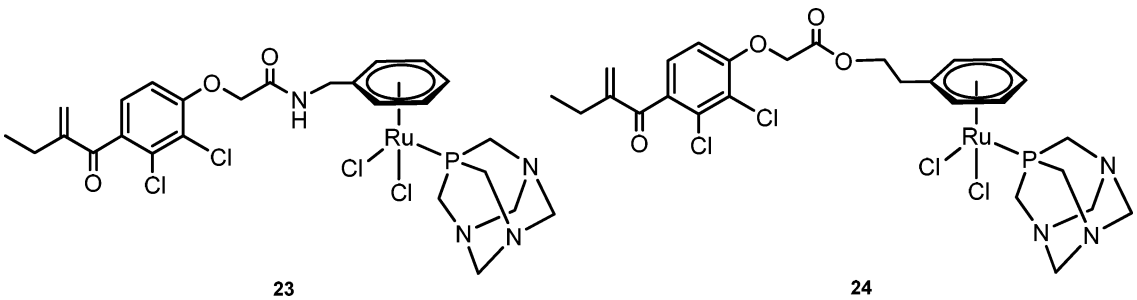


Fig. 6 RAPTA derivatives and analogues for drug delivery studies.



23 **24**

Fig. 7 Ethacrynic acid RAPTA derivatives as glutathione transferase inhibitors.

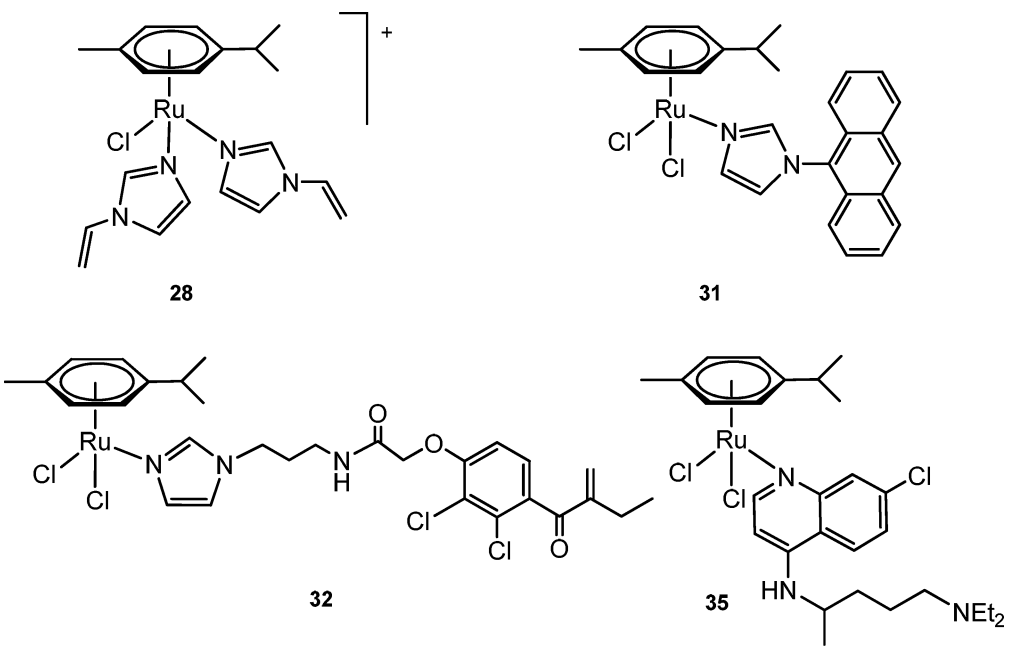
arene ligand is coupled to ethacrynic acid either by an amide or by an ester bond, have been synthesized and shown to be excellent GST P1-1 inhibitors.[70]

Apart from the complexes of the RAPTA family containing the water-soluble pta as *P*-donor ligand, mononuclear arene ruthenium complexes containing *N*-donor ligands have also been reported as anticancer agents, in particular complexes containing imidazole ligands. Inspired by the work of Keppler and Sava: the compounds [indH]*trans*-[Ru(*N*-ind)$_2$Cl$_4$] (KP1019) and [imiH]*trans*-[Ru(*N*-imi)(*S*-dmso)Cl$_4$] (NAMI-A) (see Fig. 2), which are already in clinical trials,[4] contain imidazole or indazole ligands. The low systemic toxicity of KP1019 is attributed, at least in part, to transferrin-mediated drug transport,[71] with KP1019 binding strongly to transferrin in the iron-binding pocket.[72] Its imidazolium analogue, which binds more weakly to transferrin is taken up less effectively by the cells.[73]

In an attempt to synergistically combine the arene ruthenium piano-stool characteristics of the RAPTA complexes with the imidazole ligand properties of the NAMI-A system, Dyson synthesized a whole series of arene imidazole complexes such as (η^6-*p*-MeC$_6$H$_4$Pr^i)Ru(*N*-imiR)Cl$_2$ (imiR = *N*-methylimidazole **25**, *N*-benzoylimidazole **26**), [(η^6-*p*-MeC$_6$H$_4$Pr^i)Ru(*N*-imiR)$_2$Cl]$^+$ (imiR = *N*-methylimidazole **27**, *N*-vinylimidazole **28**) (Fig. 8) and [(η^6-*p*-MeC$_6$H$_4$Pr^i)Ru(*N*-imiR)$_3$]$^{2+}$ (imiR = *N*-methylimidazole **29**), which showed essentially the same order of cytotoxicity as the RAPTA compounds toward cancer cells.[74]

Imidazole ligands also allow the introduction of substituents that endow biological function to the arene ruthenium unit. Thus, phenoxazine- and anthracene-based multidrug resistance modulator substituents have been introduced *via* an imidazole linker in (η^6-*p*-MeC$_6$H$_4$Pr^i)Ru(*N*-imiR)Cl$_2$ (imiR = *N*-phenoxbenzimidazole **30**, *N*-anthramimidazole **31**) in an effort to develop antitumour drugs that overcome multidrug resistance mechanisms; for the most powerful P-glycoprotein inhibitor **31** (Fig. 8), the inhibition of DNA synthesis is a possible mechanism of the cytotoxic action.[75]

A family of arene ruthenium complexes containing an amide of the GST inhibitor ethacrynic acid in an imidazole ligand was also synthesized by Dyson. Apart from the dichloro derivative (η^6-*p*-MeC$_6$H$_4$Pr^i)Ru(*N*-L)Cl$_2$ (L = ethacrynic *N*-imidazole propyl amide, **32**) (Fig. 8), the oxalato and 1,1-cyclobutanedicarboxylato complexes (η^6-*p*-MeC$_6$H$_4$Pr^i)Ru(*N*-L)(*O*,*O*-C$_2$O$_4$) (**33**) and (η^6-*p*-MeC$_6$H$_4$Pr^i)Ru(*N*-L)(*O*,*O*-C$_6$H$_6$O$_4$) (**34**) have been shown to inhibit GST P1-1 and its cysteine-modified mutants and to be



28 **31** **32** **35**

Fig. 8 Cytotoxic imidazole and chloroquine arene ruthenium complexes.

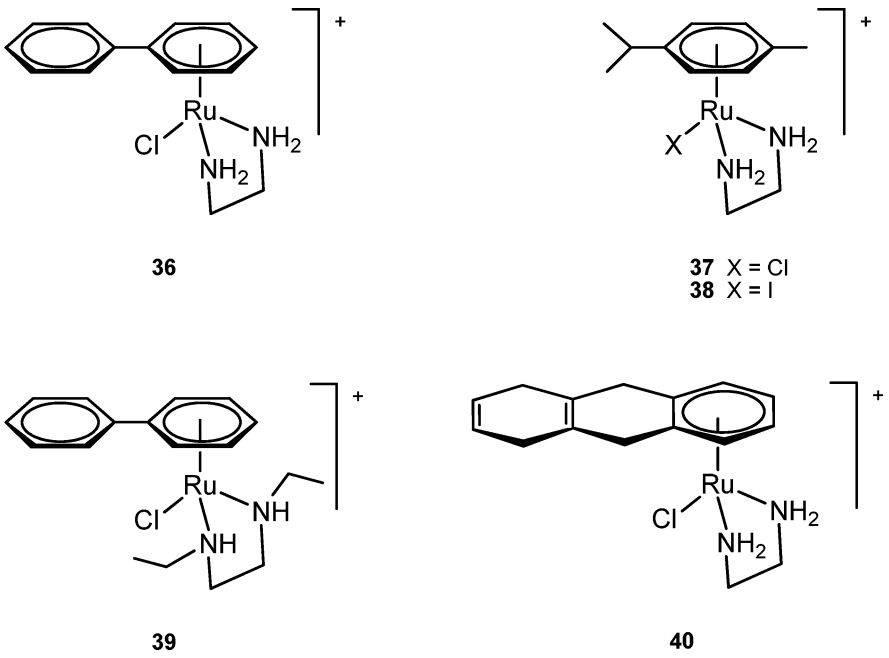


Fig. 9 Cytotoxic arene ruthenium complexes containing ethylenediamine ligands.

active against human ovarian cancer cell lines.[76] The chloroquine (clq) complex (η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*-clq)Cl_2 (**35**) (Fig. 8), recently designed by Sánchez-Delgado, not only has antimalarial properties but also a remarkable anticancer activity *in vitro*, especially towards liposarcoma cell lines.[77]

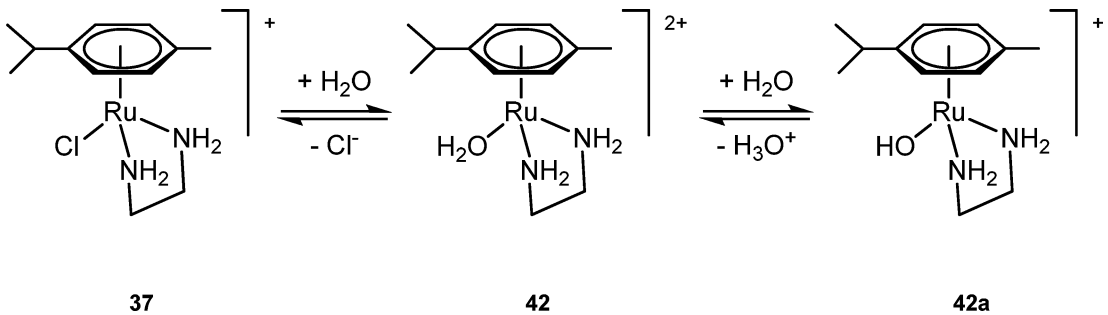
Mononuclear arene ruthenium complexes with *N*,*N*-, *N*,*O*- or *O*,*O*-chelating ligands

In contrast to the phosphine complexes of the RAPTA family that have only a low cytotoxicity *in vitro* but a high metastatic activity *in vivo*,[47] thus being inactive against primary tumours but active towards secondary metastasis tumours,[16] the cationic arene ruthenium complexes containing ethylenediamine chelating ligands reported by Sadler (Fig. 9) show very high cytotoxicities *in vitro* as hexafluorophosphate salts, and they are also very active *in vivo*.

