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Designing organometallic compounds for catalysis and therapy

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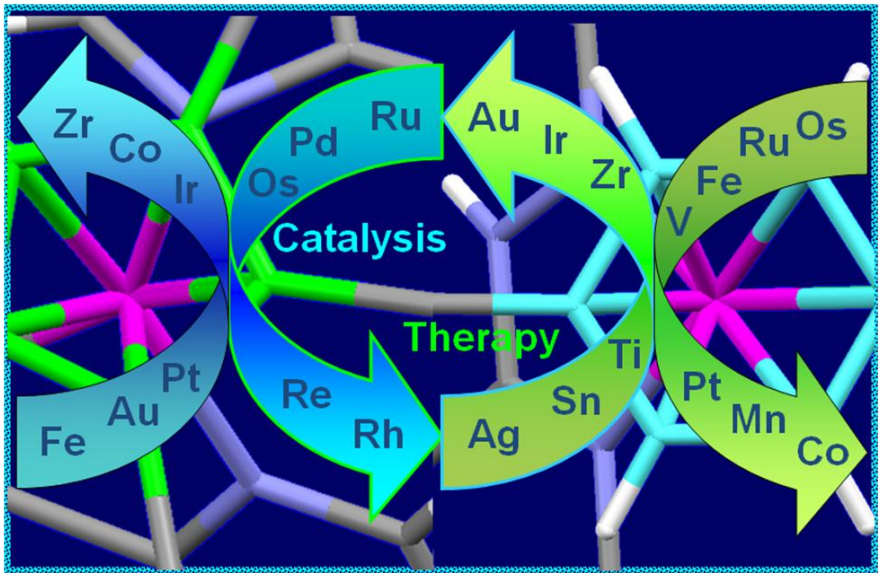

Bioorganometallic chemistry is a rapidly developing area of research. In recent years organometallic compounds have provided a rich platform for the design of effective catalysts, e.g. for olefin metathesis and transfer hydrogenation. Electronic and steric effects are used to control both the thermodynamics and kinetics of ligand substitution and redox reactions of metal ions, especially Ru^{II}*. Can similar features be incorporated into the design of targeted organometallic drugs? Such complexes offer potential for novel mechanisms of drug action through incorporation of outer-sphere recognition of targets and controlled activation features based on ligand substitution as well as metal- and ligand-based redox processes. We focus here on* η^6*-arene,* η^5*-cyclopentadienyl sandwich and half-sandwich complexes of* Fe^{II}*,* Ru^{II}*,* Os^{II} *and* Ir^{III} *with promising activity towards cancer, malaria, and other conditions.*

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1. Introduction

In recent years there have been major advances in the design of organometallic catalysts for the stereo selective control of organic syntheses. These small molecules can show high specificity for their substrates followed by stereo specific conversions into products. At the same time, structurally similar organometallic complexes offer promise as therapeutic agents, some of which are summarised in Figure 1. These compounds aim to act with a comparable specificity on selected biological targets. Design concepts for such organometallic drugs, however, are still in their infancy; mainly because an understanding of structure-activity relationships (SARs) has not yet reached a level that allows the extrapolation of general rules. Two main questions result from this observation: Firstly, how much common chemistry is there between the design of organometallic catalysts and drugs? Secondly, can some of the by-now well-understood principles of catalyst design be applied to organometallic drug design?

We will try to answer these questions by exploring the structures and thermodynamic stability of organometallic complexes currently being developed for catalysis or therapy. The strengths of metal-ligand bonds, reaction mechanisms, and the kinetics of ligand substitution reactions as well as (metal and ligand-centred) redox processes will be discussed with respect to catalytic or (mainly) anticancer activity. Critical evaluation of these insights should then allow the next steps to be taken towards optimisation of structure-activity relationships.

Assessment of optimisation of drug design is more difficult than for catalysts. In catalysis a number of observables can be defined that lead to comparability of different systems: The best candidates usually give high yields, high turn-over numbers (TON) and –frequencies (TOF), and, in the case of enantioselective catalysis, high enantiomeric excesses (ee). In the case of anticancer drugs, for example, an initial measure of efficacy is often their *in vitro* activity towards cancer cells in culture, with potency described by the IC_{50}-value, the drug concentration

which inhibits cell growth by 50% during drug application. However, the conditions under which these values are obtained (e.g. cell-line, drug application time, time of cell-recovery, assay method) can vary between different laboratories. Hence, the use of standard comparator drugs, such as Cisplatin, is helpful. If the target of a drug is known, optimisation can be based on potency towards e.g. protein binding or enzyme inhibition. For further drug development, other factors as cell uptake, organelle distribution and general metabolism then become important.

Nevertheless, interesting parallels emerge between the design of ligands for complexes with catalytic and medicinal properties.[1] However, some differences present challenges when studying physiologically-relevant chemistry of organometallic complexes. For example, catalysis is often carried out in non-aqueous media, whereas drugs operate under physiological conditions. Aqueous media for cell tests contain a range of small and large biomolecules, including O_2. Experiments can also suffer from complications due to insolubility or unexpected side reactions. In general, cellular media are more heterogeneous than those used in catalysis and the effect of water and the possibilities of 'poisoning' need to be considered in the design of new drugs, especially if they might function as 'catalytic drugs'.