Thus, [(η^6-C_6H_5Ph)Ru(*N*,*N*-en)Cl]$^+$ (**36**), [(η^6-*p*-$MeC_6H_4Pr^i$)-Ru(*N*,*N*-en)Cl]$^+$ (**37**) and [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*,*N*-en)I]$^+$ (**38**) as well as the *N*,*N*′-diethylethylenediamine ($enEt_2$) derivative [(η^6-C_6H_5Ph)Ru(*N*,*N*-$enEt_2$)Cl]$^+$ (**39**) inhibit the growth of human ovarian cancer cells (A2780) with IC_{50} values comparable to that of carboplatin (6 μM),[44] while the more hydrophobic tetrahydroanthracene (tha) derivative [(η^6-tha)Ru(*N*,*N*-en)Cl]$^+$ (**40**) is equipotent with cisplatin (0.6 μM).[78] Interestingly, arene ruthenium complexes containing two monodentate *N*-donor ligands instead of a chelating diamine ligand, such as [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*-$NCMe)_2Cl]^+$ (**41**) are almost inactive toward the A2780 cell line, and structure–activity relationships showed that the most active complexes contain a stable bidentate *N*,*N*-donor ligand, a more hydrophobic arene ligand, and a halide as exchangeable ligand.[44]

It was also shown that in water [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*,*N*-en)Cl]$^+$ (**37**) undergoes rapid aquation to give [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*,*N*-en)(H_2O)]$^{2+}$ (**42**) and [(η^6-*p*-$MeC_6H_4Pr^i$)-Ru(*N*,*N*-en)(OH)]$^+$ (**42a**); this hydrolysis can be suppressed in 0.1 M NaCl (Scheme 2).[44]

The aquation kinetics of the biphenyl, tetrahydroanthracene and dihydroanthracene complexes [(η^6-C_6H_5Ph)Ru(*N*,*N*-en)Cl]$^+$ (**36**), [(η^6-tha)Ru(*N*,*N*-en)Cl]$^+$ (**40**) and [(η^6-dha)Ru(*N*,*N*-en)Cl]$^+$ (**43**) have also been studied: The hydrolysis is essentially independent of ionic strength and increases with the size of the arene, the aquation reaction is about twenty times faster than that of cisplatin, the pK_a values of the corresponding aqua complexes (η^6-C_6H_5Ph)Ru(*N*,*N*-en)(H_2O)]$^{2+}$, [(η^6-tha)Ru(*N*,*N*-en)(H_2O)]$^{2+}$ and



Scheme 2 Hydrolysis of **37** to give **42** and **42a** in water at a 10 micromolar concentration.

$[(\eta^6\text{-dha})Ru(N,N\text{-en})(H_2O)]^{2+}$ being 7.71, 7.89 and 8.01, respectively. The reverse reactions (anation) are also very rapid on addition of 100 mM NaCl (comparable to blood plasma), reaching equilibrium after 100 to 1600 s.[79]

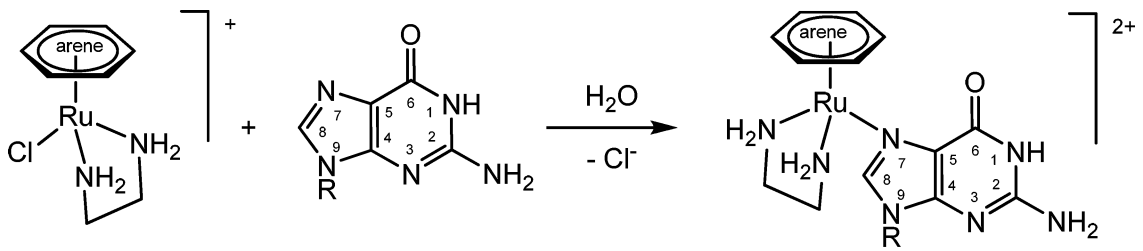
The interaction with DNA model compounds has been extensively investigated by Sadler: in contrast to RAPTA-C, where it was not possible to identify preferential DNA binding sites with the single-strand 14-mer oligonucleotide d(ATACATGGTACATA),[61] the analogous reaction of **37** shows the intermediary aqua complex **42** to ruthenate DNA specifically at guanine positions to give two G7- or G8-monoruthenated derivatives as well as a G7,G8-diruthenated DNA species.[44] The self-complementary 6-mer oligonucleotide d(CGGCCG), which exists single-stranded [ss-d(CGGCCG)] or as a duplex $[d(CGGCCG)_2]$, reveals the effect of base-pairing on the guanine ruthenation: whereas in ss-d(CGGCCG) all three guanine positions are ruthenated with arene ruthenium ethylenediamine units (with excess ruthenium only the triruthenated product being observed), in the duplex $d(CGGCCG)_2$ only G3 and G6 are ruthenated, but not G2. By comparing the *para*-cymene derivative **32**, which cannot act as a DNA intercalator, and the biphenyl derivative **36**, which is a potential DNA intercalator, indeed intercalation of the non-coordinated phenyl ring of **36** between G3 and C4 or G6 and C5 is observed in the mono- and diruthenated duplexes, together with weakening of the (G)O $\cdots$ H(en) hydrogen bonding.[80]

In a comparative study with various DNA bases, it was established that arene ruthenium ethylenediamine units bind preferentially to N7 of guanine. NMR observation of the species produced after 24 hours by reacting complex **36** with guanosine, inosine, thymidine, cytidine and adenosine revealed the base selectivity order G(N7) > I(N7) > I(N1) > T(N3) > C(N3) > A(N7) > A(N1) > G(N1), which can be rationalized in terms of hydrogen bonding attraction or repulsion.[81]

In order to gain insight into the interaction factors including covalent ruthenium–guanine bonding, hydrophobic interactions of the arene ligand and NH hydrogen bonding of the ethylenediamine ligand, complexes $[(\eta^6\text{-}C_6H_5Ph)Ru(N,N\text{-en})Cl]^+$ (**36**), $[(\eta^6\text{-tha})Ru(N,N\text{-en})Cl]^+$ (**40**) and $[(\eta^6\text{-dha})Ru(N,N\text{-en})Cl]^+$ (**43**) were reacted with the guanine derivatives 9-ethylguanine (guaEt), guanosine (guaRi) and guanosine 5′-monophosphate (guaRP), see Scheme 3; the products were studied in the solid state by X-ray crystallography and in solution by 2D NMR methods.[82] In all cases, the arene ruthenium ethylenediamine unit was found to coordinate to the N7 atom of the guanine derivative. In the crystal structure of $[(\eta^6\text{-}C_6H_5Ph)Ru(N,N\text{-en})(N7\text{-guaEt})][PF_6]_2\cdot MeOH$ (cation **44**), there is intermolecular stacking between the pendant phenyl ring and the six-membered purine ring, while strong π–π arene–nucleobase stacking is present in the crystal structures of $[(\eta^6\text{-tha})Ru(N,N\text{-en})(N7\text{-guaEt})][PF_6]_2\cdot MeOH$ (cation **45**) and $[(\eta^6\text{-dha})Ru(N,N\text{-en})(N7\text{-guaEt})][PF_6]_2\cdot 2MeOH$ (cation **46**); in $[(\eta^6\text{-}C_6H_5Ph)Ru(N,N\text{-en})(N7\text{-guaRi})][PF_6]_2\cdot 3.75H_2O$ (cation **47**) intramolecular stacking between the pendant phenyl ring and the purine five-membered ring is observed. In all these structures strong stereospecific hydrogen bonding is present between an en NH group and the C6 carbonyl group of the guanine system, suggesting that simultaneous covalent coordination, intercalation and stereospecific hydrogen bonding are involved in the DNA recognition behaviour of arene ruthenium ethylenediamine complexes.[82]

Modification of natural DNA in a cell-free medium by the arene ruthenium ethylenediamine complexes **36**, **37**, **40** and **43**, as well as the benzene parent complex $[(\eta^6\text{-}C_6H_6)Ru(N,N\text{-en})Cl]^+$ (**48**), have been studied by atomic absorption, melting behaviour, transcription mapping, circular and linear dichroism, plasmid unwinding, competitive ethidium displacement and differential pulse polarography. The results indicate that the arene ruthenium ethylenediamine units bind preferentially to guanine residues in double-helical DNA. The data are consistent with DNA binding of complexes containing biphenyl, dihydroanthracene or tetrahydridoanthracene ligands that involves combined covalent Ru–N (guanine N7) coordination and non-covalent hydrophobic interactions between the arene ligand and DNA, which may include arene intercalation and minor groove binding. In contrast, the single arene rings in the *para*-cymene and benzene complexes cannot interact with double-helical DNA by intercalation.[83]

Although arene ruthenium ethylenediamine complexes seem to target the DNA of the cancer cells, reactions with other biologically relevant molecules have been studied, in particular with amino acids and peptides, in order to see if the metal complex undergoes chemical transformations in the cell culture medium, the blood plasma, the cell membrane or in the cytoplasma before it reaches the DNA target, or if serum proteins may even be targets of these anticancer agents. The most important biomolecules studied in this respect by Sadler include the sulfur-containing amino acids *L*-cysteine and *L*-methionine, the imidazole-containing amino acid *L*-histidine, the tripeptide glutathione, an abundant intracellular thiol responsible for the detoxification of heavy transition metal ions, and the electron-transfer protein cytochrome-c.



arene = bph **36** R = ethyl *(9-ethylguanine)* **guaEt** arene = bph, R = Et **44**
arene = tha **40** R = *β-D*-ribosyl *(9-β-D-ribosylguanine)* **guaRi** arene = tha, R = Et **45**
arene = dha **43** R = *β-D*-ribosylphosphate *(guanosine 5′-monophosphate)* **guaRP** arene = dha, R = Et **46**

Scheme 3 Coordination of arene ruthenium ethylenediamine fragments to guanine derivatives (hydrogen bonding not shown).

From the reaction of the biphenyl derivative **36** with *L*-cysteine ($cysH_2$), six products have been identified by HPLC, LC-ESI-MS and NMR techniques. After 48 hours, only 50% of **36** had reacted, and the final products were the dinuclear complexes [(η^6-C_6H_5Ph)Ru(H_2O)(μN,*S*-cys)Ru(η^6-C_6H_5Ph)(*N*,*N*-en)]$^{2+}$ (**48**) and (η^6-C_6H_5Ph)Ru(H_2O)(μN,*S*-cys)(μN,*S*,*O*-cys)Ru(η^6-C_6H_5Ph) (**49**). From the analogous reaction with *L*-methionine (metH), only the sulfur-bound complex [(η^6-C_6H_5Ph)Ru(*N*,*N*-en)(*S*-metH)]$^{2+}$ (**50**) was detected, with only 27% completion after 48 hours.[84] The reaction of **36** with *L*-histidine ($hisH_2$) to give the isomeric products [(η^6-C_6H_5Ph)Ru(*N*,*N*-en)(*N1*-$hisH_2$)]$^{2+}$ (**51**) and [(η^6-C_6H_5Ph)Ru(*N*,*N*-en)(*N3*-$hisH_2$)]$^{2+}$ (**52**) was found to be slow, too; after 24 hours the equilibrium was reached with 22% completion.[85]

When the biphenyl complex **36** was reacted with glutathione (glSH), a peptide formed from the three amino acids *L*-glutamic acid, *L*-cysteine and glycine under physiologically relevant conditions (millimolar concentrations), the sulfur-bound glutathionato complex [(η^6-C_6H_5Ph)Ru(*N*,*N*-en)(*S*-glS)]$^+$ (**53**) and its oxidation product [(η^6-C_6H_5Ph)Ru(*N*,*N*-en)(*S*-glSO)]$^+$ (**54**) containing a sulfenato function were formed. When the reaction was done in an argon atmosphere, only **53** was detected, proving that **54** arises from the reaction with molecular oxygen from air.[86] A sulfenato complex of this type, [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*,*N*-en)(*S*-S(O)Pr^i)]$^+$ (**55**), was isolated from the reaction of the corresponding thiolato complex [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*,*N*-en)(*S*-SPr^i)]$^+$ (**56**) with hydrogen peroxide and structurally characterized as the iodide salt.[87] Complex **36** was also found to react with the electron transfer protein cytochrome-c to give two monoruthenated protein adducts thought to contain a ruthenium bound to the N-terminus or to a carboxylate.[85]

In the competitive reaction of **36** with glutathione and with guanosine-3′,5′-cyclic monophosphate (cGMP) under physiological conditions (pH 7, 310 K, 20 μM **36**, 250 equivalents glSH, 25 equivalents cGMP, 10 mM phosphate buffer, 22 mM NaCl), the major product was [(η^6-C_6H_5Ph)Ru(*N*,*N*-en)(*N7*-cGMP)]$^{2+}$ (**57**), but **53** and **54** were also observed.[86] The competitive reaction of **36** (0.2 mM) with cytochrome-c (0.1 mM) and with the 14-mer oligonucleotide d(ATACATGGTACATA) (0.1 mM) showed that 90% of the oligonucleotide had reacted to give mono- or diruthenated adducts.[85] These results clearly suggest that in the cells, DNA (or RNA) are the favoured targets of arene ruthenium ethylenediamine complexes.[41] Radiolabelling studies with the hexafluorophosphate salt of the fluorene (flu) complex [(η^6-flu)Ru(*N*,*N*-en)Cl]$^+$ (**58**) containing the radionucleide ^{106}Ru showed that ^{106}Ru is well distributed throughout the rat tissues 15 minutes after intravenous injection of a saline solution at a dose of 10 mg per kg to a living rat, the highest level being found in liver and kidney.[88]

In an extension to other *N*,*N*-chelating ligands, Sadler observed a loss of cytotoxicity, when in complexes of the type [(η^6-arene)Ru(*N*,*N*-en)Cl]$^+$ the σ-donor chelating ligand ethylenediamine was replaced by 2,2′-bipyridine (bipy) and its derivatives, which are also strong π-acceptors. Thus, [(η^6-C_6H_5Ph)Ru(*N*,*N*-bipy)Cl]$^+$ (**59**) is almost inactive toward human ovarian and lung cancer cell lines; it was shown that initial aquation is followed by partial arene loss.[89] Incorporation of methyl, hydroxymethyl or methylester groups in the 4,4′ positions of the bipyridine ligand did not restore the activity. However, complexes containing 2,2′-bipyridine-3,3′-diol [bipy($OH)_2$] as chelating ligand showed a dramatic increase in the anticancer activity; in aqueous solution only neutral complexes with a deprotonated chelating bipyOHO ligand are present over a pH range from 2 to 10, such as [(η^6-tha)Ru(*N*,*N*-bipyOHO)Cl]$^+$ (**60**), see Fig. 10. Complex **60** also strongly binds to 9-ethylguanine; the X-ray crystal structure analysis of [(η^6-C_6H_5Ph)Ru(*N*,*N*-bipyOHO)(guaEt)][PF_6] shows intramolecular CH–π interactions between the arene ligand and the bipy system, and DFT calculations suggest that their interactions are more stable than π–π interactions between the arene ligand and the guanine system.[90]

Arene ruthenium complexes containing *N*,*O*- and *O*,*O*-chelating ligands have also been studied by Sadler, in particular the complexes (η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*,*O*-gly)Cl (**61**), (η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*,*O*-ala)Cl (**62**), (η^6-C_6H_5Ph)Ru(*N*,*O*-pro)Cl (**63**), derived from the amino acids glycine (glyH), L-alanine (alaH), and proline (proH), the oxinato complex [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*,*O*-oxi)Cl]$^+$ (**64**) (oxiH = 8-hydroyquinoline), or complexes of the type (η^6-*p*-$MeC_6H_4Pr^i$)Ru(*O*,*O*-acacMe_2)Cl (**65**) (HacacMe_2 = 1,3-dimethylacetylacetone), see Fig. 10. They are also moderately cytotoxic and were shown to coordinate to guanine (N7 binding).[89]

Tethered arene ruthenium amine complexes of the type [η^6:η^1-$C_6H_5(CH_2)_3NH_2$]$RuCl_2$ (**66**) (Fig. 11) hydrolyze rapidly in aqueous solution to give the cationic monoaqua monochloro species but show almost no activity toward human ovarian cancer cells. The dinitrato analogue [η^6:η^1-$C_6H_5(CH_2)_3NH_2$]Ru(*O*-$NO_3)_2$ (**67**) readily ruthenates calf thymus DNA but fails to produce stop sites on pSP73KP plasmid DNA during DNA transcription by an RNA polymerase, suggesting only monofunctional DNA adducts to be formed; this may explain the low cytotoxicities of this type of complex.[91]

Given their inherent catalytic potential, organometallic molecules may lead to potentially new mechanisms of drug action, as compared to purely organic molecules. Sadler showed that the presence of both an iodo ligand and a *para*-substituted phenylazopyridine chelating ligand in the complexes [(η^6-C_6H_5Ph)Ru(*N*,*N*-azpyR)I]$^+$ (R = NMe_2 **68**, R = OH **69**) (Fig. 11) and [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*,*N*-azpyR)Cl]$^+$ (R = NMe_2 **70**, R = OH **71**) confers a remarkable inertness toward ligand substitution (aquation) to these systems. Surprisingly, despite this inertness, these complexes have been found to be highly cytotoxic toward human ovarian (A2780) and human lung (A549) cancer cells (IC_{50} 2–6 μM). Fluorescence trapping experiments suggested that the cytotoxicity arises from an increase in reactive oxygen species (ROS) such as the superoxide radical anion, the hydroxyl and hydroperoxyl radicals, hydrogen peroxide and singlet oxygen, which is due to the depletion of glutathione (glSH), a ROS scavenger, presumably caused by the oxidative coupling of glSH to give glutathione disuldide (glS–Sgl) catalyzed by **68**–**71**.[92]

The paullone-derived ruthenium complexes [(η^6-*p*-$MeC_6H_4Pr^i$)Ru(*N*,*N*-pauR)Cl]$^+$ [R = C_6H_4OH **72**, R = $C_5HNMe(CH_2OH)OH$ **73**] and their osmium analogues **74** and **75** (Fig. 12), synthesized recently by Keppler in the form of their chloride salts, have been found to show a very high antiproliferative activity in three human cancer cell lines, the IC_{50} values being in the submicromolar concentration range.[93]

The fact that in this case ruthenium and osmium analogues do not differ in their biological activity has been interpreted in terms of a different mechanism to those accepted for arene ruthenium