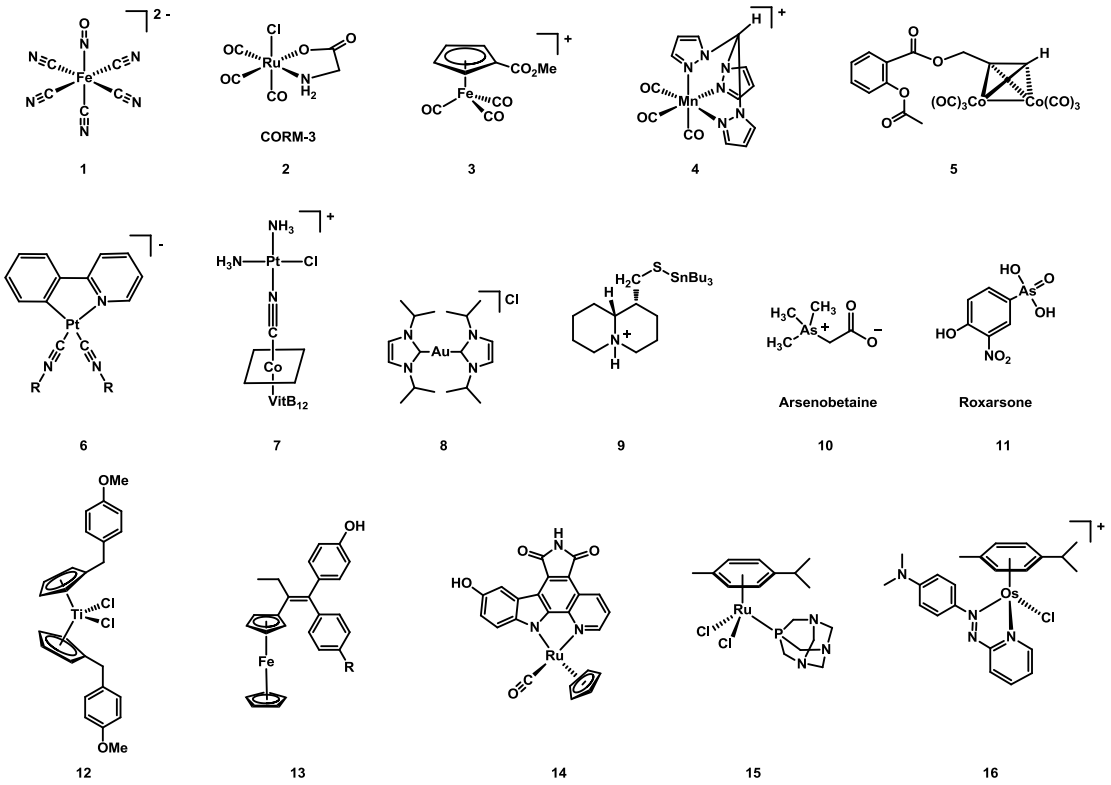


Figure 1 Some examples of biologically-active organometallic complexes.

The most studied organometallic catalysts are probably those with carbon monoxide ligands. For example, carbonyl complexes of Rh, Ir or Co are widely used as catalysts for the synthesis of basic organic compounds such as methanol or acetic acid.[2, 3] Intriguingly, carbon monoxide is a natural metabolite in the body, arising from heme metabolism, and also (along with NO, e.g. in **1** Figure 1) is an important signalling molecule. CO can bind strongly to certain heme proteins, forming stable Fe-CO bonds. This natural metal-carbon bond and its diverse properties have stimulated attempts to design metal-CO complexes which can deliver CO to specific tissues, with the prospect of a new class of drugs, CO releasing molecules ("CORMs", **2-5**). Early candidates such as $[Ru(CO)_3(Gly)Cl]$ (**2**, CORM-3)[4] are being supplemented by CO complexes of Fe (**3**), Mn (**4**)[5] and Co (**5**) as well as boron precursors. A critical requirement is that the complexes are stable enough in the blood to be transported to their biological target and only then are triggered to release the CO. The research field of carbon monoxide releasing molecules is now well established,[5-8] and we might expect to see clinical trials of a CORM in the near future.

Along with cyclometallated platinum compounds (e.g. **6**), other precious metals like gold (**7**) are used in organometallic drug design, not only for cancer chemotherapy, but also against inflammatory diseases.[9] Compounds of the main group metals tin and arsenic have long been known for their hazards as well as their therapeutic potential, and organotin compounds like tri-n-butyltin(IV)lupinylsulfide hydrogen fumarate (IST-FS 35, **9**) are under development as anti-cancer agents.[10-12] The arsenic containing compound arsenobetaine (**10**) is a natural metabolite in marine organisms and Roxarsone (**11**) is used for growth promotion in poultry. Examples of sandwich complexes (**12**, **13**), and half-sandwich structures (**14 - 16**) will be discussed in greater detail below. Generally, the challenge is to identify the active pharmacophores (the steric and electronic features necessary for recognition of specific biological targets and for triggering the biological response), including the roles of both the metal ion and the ligands. This, in turn, will lead to a better understanding of structure-activity-relationships (SAR) for the most promising organometallic drug candidates. As will be described in the following sections, SARs are currently being developed for promising metal-arene and metal-cyclopentadienyl anticancer complexes.

1.1 Why organometallic compounds?

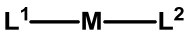
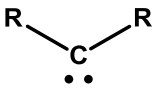
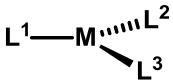
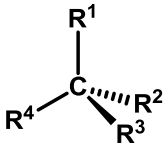
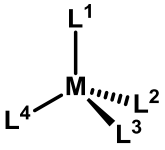
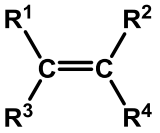
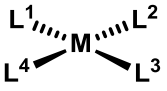
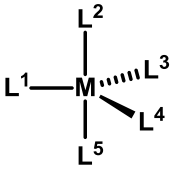
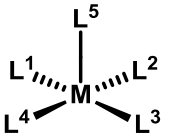
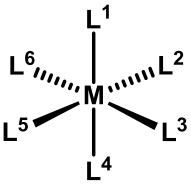
Metallodrugs behave differently from the majority of organic medicinal compounds.[13] The disparity becomes evident when, for example, the three-dimensional structures of both types of molecules are considered (Table 1).

Firstly, organic compounds consist of carbon skeletons which have only small differences in electronegativity and are therefore highly stable (ΔH^0_{CC} = 250 – 500 $kJ \cdot mol^{-1}$). As a result, the synthesis of complicated natural products or even smaller but sophisticated organic molecules commonly involves a large number of reaction steps, protection-group strategies and elaborate purification procedures. Metal-ligand bonds (coordination bonds, M-L), on the contrary, can be much weaker (50 – 150

kJ·mol^{-1})[14, 15] and thus more labile, which allows easier synthesis in fewer steps but with a greater variety of elements, substituents, geometries and charge distributions.

Secondly, the sp, sp^2 and sp^3 hybridisation of carbon centres provides mainly linear, trigonal and tetrahedral geometries (Table 1), whereas metal ions can adopt coordination numbers between 2 and 10, with 4, 5 and 6 being most common for transition metal ions. This repertoire already introduces extra structural diversity for potential use in drug design, but stereochemistry in particular is a striking example of multifariousness: while a tetrahedral carbon atom with four different substituents exists as a single pair of enantiomers; equally small octahedral metal complexes with six different ligands can form 15 enantiomeric pairs.

Table 1 Common geometries of organic and inorganic/organometallic molecules.

Number of bonds	Geometries for organic structures	Geometries in transition metal complexes
(coordination number)	'coordination' numbers 2 – 4	coordination numbers 2 – 10 (most common 4-6)
2	**linear** (sp hybridization): 	**linear:** 
	bent (sp^2, carbene): 	**trigonal-planar:** 
3	**tetrahedral** (sp^3): 1 enantiomeric pair 	**tetrahedral:** 1 enantiomeric pair 
4	**'trigonal'-planar** (sp^2): 	**square-planar** 
		trigonal-bipyramidal 
5	not observed!	**square-pyramidal** 
		octahedral: 15 enantiomeric pairs
6	not observed!	

In organometallic complexes it is the metal-carbon (M-C) bonds that endow these coordination compounds with special properties. On the one hand they have high *trans* effects and *trans* influences: the lability of bonds to other ligands (M-L) in the complex can be greatly influenced by the presence of M-C bonds. On the other hand, π-bonded aromatic arene and cyclopentadienyl ligands can act both as electron donors and π -acceptors. These ligands can therefore modify the donor/acceptor behaviour (and reactivity) of other ligands in the complex.

In this article, most of our examples are (pseudo) octahedral organometallic complexes in which one face of the octahedron (3 coordination sites) is occupied by 6 carbon atoms from an arene ring (η^6-hapticity) or 5 C-atoms from a cyclopentadienyl ring (η^5-hapticity). Release of these rings can therefore generate 3 coordination sites, although ring slippage is possible, which leads to fewer positions becoming available (e.g. η^4-arene rings occupy 2 coordination sites). Such changes depend not only on thermodynamics, i.e. the strengths of the coordination bonds, but also on kinetics: the making and breaking of M-L bonds, which can occur on an enormous range of timescales, ranging from nanoseconds to years.

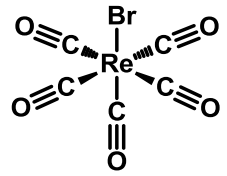
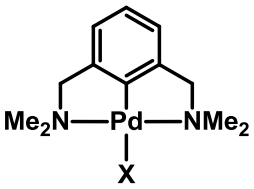
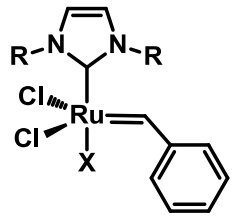
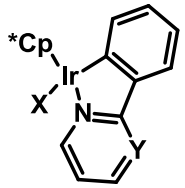
As a result of their diverse reactivity, coordination compounds are subject to modification during uptake and transport inside the human body and are therefore almost always 'pro-dugs'. Thus, the ligands must be chosen carefully, as they may possess different functions. Not only can they influence the reactivity of the metal or be involved in redox reactions, but also greatly affect the absorption and delivery of the complex, and sometimes even the specificity of target recognition. These non-covalent interactions with target sites (e.g. membranes, DNA and proteins) occur through weaker interactions such as hydrogen bonding (20 – 60 $kJ \cdot mol^{-1}$) and van der Waal's interactions (<50 $kJ \cdot mol^{-1}$). If the ligands are released through substitution reactions, they may be involved independently in biological activity. However, through transport by a metal ion they may reach target sites which otherwise would be inaccessible to the free ligand alone. The involvement of metal compounds (including

those in pre-clinical development) in cellular redox processes has recently been reviewed.[16]

First we describe briefly the design features which have been incorporated into organometallic catalysts. Similar to medicinal compounds, they not only demand general stability of the active species under the various reaction conditions, but also tune-ability for substrate recognition, binding and product release. The design process requires consideration of the three dimensional structures of the complexes (see above and Table 1), as well as their thermodynamic and kinetic properties.

2 Design features in catalysts

Table 2 Examples of organometallic homogenous catalysts.

Catalyst	Reactions catalysed	Design features
 **17**	C-C, C-N, C-O and C-S bond formation reactions (e.g. *Friedel-Crafts*, *Mukayama* aldol)	Low-spin d^6 complex is symmetrical, can form dimeric species with bridging CO ligands; thus many transformations are possible.
 **18**	C-C-cross-coupling reactions (e.g. *Heck*, *Suzuki-Miyaura*, *Sonogashira*), *Michael*-reacitons, Aldol-reactions, Allylations of aldehydes and imines	Labile ligand X as leaving group can be substituted by the substrate. Reduction of Pd can break Pd-C bond and deliver defined Pd^0 particles which act as heterogeneous catalysts.
 **19**	*Grubbs'* catalyst: C=C-bond metathesis	Sterically demanding carbene blocks part of the coordination sphere and tunes lability of X, the leaving group.
 **20**	Oxidation of H_2O	Cp* occupies 3 coordination sites, only one (X) is accessible for the substrate through ligand exchange.

Some well-known examples of organometallic catalysts are given in Table 2. While simpler, mainly symmetrical complexes (e.g. **17** (Re), **18** (Pd), can catalyse many different reactions,[17] complexes **19** (Ru) and **20** (Ir) are designed to perform one particular reaction with high selectivity.[18, 19] Complex **20**, for example, is one of only a few homogeneous catalysts for the oxidation of water to dioxygen. Most are not stable in the high oxidation states required during the catalytic cycle. Successive improvements in the design of organometallic iridium precursor complexes for water oxidation by Crabtree et al. are illustrated in Figure 2. These have resulted in half-sandwich complexes of the type [Cp*Ir(N-C)X] (**20**)[20], $[Ir(N\text{-}C)_2(H_2O)_2]X$ (**21**) [20], and [Cp*Ir(C-C)X]X (**22**)[21] which are robust and activated by Ce^{IV}.

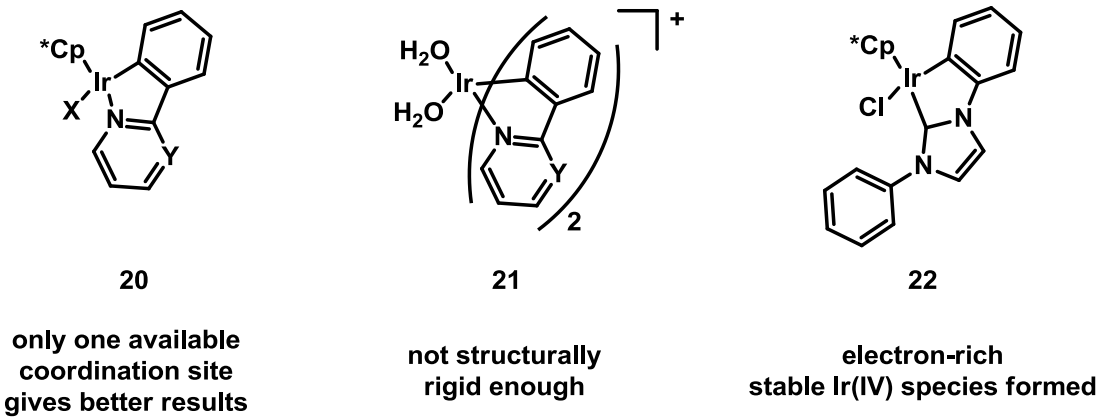


Figure 2 Successive development of Ir^{III} water oxidation catalysts.