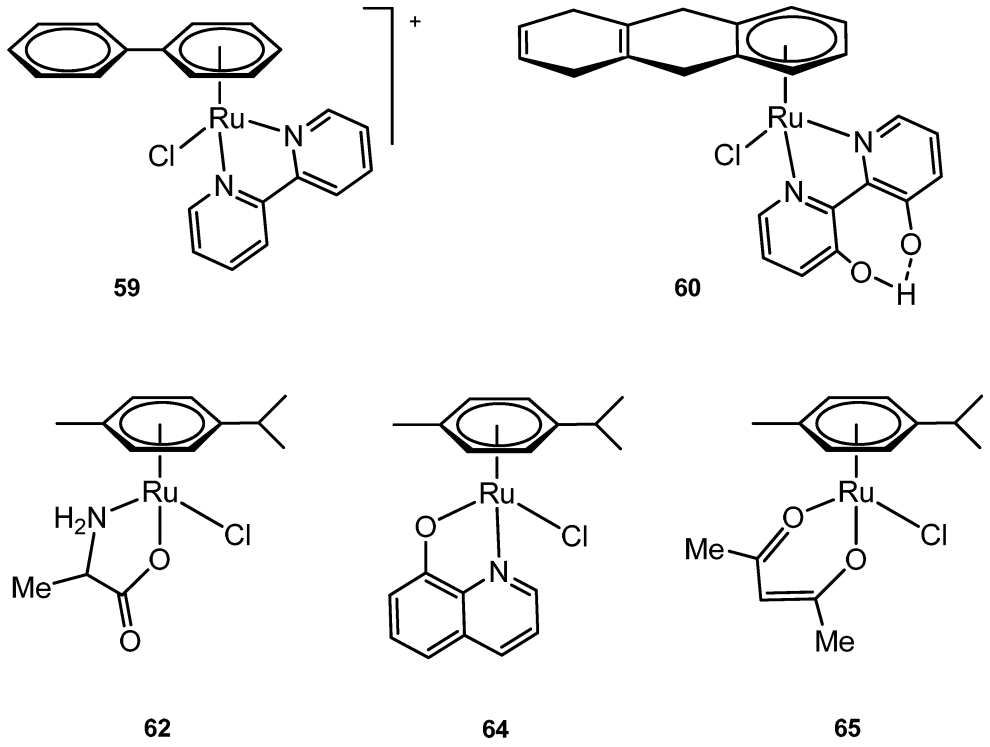


Fig. 10 Cytotoxic arene ruthenium complexes with *N*,*N*-, *N*,*O*- or *O*,*O*-chelating ligands.

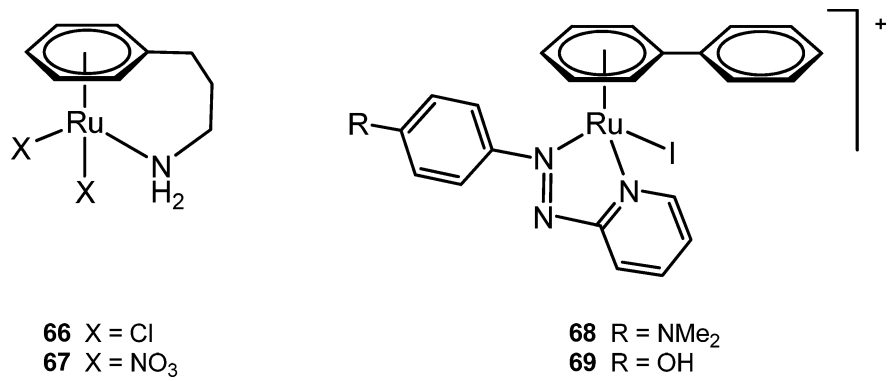


Fig. 11 Arene ruthenium complexes with tethered amine or with azopyridine ligands.

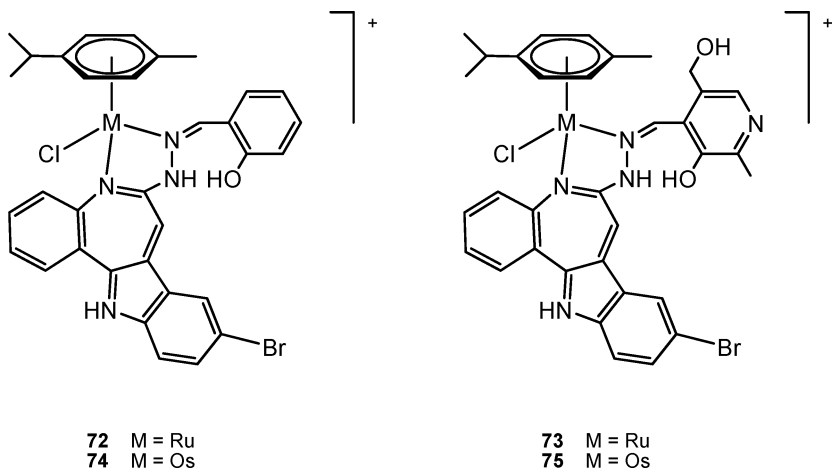


Fig. 12 Arene ruthenium and osmium complexes containing paullone-derived *N*,*N*-ligands.

complexes (covalent DNA binding after hydrolysis), either by non-covalent DNA binding (intercalation) or by interactions with proteins.[93]

Multinuclear arene ruthenium complexes and clusters

In platinum chemistry, trinuclear complexes such as [*trans*, *trans*,*trans*-$(NH_3)_2Pt(Cl)(CH_2)_6NH_2Pt(NH_3)_2NH_2(CH_2)_6NH_2Pt$-$(NH_3)_2(Cl)][NO_3]_4$ (termed BBR3464) were found to show a higher cytotoxicity than cisplatin *in vitro* and,[94] despite clinical failure in phase II,[95] gave rise to expectations that multinuclearity could considerably improve the curative activity of anticancer drugs. This multinuclearity concept was recently transferred to ruthenium.[96]

The dinuclear arene ruthenium complexes (η^6-*p*-$MeC_6H_4Pr^i$)-$Ru(O,O\text{-}C_6H_5O_2N(CH_2)_nNC_6H_5O_2\text{-}OO)Ru(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)$ (**76–81**) containing a pyridone-derived linker (Fig. 13) reported by Hartinger show interesting cytotoxic effects against human ovarian (A2780) and colon (SW480) cancer cell lines. A pronounced influence of the spacer length and cytotoxicity was found (A2780 IC_{50} 25 μM for **77**, 30 μM for **79**, 1,5 μM for **81**, SW480 IC_{50} 62 μM for **77**, 26 μM for **79**, 0.3 μM for **81**), the cytotoxicity being correlated with lipophilicity and water solubility.[97,98] This is in line with the cytotoxicities we found for dinuclear ferrocenyl pyridine arene ruthenium complexes [(η^6-*p*-$MeC_6H_4Pr^i$)$RuCl_2]_2(NC_5H_4OOCC_5H_4FeC_5H_4COOC_5H_4N)$ (**82**) (Fig. 14) and [(η^6-C_6Me_6)$RuCl_2]_2(NC_5H_4OOCC_5H_4FeC_5H_4COO$-$C_5H_4N)$ (**83**).[99]

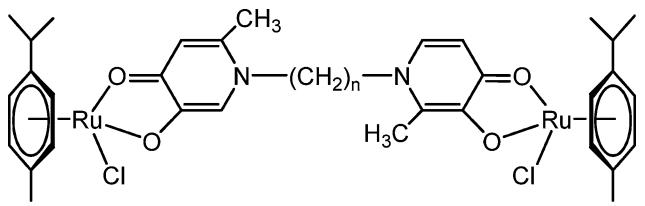


Fig. 13 Dinuclear arene ruthenium complexes with pyridone-based *O*,*O*:*O*,*O*-bridges.

A detailed structure–activity relationship was established based on water/octanol partition and hydrolytic stability, showing that only the most lipophilic long chain (n = 12) complex **81** is highly active.[100] The reactivity of representative complexes towards proteins and nucleotides was also studied: Complex **77** forms a 1:1 adduct with transferrin, but not with other relevant proteins like ubiquitin or cytochrome-c, and complexes **79** and **80** react with guanosine 5′-monophosphate (guaRP) and with adenosine 5′-monophosphate (adeRP), but not with other nucleotides; the reaction of **79** and **80** with calf thymus DNA resulted in a high degree of ruthenation. These results demonstrate that both proteins and nucleobases are potential targets for these arene ruthenium complexes.[100]

Dinuclear arene ruthenium complexes containing 2,3-bis(2-pyridyl)pyrazine (dpp) as doubly chelating ligands in the *N*,*N*:*N*,*N*-bridge have been synthesized and studied for photoactivation by Sadler in view of their potential for photodynamic therapy: while the benzene and indane derivatives [{(η^6-C_6H_6)RuCl}$_2$(*N*,*N*:*N*,*N*-dpp)]$^{2+}$ (**84**) and [{(η^6-C_9H_{10})RuCl}$_2$(*N*,*N*:*N*,*N*-dpp)]$^{2+}$ (**85**) (Fig. 14) readily undergo arene loss upon UV irradiation, the *para*-cymene and hexamethylbenzene derivatives [{(η^6-*p*-$MeC_6H_4Pr^i$)RuCl}$_2$(*N*,*N*:*N*,*N*-dpp)]$^{2+}$ (**86**) and [{(η^6-C_6Me_6)RuCl}$_2$(*N*,*N*:*N*,*N*-dpp)]$^{2+}$ (**87**) do not. The photochemistry of the indane derivative **85** was studied in detail. In water, aquation occurs in the dark; UV or visible light leads to a dissociation of the indane ligand, visualized by its fluorescence, and to the formation of strong diruthenium DNA adducts. These complexes therefore have the potential to combine both photoinduced cell death and fluorescence imaging of the location and the efficiency of the photoactivation process.[101]