Stabilization of the Ir^{IV} oxidation state is necessary for the catalytic conversion of water to oxygen and demands a highly electron-rich environment. This is provided by the strong electron donor Cp*, assisted by a C,C-donor which contains an NHC-fragment and a cyclometallating *N*-phenyl group (**22**). This example shows how fine tuning of the electronic properties around a metal centre can optimise catalyst performance, a principle which can also be applied to drug design.

2.1 Well developed Ru catalysts: Grubbs - a guide for drug design?

The successful development of Grubbs' catalyst for olefin metathesis (**19**, Table 2) was achieved through the careful examination of the structure-activity relationships and mechanistic studies of the organometallic complexes. Olefin metathesis involves the interconversion of an olefin and a metal alkylidene via a metallacyclobutane intermediate and [2+2] cycloadditions and cycloreversions.[22, 23] The scope of reactions encompasses ring-opening metathesis polymerization (ROMP), ring-closing metathesis (RCM), acyclic diene metathesis polymerization (ADMP), ring opening metathesis (ROM) and cross- metathesis (CM or XMET). The structures of the catalysts are shown in Figure 3.

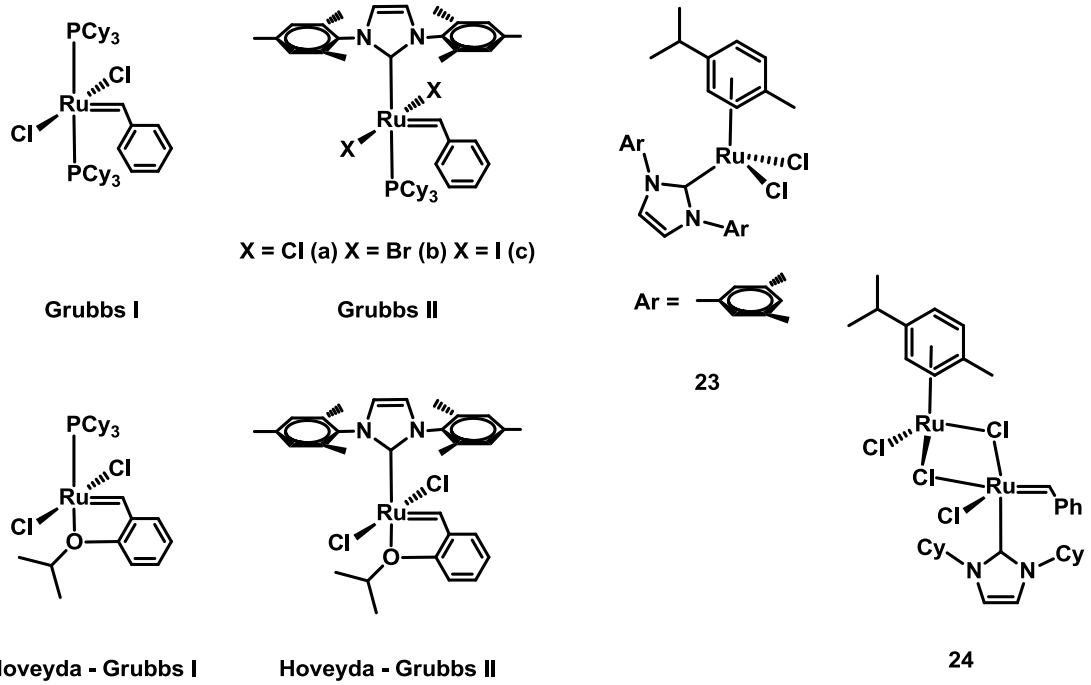


Figure 3 First and second generation of the Grubbs and the Hoveyda-Grubbs catalyst as well as two examples of recent approaches to develop these further (Cy = cyclohexyl).

An important achievement in catalyst development was tolerance to a broad range of substrates, which was determined by the choice of the metal centre. Ruthenium, in comparison to the early transition metals, results in a remarkable functional group tolerance. Because it seems to react preferentially with carbon-

carbon double bonds over most other species, these catalysts are unusually stable toward alcohols, amides, aldehydes, and carboxylic acids.

For olefin metathesis to occur at least one of the ancillary ligands needs to be labile enough for catalyst activation. Catalyst activity increases with larger and more electron-donating phosphines, and the steric bulk of the ligands can also contribute to phosphine dissociation by destabilizing the crowded bis(phosphine) olefin complex. Perhaps even more importantly, δ-donation helps stabilise the 14-electron metallacyclobutane intermediate. The catalytic activity of these complexes increases with the basicity of the phosphines in the order $PPh_3 < PPri_3 < PCy_3$.[22]

In contrast to the trend for phosphines, the halide ligands correlate with decreasing activity as they become larger and more strongly electron-donating, in the order Cl > Br > I. Since the incoming olefin may initially bind *trans* to a halide, a more electron-donating halide should weaken the ruthenium-olefin bond and disfavour olefin coordination.

The overall activity also depends on catalyst initiation and thus on the nature of the alkylidene moiety. In general, alkyl-substituted alkylidenes display more efficient initiation than the methylidene complex. However, the phenyl substituted alkylidenes are preferred as the phenyl group is electron withdrawing, and its steric demand may assist phosphine dissociation.[22]

Mechanistic studies have identified a number of factors that contribute to catalyst activity. A crucial finding is that $(PCy_3)_2Cl_2Ru{=}CHPh$ forms a highly active mono(phosphine) intermediate during the catalytic cycle. This intermediate became a starting point for a design motif in the development of improved catalysts. In the 'second generation' catalysts, one of the phosphine substituents is replaced by a *N*-heterocyclic carbene (NHC) ligand. Compared to phosphines, NHC ligands are strong Lewis bases, acting as excellent σ-donors and poor π-acceptors, and afford metal-carbon bonds that are usually less labile than the related metal-phosphine bonds. In the 'second generation' complexes (**Grubbs II**, Figure 3), the carbene ligand enhances the dissociation of the more labile *trans* phosphine from the metal centre. Moreover,

by virtue of its steric bulk and electron-donating properties, the NHC can stabilize the electron-deficient intermediates more effectively and promote olefin metathesis.[24] In general, halido ligands have a significant impact on the initiation rates of second-generation catalysts. For example, the dibromido complex **Grubbs IIb** and diiodido complex **Grubbs IIc** initiate 3 and 250 times faster, respectively, than the dichlorido parent compound **(Grubbs IIa)**.[25] This initiation rate enhancement is attributed largely to the increased steric bulk of bromido or iodido ligands. However, despite the increased initiation efficiency, olefin metathesis activity of **IIb** and **IIc** is comparable to, or even lower, than **IIa** due to slower turnover rates.

The second generation catalysts have become more successful as they combine the best characteristics of early and late metal centres into a single species. These small changes in the steric and electronic character of the ligands combine to influence olefin binding, phosphine dissociation, and the stability of intermediates, which results in large variations of catalyst activity.

The half-sandwich η^6-arene ruthenium complex **23** was one of the first bearing the NHC ancillary ligands to be reported and outperformed phosphine-containing analogues in the ring-closing methathesis (RCM) of diethyl diallymalonate, but required the *in situ* generation of alkylidene species. Alternatively, visible light irradiation resulted in *p*-cymene loss and subsequent generation of a highly active and coordinatively unsaturated species which is believed to trigger metathesis. Interestingly, preformed **23** can also be obtained *in situ* as a three component system (i.e. $[RuCl_2(p\text{-cymene})]_2$/NHC-precursor salt/base) and is equally catalytically efficient. More recently it was shown to promote the cross-metathesis of functionalized styrenes efficiently as well as the ring-closing metathesis (RCM) of dimethallyl tosylamide. The homobimetallic complex **24** was also isolated and shown to be efficient in RCM and ring-opening methathesis polymerisation (ROMP) transformations.[26, 27]

We use the Ru^{II} Grubbs-type metathesis catalysts of the class $[Ru(NHC)(X{=}CR)Cl_2]^+$ (**19**, Table 2 and Figure 4) as examples of highly successful

organometallic catalysts and compare their features with those of Ru^{II} arene anti-cancer complexes. The structures discussed are illustrated in Figure 4.

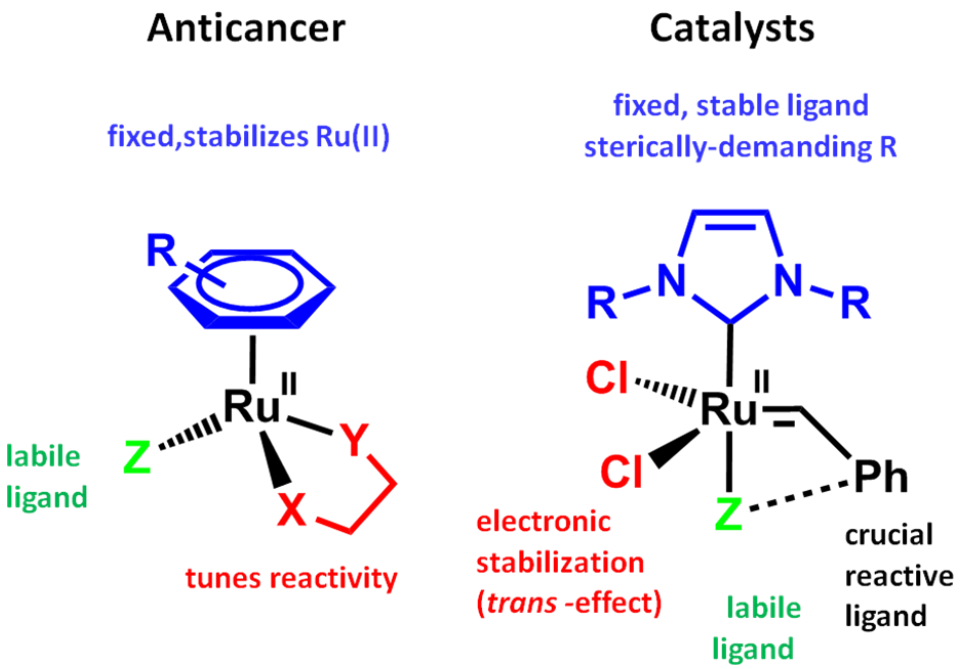


Figure 4 Comparison of design features in Ru^{II} metathesis catalyst **19** (Table 2) with those in mono-functional Ru^{II} arene anticancer complexes.

Both complexes contain ruthenium in the +2 oxidation state, stabilised by one large ligand (carbene vs. arene). The ligand set in both complexes is completed by labile ligands that can act as leaving groups (Z) and ligands which have an active role in the performed reaction.

In the catalyst, Ru, as opposed to early transition metals, allows for a large functional group tolerance. However, the properties of the complexes are determined by the different ligands and their lability towards substitution. Both electronic and steric features are important for this, as the donor/acceptor abilities define not only the strengths of the Ru-ligand bonds (thermodynamics) but also rates of ligand exchange (kinetics). For example, the carbene ligand acts as a strong electron donor towards the metal centre and is itself usually inert towards substitution. Its strong *trans*-effect, however, influences the lability of the leaving group X, while the bulky substituents with their steric demands affect the exchange rate of substrate and product molecules. Both of these substitutions account for the rate of the reaction at the metal centre and

therefore the activity of the catalyst. Furthermore, the chloride ligands, which also have a *trans*-effect, stabilise the reactive ligands at the metal centre electronically. Their role is therefore as crucial, because the strength of the Ru=C bonds also adds to the overall rate of the metathesis reaction. Thus, it can be concluded that all parts of this metal complex have a defined role and contribute to the performance of the molecule as a catalyst.

A recent study by Ott et al. has addressed the question as to whether ruthenium complexes like the Grubbs' catalyst can also "trigger biological effects".[28] The commercially available catalysts Grubbs I and Grubbs IIa and their Hoveyda-Grubbs analogues (Figure 3) were screened in several biological assays including inhibition of thioredoxin reductase and cathepsin B, as well as antiproliferative activity in MCF-7 and HT-29 cell lines. The Hoveyda-Grubbs II complex HG2 proved to be the most promising compound, allowing the conclusion that the stabilising NHC as well as the tuned lability of the *trans*-ligand are crucial for their biological behaviour.

To reach a level of understanding similar to that for the catalytic activity of Grubbs' and related catalysts is one aim in the design of ruthenium arene anticancer complexes. Below we will discuss how the different ligands influence the biological activity of these compounds and lead to the rational design of novel metal-based drugs.