We found the water-soluble chloride or tetrafluoroborate salts of the cationic trinuclear arene ruthenium clusters [(η^6-$C_6Me_6)_2(\eta^6\text{-}C_6H_6)Ru_3(\mu_2\text{-}H)_3(\mu_3\text{-}O)]^+$ (**88**) and [(η^6-C_6Me_6)(η^6-*p*-$MeC_6H_4Pr^i$)(η^6-C_6H_6)$Ru_3(\mu_2$-H)$_3(\mu_3$-O)]$^+$ (**89**) to be cytotoxic against human ovarian cancer cells with A2780 IC_{50} values of 9.8 and 9.1 μM, respectively, while the tetranuclear cluster cations [(η^6-$C_6H_6)_4Ru_4(\mu_3\text{-}H)_4]^{2+}$ (**90**) and [(η^6-$C_6H_5Me)_4Ru_4(\mu_3$-H)$_4]^{2+}$ (**91**), which contain the same type of ligands and are of comparable size, do not display significant cytotoxicities. This striking difference between Ru_3 and Ru_4 cluster cations (Fig. 15), all of which are

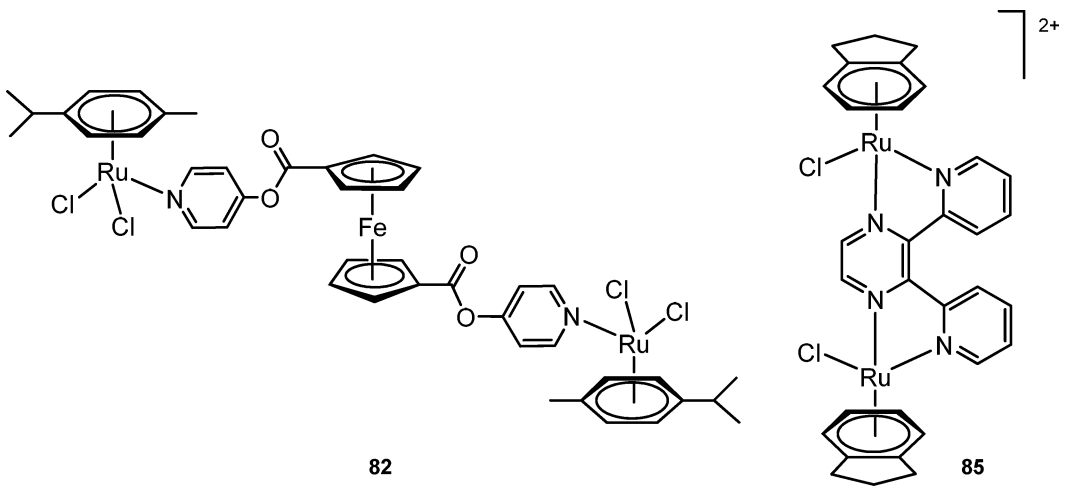


Fig. 14 Dinuclear arene ruthenium complexes with nitrogen-containing bridging ligands.

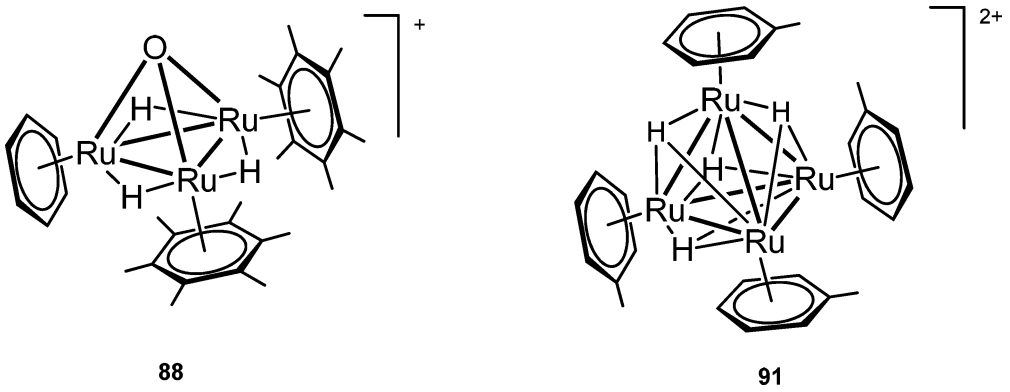


Fig. 15 Tri- and tetranuclear arene ruthenium cluster cations.

stable in aqueous solution, was explained by supramolecular interactions with biomolecules possible for the trinuclear clusters thanks to an open hydrophobic pocket spanned by the three arene ligands and to the hydrophilic oxo cap prone to form hydrogen bonds, both of which are not possible for the tetranuclear clusters.[102]

Tetranuclear arene ruthenium complexes containing the porphyrin scaffold have been designed for photodynamic therapy (PDT) in an effort to combine the photodynamic action of porphyrin (presumably by singlet oxygen production) with the cytotoxicity of ruthenium (presumably by DNA denaturation). With 5,10,15,20-tetra(4-pyridyl)porphyrin (4-tpp) as the central unit, the *para*-cymene and toluene derivatives $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)RuCl_2]_4$(4-tpp) (**92**) and $[(\eta^6\text{-}C_6H_5Me)RuCl_2]_4$(4-tpp) (**93**) (Fig. 16) proved to be very efficient against ME300 and ME275 melanoma cells: While the cytotoxicities in the dark are not very high (IC_{50} > 80 μM), the complexes become very cytotoxic upon exposure to light (laser 652 nm), a light dose of 5 J/cm^2 already causes a phototoxicity of 60–80%, and a light dose of 30 J/cm^2 90–95%. The ruthenation of the porphyrin system not only increases the solubility of the system (in aqueous solution aquation of the chloro ligands being possible), but also the selectivity for cancer cells. Fluorescence microscopy revealed that, while the ruthenated 4-tpp derivatives **92** and **93** are internalized in ME300 cells, the isoelectronic pentamethylcyclopentadienyl (Cp*) rhodium derivative $[(\eta^5\text{-}C_5Me_5)RhCl_2]_4$(4-tpp) (**94**) does not enter the cells under the same conditions (24 hours incubation in the dark).[103]

The isomeric 5,10,15,20-tetra(3-pyridyl)porphyrin (3-tpp) *para*-cymene and toluene derivatives $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)RuCl_2]_4$(3-tpp) (**95**) and $[(\eta^6\text{-}C_6H_5Me)RuCl_2]_4$(3-tpp) (**96**) (Fig. 16) turned out to be even more efficient, very low light doses (less than 0.5 J/cm^2) and low concentrations (5 μM) are required to induce cell death. The reason for higher efficiency is presumably a less pronounced aggregation of the complexes in the cell cytoplasma. Fluorescence microscopy revealed a homogeneous distribution in the cytoplasma for the 3-tpp derivative **96**, while for the 4-tpp derivative **93** an accumulation of red spots (porphyrin fluorescence) in the cytoplasma is observed.[104]

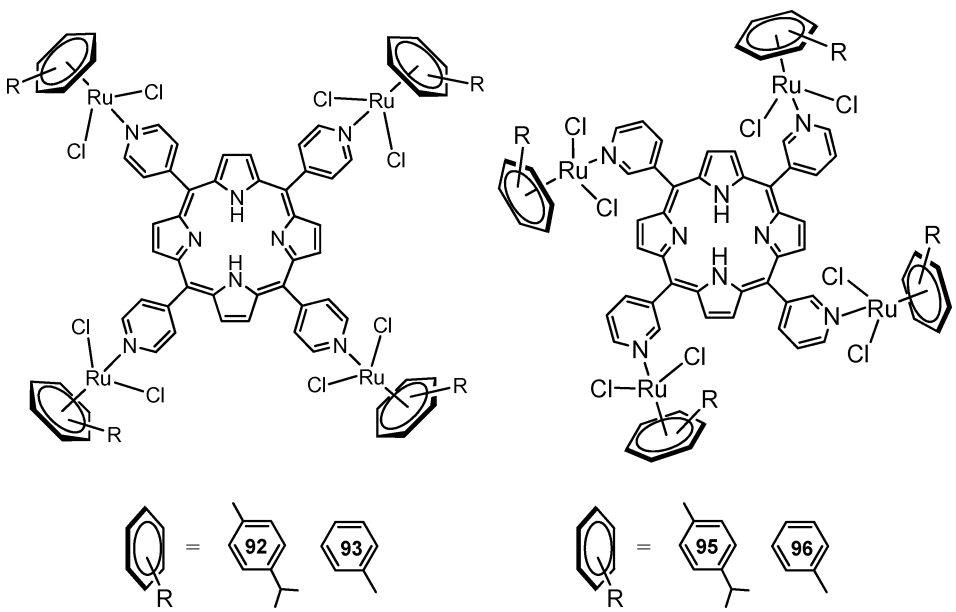


Fig. 16 Tetranuclear arene ruthenium complexes containing a porphyrin scaffold for PDT.

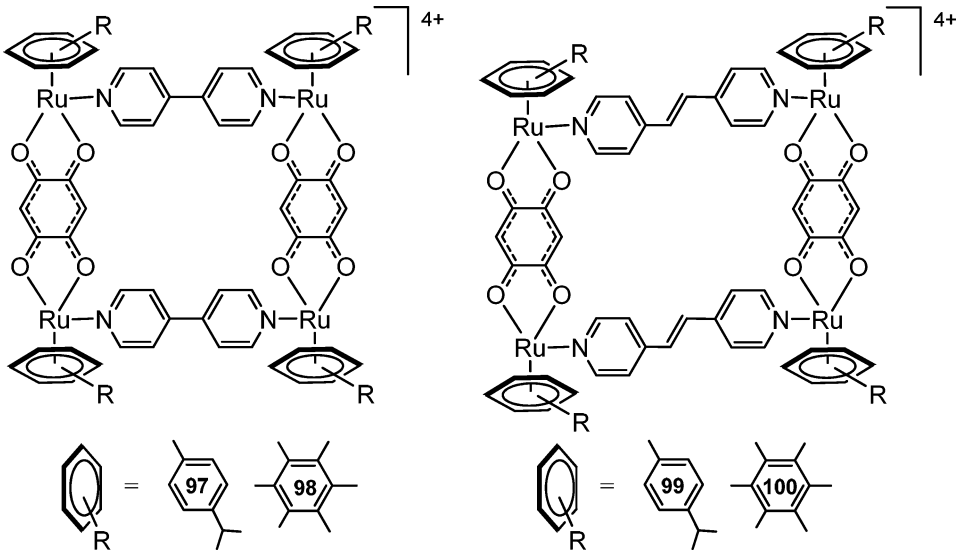


Fig. 17 Tetranuclear arene ruthenium complex cations with rectangular geometry.