2.2 Transfer-hydrogenation catalysis

Catalytic transfer hydrogenation can be achieved with the aid of an organic molecule which acts as the hydrogen donor in the presence of a transition metal catalyst.[29] The turning point in the development of catalysts for this reaction came when Noyori and co-workers discovered that organometallic half-sandwich complexes of the type [(η^6-arene)]Ru^{II}H[TsDPEN] (with TsDPEN = N-(*p*-toluene-sulfonyly-1,2-diphenylethylenediamine) complex (**25**, Figure 5) are excellent

catalysts for the asymmetric reduction of aromatic ketones, affording enantiomeric excesses (ee) of up to 99%.[30-32]

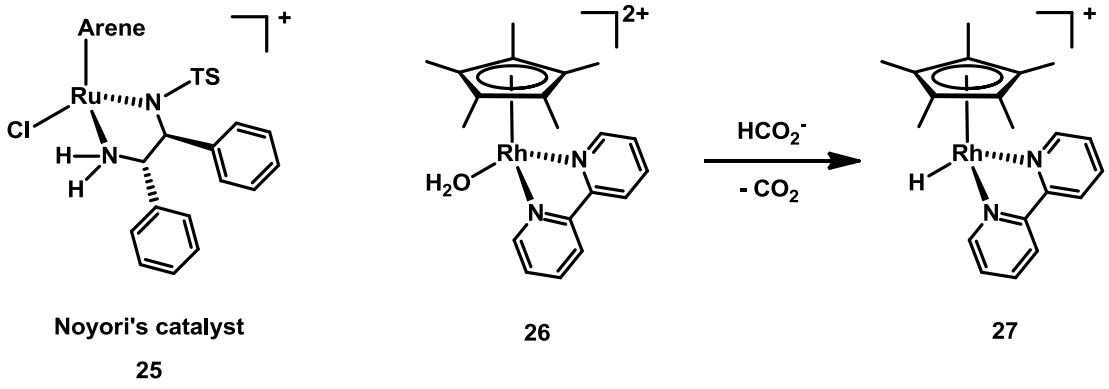


Figure 5 Noyori's transfer hydrogenation catalyst (**25**). Generation of a Rh-H intermediate (**27**) by reaction of an Rh-aqua complex (**26**) with formate followed by CO_2 release.

The Ru-H and the protic N-H hydrogen atoms are simultaneously transferred to the carbonyl function of the ketone via a 6-membered transition state, thereby forming an alcohol product directly. Noyori et al. further noted that the arene in these half-sandwich Ru^{II} catalysts confers the following advantages. It coordinates in an η^6-fashion, which in an octahedral complex leaves three vacant coordination sites; it is also a weak electron donor that generates unique reactivity at the metal centre, and it is inherently flexible with regard to the variety of possible substituents on the aromatic ring.[33]

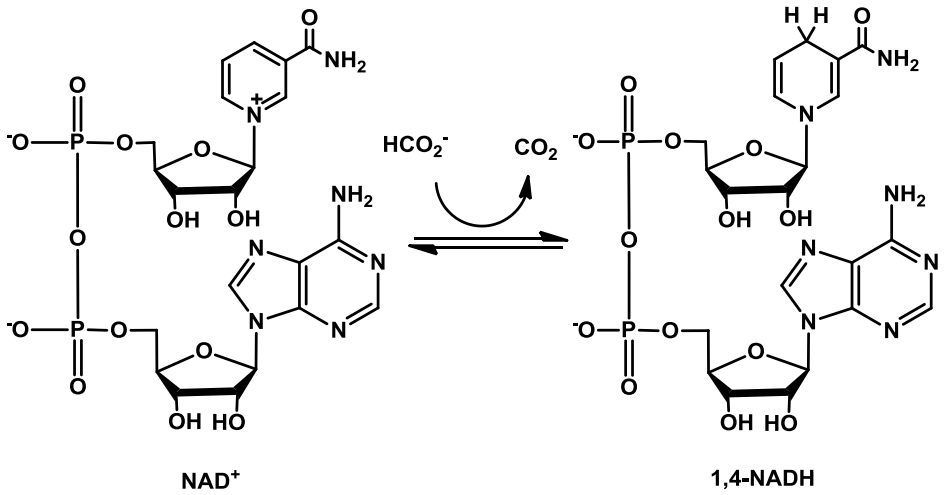


Figure 6 The conversion of NAD^+ to NADH.

Steckhan et al. have used the Rh^{III} complex $[(\eta^5\text{-Cp*})Rh(bipy)(OH_2)]^{2+}$ (**26**) as a pre-catalyst for the regioselective reduction of NAD^+ to 1,4-NADH, using sodium formate as the hydrogen source in aqueous solution.[34, 35] The conversion of NAD^+ to NADH (Figure 6) by enzymatic processes has been extensively investigated,[36-38] but the use of some redox enzymes is limited by the need to regenerate this cofactor. Hence, there is potential for platinum-group metals, especially Ru^{II}, Rh^{III} and Ir^{III}, to act as transfer-hydrogenation catalysts for this conversion. This is mainly because arene or cyclopentadienyl complexes of these metal ions can readily form hydrido species, some of which are relatively stable.[39-41] The formation of $[Cp^*Rh(bipy)H]^+$ (**27**), where bipy is bipyridine, for example, is the rate-limiting step in the reduction of ketones and aldehydes to alcohols in water at pH 7 in the presence of sodium formate as hydride donor at ambient temperature.[42] Therefore, hydrido complexes are key intermediates, usually generated by reaction of formate with a metal complex, with concomitant CO_2 release.[43]

Following from this, Fish et al. studied the kinetics and mechanism of this catalytic reduction. They demonstrated with a variety of 3-pyridinium NAD^+ models[44-47] that the pyrophosphate and adenosine groups in NAD^+ are not essential, and do not contribute to the rate of hydride transfer.[45] The reduction is regioselective, giving the 1,4-NADH isomer, and can drive enzymatic reactions that rely on NADH as cofactor. One example is the reduction of 3-methylcyclohexanone (catalyzed by alcohol dehydrogenase from *Thermus sp*.), which can be achieved with an enantiomeric excess of up to 97%.[48]

Regioselective hydride transfer from $[Cp^*Rh(bipy)H]$ (**27b**) to NAD^+ to give 1,4-NADH appears to be a consequence of the ability of the amide to coordinate to the ring-slipped Cp*Rh centre. This process acts in concert with hydride transfer to form a 6-membered ring as part of a reversible transition state (**28a/b**, Figure 7). Finally, displacement of the 1,4-dihydrido product by H_2O completes the catalytic cycle.[47]