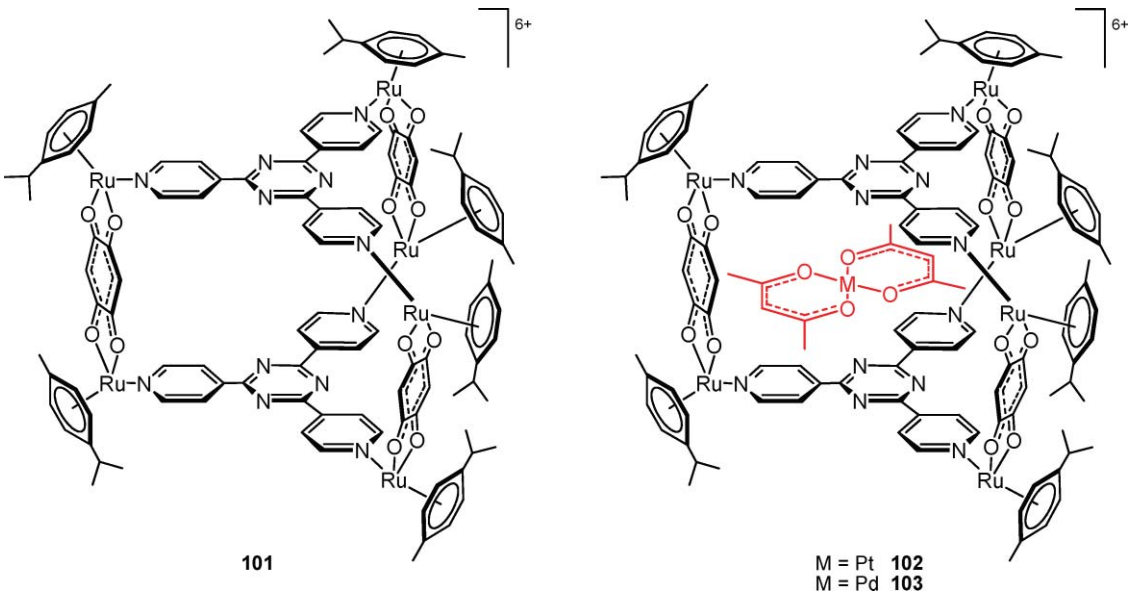
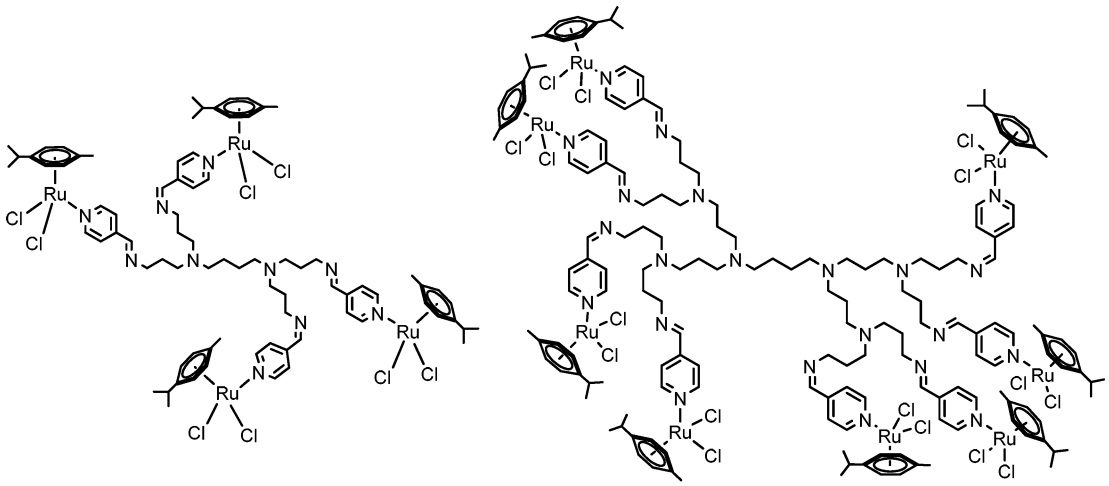


Fig. 18 Hexanuclear arene ruthenium complex cations with cage-like geometry.

We also synthesized rectangular tetranuclear arene ruthenium complex cations incorporating 2,5-dihydroxy-1,4-benzoquinato (dbq) and dipyridyl linkers, which proved to be active against human ovarian (A2780) cancer cells, showing a pronounced size effect: while the smaller rectangles containing bipyridine (bipy) bridges, $[\{(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)Ru\}_4(N,N\text{-bipy})_2(O,O{:}O,O\text{-dbq})_2]^{4+}$ (**97**) and $[\{(\eta^6\text{-}C_6Me_6)Ru\}_4(N,N\text{-bipy})_2(O,O{:}O,O\text{-dbq})_2]^{4+}$ (**98**) are only moderately cytotoxic (IC_{50} 66 and 27 μM, respectively), the larger rectangles containing 1,2-bis(4-pyridyl)ethane (bpe) bridges, $[\{(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)Ru\}_4(N,N\text{-bpe})_2(O,O{:}O,O\text{-dbq})_2]^{4+}$ (**99**) and $[\{(\eta^6\text{-}C_6Me_6)Ru\}_4(N,N\text{-bipy})_2(O,O{:}O,O\text{-bpe})_2]^{4+}$ (**100**) (Fig. 17) show good cytotoxicities (IC_{50} 6 and 4 μM, respectively).[105]

Hexanuclear arene ruthenium complexes that form hexacationic cages have been synthesized using dbq bridges and tripodal 2,4,6-tris(pyridin-4-yl)1,3,5-triazine (tpt) linkers: the cation $[\{(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)Ru\}_6(N,N,N\text{-tpt})_2(O,O{:}O,O\text{-dbq})_3]^{6+}$ (**101**) forms by self-assembly from tpt and the dinuclear precursor $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)Ru]_2(O,O{:}O,O\text{-dbq})_2Cl_2$ in the presence of silver triflate and crystallizes as the triflate salt. If the synthesis is done in the presence of platinum or palladium bisacetylacetonate, the planar complex is encapsulated in the hexaruthenium cage to give the carciplex systems $[(acac)_2M\subset\{(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)Ru\}_6(N,N,N\text{-tpt})_2(O,O{:}O,O\text{-dbq})_3]^{6+}$ (M = Pt **102**, M = Pd **103**), for which we coined the term "complex-in-a-complex" systems (Fig. 18), accessible as triflate salts.[106]

All these systems are active against human ovarian (A2780) cancer cells: The empty hexaruthenium cage **101** has already an IC_{50} value of 23 μM, by using the platinum-containing cage **102** the cytotoxicity doubles (IC_{50} 12 μM), and by using the palladium-containing cage **103** the activity goes up by a factor of twenty (IC_{50} 1 μM), while free $Pt(acac)_2$ and $Pd(acac)_2$ are completely inactive due to their insolubility in water. The working hypothesis of a "Trojan Horse" strategy to deliver a hydrophobic



104 **106**

Fig. 19 Dentritic arene ruthenium complex cations of 1st and 2nd generation.

metal-containing host to a cancer cell by a water-soluble cage molecule produces a synergistic effect, because the cage molecule itself is cytotoxic, and the cytotoxicity is increased by the release of the encapsulated active complex.[106]

The tetra- and octanuclear arene ruthenium complexes [(η^6-*p*-$MeC_6H_4Pr^i$)$RuCl_2$]$_4$(*N:N:N:N*-dendG^1) (**104**), [(η^6-C_6Me_6)-$RuCl_2$]$_4$(*N:N:N:N*-dendG^1) (**105**), [(η^6-*p*-$MeC_6H_4Pr^i$)$RuCl_2$]$_8$-(*N:N:N:N:N:N:N:N*-dendG^2) (**106**) and [(η^6-*p*-C_6Me_6)$RuCl_2$]$_8$-(*N:N:N:N:N:N:N:N*-dendG^2) (**107**), containing the first or second generation of a diaminobutane-based dendrimer with four (dendG^1) or eight (dendG^2) iminopyridyl dendrons (Fig. 19), were found to be moderately cytotoxic (IC_{50} 20–43 μM) without, however, a pronounced size effect.[107]

Outlook

Water-soluble arene ruthenium complexes are an emerging class of molecules for the design of anticancer drugs, not only because of the supposed affinity of cancer cells for the iron homologue ruthenium and because of the low systemic toxicity of many ruthenium compounds, but also because arene ruthenium complexes combine lipophilicity and hydrophilicity, which is very important for their transport in biological media. Since not only additive but synergistic effects have been observed with multinuclear arene ruthenium complexes, and since coupling with molecules of distinct biological function allows targeted chemotherapy, cationic arene ruthenium cage molecules containing other drugs seem to be one of the promising strategies for the development of synergistic anticancer drugs.

References

1 American Cancer Society, Global Cancer - Facts & Figures 2007, p. 1.
2 Council of the European Union, Press release of the 2876th council meeting on employment, social policy, health and consumer affairs, document 10414/08, p. 20.
3 Robert Koch Institut, Federal Health Reporting, Cancer in Germany 2003-2004, sixth edition 2008, p. 18.
4 I. Bratsos, St. Jedner, T. Gianferrara and E. Alessio, *Chimia*, 2007, **61**, 692.
5 B. Rosenberg, L. Vancamp, J. E. Trosko and V. H. Mansour, *Nature*, 1969, **222**, 385.
6 Wee Han Ang and P. J. Dyson, *Eur. J. Inorg. Chem.*, 2006, 4003.
7 J. Reedijk, *Chem. Commun.*, 1996, 801.
8 U. M. Ohndorf, M. A. Rould, Q. He, C. O. Pabo and S. J. Lippard, *Nature*, 1999, **399**, 708.
9 J. Reedijk, *Eur. J. Inorg. Chem.*, 2009, 1303.
10 E. Wong and C. M. Giandomenico, *Chem. Rev.*, 1999, **99**, 2451.
11 M. Galanski, M. A. Jakupec and B. K. Keppler, *Curr. Med. Chem.*, 2005, **12**, 2075.
12 S. B. Fricker, *Dalton Trans.*, 2007, 4903.
13 J. Reedijk, *Platinum Met. Rev.*, 2008, **52**, 2.
14 C. S. Allardayce and P. J. Dyson, *Platinum Met. Rev.*, 2001, **45**, 62.
15 J. Kostova, *Curr. Med. Chem.*, 2006, **13**, 1085.
16 P. J. Dyson and G. Sava, *Dalton Trans.*, 2006, 1929.
17 C. S. Allardyce, A. Dorcier, C. Scolaro and P. J. Dyson, *Appl. Organomet. Chem.*, 2005, **19**, 1.
18 J. R. Durig, J. Danneman, W. D. Behnke and E. E. Mercer, *Chem.-Biol. Interact.*, 1976, **13**, 287.
19 M. J. Clarke, *Met. Ions Biol. Syst.*, 1980, **11**, 231.
20 M. J. Clarke, F. Zhu and D. R. Frasca, *Chem. Rev.*, 1999, **99**, 2511.
21 O. Novakova, J. Kasparkova, O. Vrana, P. M. Vanvliet, J. Reedijk and V. Brabec, *Biochemistry*, 1995, **34**, 12369.
22 P. M. Vanvliet, S. M. S. Toekimin, J. G. Haasnoot, J. Reedijk, O. Novakova, O. Vrana and V. Brabec, *Inorg. Chim. Acta*, 1995, **231**, 57.
23 R. Vilaplana, M. Romero, M. Quiros, J. Salas and F. Gonzalez-Vilches, *Met.-Based Drugs*, 1995, **2**, 211.
24 D. Chatterjee, A. Mitra and G. S. De, *Platinum Met. Rev.*, 2006, **50**, 2.
25 E. Alessio, G. Mestroni, G. Nardin, W. M. Attia, M. Calligaris, G. Sava and S. Zorzet, *Inorg. Chem.*, 1988, **27**, 4099.
26 G. Sava, S. Pacor, S. Zorzet, E. Alessio and G. Mestroni, *Pharmacol. Res.*, 1989, **21**, 617.
27 M. Coluccia, G. Sava, F. Loseto, A. Nassi, A. Bocarelli, D. Giordano, E. Alessio and G. Mestroni, *Eur. J. Cancer*, 1993, **29**, 1873.
28 A. H. Velders, H. Kooijman, A. L. Spek, J. G. Haasnoot, D. de Vos and J. Reedijk, *Inorg. Chem.*, 2000, **39**, 2966.
29 A. C. G. Hotze, S. E. Caspers, D. de Vos, H. Kooijman, A. L. Spek, A. Flamigni, M. Bacac, G. Sava, J. G. Haasnoot and J. Reedijk, *JBIC, J. Biol. Inorg. Chem.*, 2004, **9**, 354.
30 A. D. Kelman, M. J. Clarke, S. D. Edmonds and H. J. Peresie, *J. Clin. Hematol. Oncol.*, 1977, **7**, 274.
31 M. J. Clarke, *Coord. Chem. Rev.*, 2003, **236**, 209.
32 H. Keller and B. Keppler, *US Patent*, 1989, 4843069; cf. B. K. Keppler, K.-G. Lipponer, B. Stenzel and F. Kratzin, in, B. K. Keppler (editor), *Metal Complexes in Cancer Chemotherapy*, VCH, Weinheim, 1993, p. 187.
33 G. Mestroni, E. Alessio and G. Sava, *Int. Patent*, 1998, PCT C 07F 15/00, A61K 31/28, WO 98/00431; cf. G. Mestroni, E. Alessio, G.