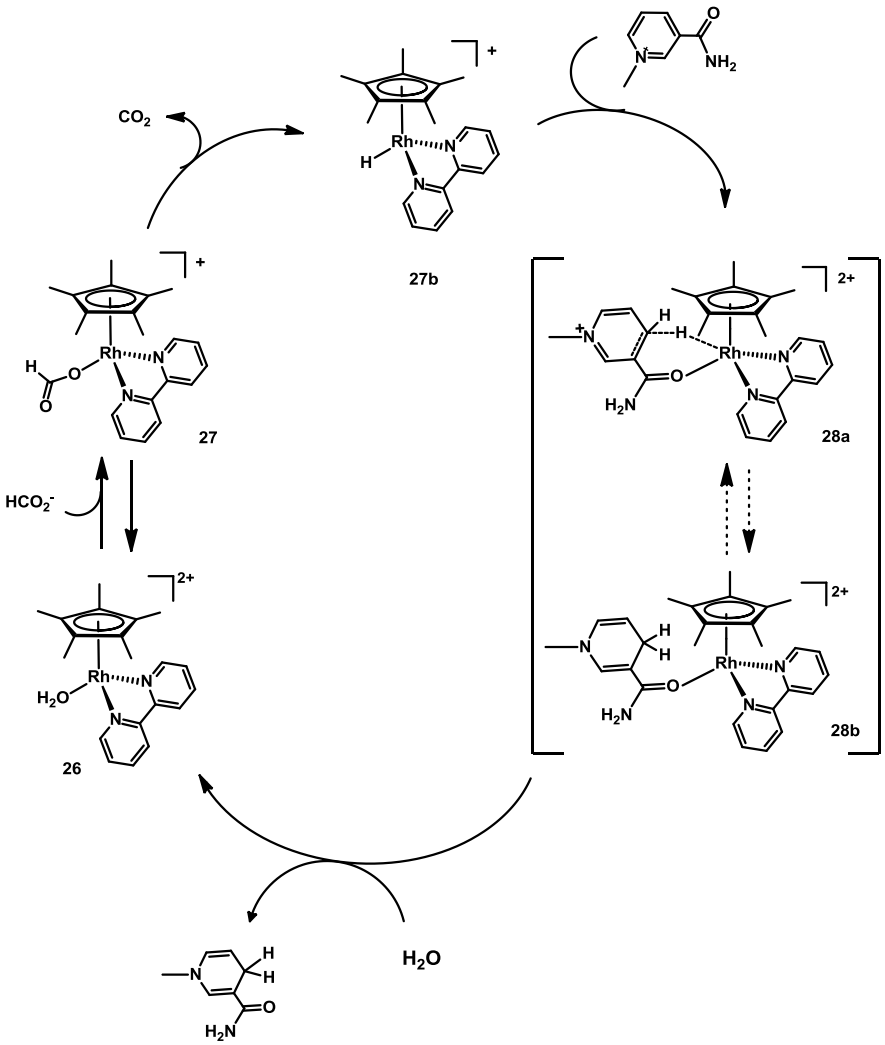


Figure 7 Catalytic cycle for transfer hydrogenation of NAD^+ with a Cp*Rh catalyst (adapted from Lo[47]).

There is strong evidence for the formation of a partial Rh-O bond in the transition state (**28a/b**).[49] Phosphite can replace formate as the hydride source while preserving the properties of the catalyst $[Cp^*Rh(bipy)(OH_2)]^{2+}$ (**26**) itself.[50]

In nature, oxidoreductases, such as horse liver alcohol dehydrogenase (ADH), catalyse the reduction of carbonyl compounds to alcohols by transfer hydrogenation, commonly using the reduced coenzyme NADH, or NADPH. Such biochemical reactions are normally highly stereoselective.[51] Photochemical *in situ* NADH regeneration can be achieved with high catalytic activity through the electrochemical mediation of $[Cp^*Rh(bipy)(OH_2)]^{2+}$ (**26**, Figure 5) in the presence of SiO_2-supported quantum dots (CdS mono crystals) as visible-light absorbing photosensitizers. Such a regeneration has been coupled with the enzymatic reaction of glutamate

dehydrogenase to convert α-ketoglutarate to L-glutamate.[52] A visible-light-driven transfer hydrogenation of carbonyl and C=C compounds has been developed by coupling CdS nanoparticles with iridium complexes, $[Cp^*Ir(bipy\text{-}R)OH_2]^{2+}$ (R = OH, or OMe on the *para*-positions), (**29** Figure 8) giving high activity, excellent selectivity and a unique pH-dependent catalytic activity.[53]

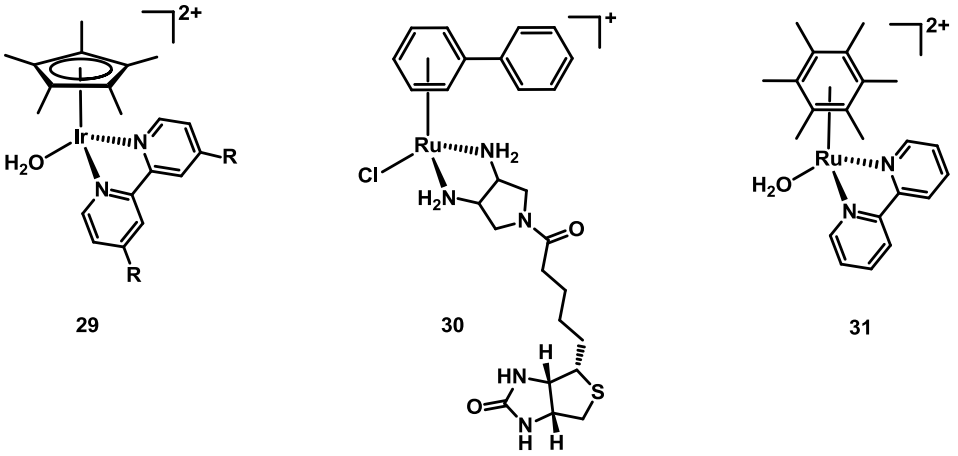


Figure 8 Examples of transfer hydrogenation catalysts.