Sava, S. Pacor and M. Coluccia, in, B. K. Keppler (editor), *Metal Complexes in Cancer Chemotherapy*, VCH, Weinheim, 1993, p. 157.
34 G. Sava, A. Bergamo, S. Zorzet, B. Gava, C. Casrsa, M. Cocchietto, A. Furlani, V. Scarcia, B. Serli, E. Iengo, E. Alessio and G. Mestroni, *Eur. J. Cancer*, 2002, **38**, 427.
35 B. Cornils and W. A. Herrmann (editors), *Aqueous-Phase Organometallic Catalysis*, Wiley-VCH, Weinheim, 1998.
36 F. Joó, *Aqueous Organometallic Catalysis*, Kluwer, Dordrecht, 2001.
37 G. Jaouen (editor), *Bioorganometallics*, Wiley-VCH, Weinheim, 2006.
38 C. G. Hartinger and P. J. Dyson, *Chem. Soc. Rev.*, 2009, **38**, 391.
39 G. Jaouen and A. Vessières, *Pure Appl. Chem.*, 1985, **57**, 1865.
40 P. J. Dyson, *Chimia*, 2007, **61**, 698.
41 S. J. Dougan and P. J. Sadler, *Chimia*, 2007, **61**, 704.
42 L. D. Dale, J. H. Tocher, T. M. Dyson, D. I. Edwards and D. A. Tocher, *Anti-Cancer Drug Design*, 1992, **7**, 3.
43 C. S. Allardyce, P. J. Dyson, D. J. Ellis and S. L. Heath, *Chem. Commun.*, 2001, 1396.
44 R. E. Morris, R. E. Aird, P. d. S. Murdoch, H. Chen, J. Cummings, N. D. Hughes, S. Pearsons, A. Parkin, G. Boyd, D. I. Jodrell and P. J. Sadler, *J. Med. Chem.*, 2001, **44**, 3616.
45 M. Melchart and P. J. Sadler, in, G. Jaouen (editor), *Bioorganometallics*, Wiley-VCH, Weinheim, 2006, p. 39.
46 L. Ronconi and P. J. Sadler, *Coord. Chem. Rev.*, 2007, **251**, 1633.
47 C. Scolaro, A. Bergamo, L. Brescacin, R. Delfino, M. Cocchietto, G. Laurenczy, T. J. Geldbach, G. Sava and P. J. Dyson, *J. Med. Chem.*, 2005, **48**, 4161.
48 C. S. Allardyce, P. J. Dyson, D. E. Ellis, P. A. Salter and R. Scopelliti, *J. Organomet. Chem.*, 2003, **668**, 35.
49 W. H. Ang, E. Daldini, C. Scolaro, R. Scopelliti, L. Juillerat-Jeanneret and P. J. Dyson, *Inorg. Chem.*, 2006, **45**, 9006.
50 C. A. Vock, A. K. Renfrew, R. Scopelliti, L. Juillerat-Jeanneret and P. J. Dyson, *Eur. J. Inorg. Chem.*, 2008, 1661.
51 A. K. Renfrew, A. D. Phillips, A. E. Egger, C. G. Hartinger, S. S. Bosquain, A. A. Nazarov, B. K. Keppler, L. Gonsalvi, M. Peruzzini and P. J. Dyson, *Organometallics*, 2009, **28**, 1165.
52 C. Scolaro, T. J. Geldbach, S. Rochat, A. Dorcier, C. Gossens, A. Bergamo, M. Cocchietto, I. Tavernelli, G. Sava, U. Rothlisberger and P. J. Dyson, *Organometallics*, 2006, **25**, 756.
53 B. Serli, E. Zangrando, T. Gianferrara, C. Scolaro, P. J. Dyson, A. Bergamo and E. Alessio, *Eur. J. Inorg. Chem.*, 2005, 3423.
54 I. Bratsos, S. Jedner, A. Bergamo, G. Sava, T. Gianferrara, E. Zangrando and Enzo Alessio, *J. Inorg. Biochem.*, 2008, **102**, 1120.
55 B. Dutta, C. Scolaro, R. Scopelliti, P. J. Dyson and K. Severin, *Organometallics*, 2008, **27**, 1355.
56 W. H. Ang, Z. Grote, R. Scopelliti, L. Juillerat-Jeanneret and K. Severin, *J. Organomet. Chem.*, 2009, **694**, 968.
57 A. Dorcier, W. H. Ang, S. Bolaño, L. Gonsalvi, L. Juillerat-Jeanneret, G. Laurenczy, M. Peruzzini, A. D. Phillips, F. Zanobini and P. J. Dyson, *Organometallics*, 2006, **25**, 4090.
58 C. Scolaro, C. G. Hartinger, C. S. Allardyce, B. K. Keppler and P. J. Dyson, *J. Inorg. Biochem.*, 2008, **102**, 1743.
59 M. Groessl, C. G. Hartinger, P. J. Dyson and B. K. Keppler, *J. Inorg. Biochem.*, 2008, **102**, 1060.
60 A. Dorcier, C. G. Hartinger, R. Scopelliti, R. H. Fish, B. K. Keppler and P. J. Dyson, *J. Inorg. Biochem.*, 2008, **102**, 1066.
61 A. Dorcier, P. J. Dyson, C. Goessens, U. Rothlisberger, R. Scopelliti and I. Tavernelli, *Organometallics*, 2005, **24**, 2114.
62 I. W. McNae, K. Fishburne, A. Habtemariam, T. M. Hunter, M. Melchart, F. Wang, M. D. Walkinshaw and P. J. Sadler, *Chem. Commun.*, 2004, 1786.
63 A. Casini, G. Mastrobuoni, W. H. Ang, C. Gabbiani, G. Pieraccini, G. Moneti, P. J. Dyson and L. Messori, *ChemMedChem*, 2007, **2**, 631.
64 A. Casini, C. Gabbiani, F. Sorrentino, M. P. Rigobello, A. Bindoli, T. J. Geldbach, A. Marrone, N. Re, C. G. Hartinger, P. J. Dyson and L. Messori, *J. Med. Chem.*, 2008, **51**, 6773.
65 S. Chatterjee, S. Kundu, A. Bhattacharyya, C. G. Hertinger and P. J. Dyson, *JBIC, J. Biol. Inorg. Chem.*, 2008, **13**, 1149.
66 W. H. Ang, E. Daldini, L. Juillerat-Jeanneret and P. J. Dyson, *Inorg. Chem.*, 2007, **46**, 9048.
67 I. Berger, M. Hanif, A. A. Nazarov, C. G. Hartinger, R. O. John, M. L. Kuznetsov, M. Groessl, F. Schmitt, O. Zava, F. Biba, V. B. Arion, M. Galanski, M. A. Jakupec, L. Juillerat-Jeanneret, P. J. Dyson and B. K. Keppler, *Chem.–Eur. J.*, 2008, **14**, 9046.
68 D. M. Townsend, V. L. Findlay and K. D. Tew, *Methods Enzymol.*, 2005, **401**, 287.
69 H. W. Lo and F. Ali-Osman, *Curr. Opin. Pharmacol.*, 2007, **7**, 367.
70 W. H. Ang, L. J. Parker, A. De Luca, L. Juillerat-Jeanneret, C. J. Morton, M. Lo Bello, M. W. Parker and P. J. Dyson, *Angew. Chem., Int. Ed.*, 2009, **48**, 3854.
71 A. R. Timerbaev, C. G. Hartinger, S. S. Aleksenko and B. K. Keppler, *Chem. Rev.*, 2006, **106**, 2224.
72 F. Kratz, M. Hartmann, B. K. Keppler and L. Messori, *J. Biol. Chem.*, 1994, **269**, 2581.
73 S. Kapitza, M. Pongratz, M. A. Jakupec, P. Heffeter, W. Berger, L. Lackinger and B. K. Keppler, *J. Cancer Res. Clin. Oncol.*, 2005, **131**, 101.
74 C. A. Vock, C. Scolaro, A. D. Phillips, R. Scopelleti, G. Sava and P. J. Dyson, *J. Med. Chem.*, 2006, **49**, 5552.
75 C. A. Vock, W. H. Ang, C. Scolaro, A. D. Phillips, L. Lagopoulos, L. Juillerat-Jeanneret, G. Sava, R. Scopelleti and P. J. Dyson, *J. Med. Chem.*, 2007, **50**, 2166.
76 W. H. Ang, A. De Luca, C. Chapuis-Bernasconi, L. Jeuillerat-Jeanneret, M. Lo Bello and P. J. Dyson, *ChemMedChem*, 2007, **2**, 1799.
77 C. S. K. Rajapakse, A. Martínez, B. Naoulou, A. A. Jarzecki, L. Suárez, C. Deregnaucourt, V. Sinou, J. Schrével, E. Musi, G. Ambrosini, G. K. Schwartz and R. A. Sánchez-Delgado, *Inorg. Chem.*, 2009, **48**, 1122.
78 R. E. Aird, J. Cummings, A. A. Ritchie, M. Muir, R. E. Morris, H. Chen, P. J. Sadler and D. I. Jodrell, *Br. J. Cancer*, 2002, **86**, 1652.
79 F. Wang, H. Chen, S. Parsons, I. D. H. Oswald, J. E. Davidson and P. J. Sadler, *Chem.–Eur. J.*, 2003, **9**, 5810.
80 H.-K. Liu, F. Wang, J. A. Perkinson, J. Bella and P. J. Sadler, *Chem.–Eur. J.*, 2006, **12**, 6151.
81 H. Chen, J. A. Parkinson, R. E. Morris and P. J. Sadler, *J. Am. Chem. Soc.*, 2003, **125**, 173.
82 H. Chen, J. A. Parkinson, S. Parsons, R. A. Coxall, R. O. Gould and P. J. Sadler, *J. Am. Chem. Soc.*, 2002, **124**, 3064.
83 O. Novakova, H. Chen, O. Vrana, A. Rodger, P. J. Sadler and V. Brabec, *Biochemistry*, 2003, **42**, 11544.
84 F. Wang, H. Chen, J. A. Parkinson, P. d. S. Murdoch and P. J. Sadler, *Inorg. Chem.*, 2002, **41**, 4509.
85 F. Wang, J. Bella, J. A. Parkinson and P. J. Sadler, *JBIC, J. Biol. Inorg. Chem.*, 2005, **10**, 147.
86 F. Wang, J. Xu, A. Habtemariam, J. Bella and P. J. Sadler, *J. Am. Chem. Soc.*, 2005, **127**, 17734.
87 H. Petzold, J. Xu and P. J. Sadler, *Angew. Chem., Int. Ed.*, 2008, **47**, 3008.
88 J. D. Hoeschele, A. Habtemariam, J. Muir and P. J. Sadler, *Dalton Trans.*, 2007, 4974.
89 A. Habtemariam, M. Melchart, R. Fernández, S. Parsons, I. D. H. Oswald, A. Parkin, F. P. A. Fabbiani, J. E. Davidson, A. Dawson, R. E. Aird, D. I. Jodrell and P. J. Sadler, *J. Med. Chem.*, 2006, **49**, 6858.
90 T. Bugarcic, A. Habtemariam, J. Stepankova, P. Heringova, J. Kasparkova, R. J. Deeth, R. D. L. Johnstone, A. Prescimone, A. Parkin, S. Persons, V. Brabec and P. J. Sadler, *Inorg. Chem.*, 2008, **47**, 11470.
91 M. Melchart, A. Habtemariam, O. Novakova, S. A. Moggach, F. P. A. Fabbiani, S. Parsons, V. Brabec and P. J. Sadler, *Inorg. Chem.*, 2007, **46**, 8950.
92 S. J. Dougan, A. Habtemariam, S. E. McHale, S. Parsons and P. J. Sadler, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 11628.
93 W. F. Schmid, R. O. John, V. B. Arion, M. A. Jakupec and B. K. Keppler, *Organometallics*, 2007, **26**, 6643.
94 C. Manzotti, G. Pratesi, E. Menta, R. D. Domenico, E. Cavaletti, H. H. Fiebig, L. R. Kelland, N. Farrell, D. Polizzi, R. Supino, G. Pezzoni and F. Zunino, *Clin. Cancer Res.*, 2000, **6**, 2626.
95 T. A. Hensing, N. H. Hanna, H. H. Gillenwater, M. G. Camboni, C. Allievi and M. A. Socinski, *Anti-Cancer Drugs*, 2006, **17**, 697.
96 P. J. Dyson, *Nature*, 2009, **458**, 389.
97 M.-G. Mendoza-Ferri, C. G. Hartinger, R. E. Eichinger, N. Stolyarova, K. Severin, M. A. Jakupec, A. A. Nazarov and B. K. Keppler, *Organometallics*, 2008, **27**, 2405.
98 M.-G. Mendoza-Ferri, C. G. Hartinger, A. A. Nazarov, W. Kandioller, K. Severin and B. K. Keppler, *Appl. Organomet. Chem.*, 2008, **22**, 326.
99 M. Auzias, B. Therrien, G. Süss-Fink, P. Štěpnička, W. H. Ang and P. J. Dyson, *Inorg. Chem.*, 2008, **47**, 578.
100 M.-G. Mendoza-Ferri, C. G. Hartinger, M. A. Mendoza, M. Groessl, A. E. Egger, R. E. Eichinger, J. P. Mangrum, N. P. Farrell, M.

Maruszak, P. J. Bednarski, F. Klein, M. A. Jakupec, A. A. Nazarov, K. Severin and B. K. Keppler, *J. Med. Chem.*, 2009, **52**, 916.
101 S. W. Magennis, A. Habtemariam, O. Novakova, J. B. Henry, S. Meier, S. Parsons, I. D. H. Oswald, V. Brabec and P. J. Sadler, *Inorg. Chem.*, 2007, **46**, 5059.
102 B. Therrien, W. H. Ang, F. Chérioux, L. Vieille-Petit, L. Juillerat-Jeanneret, G. Süss-Fink and P. J. Dyson, *J. Cluster Sci.*, 2007, **18**, 741.
103 F. Schmitt, P. Govindaswamy, G. Süss-Fink, W. H. Ang, P. J. Dyson, L. Juillerat-Jeanneret and B. Therrien, *J. Med. Chem.*, 2008, **51**, 1811.
104 F. Schmitt, P. Govindaswamy, O. Zava, G. Süss-Fink, L. Juillerat-Jeanneret and B. Therrien, *JBIC, J. Biol. Inorg. Chem.*, 2009, **14**, 101.
105 J. Mattsson, P. Govindaswamy, A. K. Renfrew, P. J. Dyson, P. Štěpnička, G. Süss-Fink and B. Therrien, *Organometallics*, 2009, **28**, 4350.
106 B. Therrien, G. Süss-Fink, P. Govindaswamy, A. K. Renfrew and P. J. Dyson, *Angew. Chem., Int. Ed.*, 2008, **47**, 3773.
107 P. Govender, N. C. Antonels, J. Mattsson, A. K. Renfrew, P. J. Dyson, J. R. Moss, B. Therrien and G. S. Smith, *J. Organomet. Chem.*, 2009, **694**, 3470.