Artificial metalloenzymes have been generated by incorporating an organometallic catalyst fragment such as $\{Cp^*IrCl\}^+$, $\{Cp^*RhCl\}^+$ or $\{\eta^6\text{-(arene)}RuCl\}^+$, bearing a biotin group attached to the chelating ligand (biot-*p*-L in **30**) within a host protein.[54] The resulting hybrid can act as a transfer hydrogenation catalyst. The Ru conjugates are generally more active and selective than either Rh or Ir complexes. Ruthenium arene conjugates can catalyse the transfer hydrogenation of prochiral ketones (up to 97% ee for aryl-alkyl ketones and up to 90% ee for dialkyl ketones). The arene ligand plays a significant role in determining the enantioselectivity of $[(\eta^6\text{-biphenyl})Ru(biot\text{-}p\text{-}L)Cl]^+$ and $[(\eta^6\text{-}p\text{-cymene})Ru(biot\text{-}p\text{-}L)Cl]^+$.[55] For Ru^{II} arene complexes that possess a chiral 2-amino alcohol or a related ligand, a theoretical study has shown that the enantioselectivity originates not only from the chiral geometry of the five-membered chelate ring, but also from CH/π attractions between the η^6-arene ligand and the carbonyl aryl substituent.[56]

The Ru^{II} aqua complex $[(\eta^6\text{-}C_6Me_6)Ru^{II}(bipy)(OH_2)]^{2+}$ (**31**) can act as a catalyst precursor for pH-dependent transfer hydrogenation with formate as the hydride donor in H_2O. The rate of the transfer hydrogenation is a maximum around pH 4.0. The corresponding formato complex $[(\eta^6\text{-}C_6Me_6)Ru^{II}(bipy)(HCOO)]^{+}$ forms from **31** as an intermediate for β-H elimination and the hydrido complex $[(\eta^6\text{-}C_6Me_6)Ru^{II}(bipy)H]^{+}$ finally acts as the catalyst for the transfer hydrogenation.[57]

Cationic Ru^{II} arene aqua complexes containing chiral *N,N*-chelating ligands can catalyse the transfer hydrogenation of prochiral aryl ketones and imines in aqueous solution to give the corresponding alcohols and amines with good conversion and enantioselectivity.[58]

Anticancer complexes such as $[(\eta^6\text{-arene})Ru(en)Cl]^{+}$ (**32**) can catalyse the regioselective reduction of NAD^{+} by formate in water. The mechanism, as displayed in Figure 9, proceeds via the aqua complex **33** to give the biologically-relevant 1,4-NADH isomer under physiological conditions (37°C, pH 7.2) through the formation of the hydrido complex (**35**).[59] The slow step in the cycle is the loss of CO_2 by the formate adduct $[(\eta^6\text{-arene})Ru(en)(formate)]^{+}$ (**34**). Lung cancer cells (A549), for example, are remarkably tolerant to formate even at millimolar concentrations, but the sluggishness of the reaction and the requirement for the presence of a large excess of formate inside the cells make it implausible that such a system could provide the basis for an active catalytic drug.[59] A more effective and biologically-compatible hydride source must be found if the development of Ru^{II} arene complexes as hydrogenation catalytic drugs is to advance further.

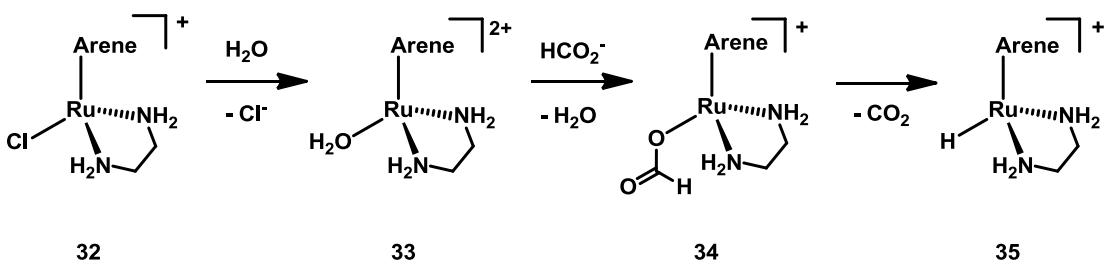


Figure 9 Arene anticancer complexes as transfer hydrogenation catalysts.

Unexpectedly however, recent work in our laboratory has shown that hydride transfer from NADH to Ru^{II} arene diimine and Ir^{III} cyclopentadienyl dimine complexes is facile and can drive catalytic transfer hydrogenation reactions.[60] Such Ir^{III} complexes can act as robust catalysts for the production of H_2 in water.[61] These findings suggest that organometallic complexes might interfere in NADH (and NAD(P)H) signalling pathways in cells and in the redox balance, so introducing a novel mechanism of drug activity.[62, 63]

Ruthenium(II) complexes of N-heterocyclic carbenes are also effective catalyst precursors for the reduction of aliphatic aldehydes via hydrogen transfer reactions using 2-propanol as the hydride source. However, the reactions require high temperature and basic conditions and are therefore not compatible with biological environments.[64] The activity of water-soluble cyclopentadienyl complexes with phenanthroline or its 5-substituted analogues as chelating ligands follow the order Rh^{III} >> Ir^{III} >Ru^{II} for the regeneration of NADH from NAD^+ using sodium formate as the hydride donor in the enzymatic reduction of ketones.[58] A trinuclear Ru^{II} complex, $[RuCl(TPA\text{-}\{phenRuCl(hmb)\}_2\text{-}H^+)](PF_6)_2$, (phen = phenanthroline, TPA = tris(2-pyridylmethyl)amine, hmb = hexamethyl benzene) is a more efficient catalyst for transfer hydrogenation of ketones with formic acid than the corresponding mononuclear Ru^{II} complex. The two arene-ruthenium complexes are brought into close proximity by the central Ru(TPA) moiety, so enhancing the interaction of substrate with the Ru-formato complex.[65]

3 Design and biological activity of metallocenes

The anticancer activity of a wide range of metallocenes was investigated in the late 1970s and the 1980s by Köpf and Köpf-Maier.[66-70] In particular, titanocene dichloride [Cp_2TiCl_2] emerged as a candidate for clinical trials.[71] Additionally, this section focusses on the successful design of ferrocene derivatives for treatment of malaria (Ferroquin) and breast cancer (Ferrocifens).

3.1 Titanocene-based anticancer complexes

Titanocene dichloride, [Cp_2TiCl_2], is one example of an organometallic "sandwich" compound with poor *in vitro* but good *in vivo* anti-cancer activity.[72, 73] This complex entered clinical trials, but its efficacy was too low to proceed further than phase II.[74, 75] It undergoes rapid aquation in water and the resulting aqua adducts are acidic, leading to formation of hydroxido- and oxido-bridged oligomers.[76-78] From these, after dissociation of the cyclopentadienyl (Cp) ligand, the stable, insoluble and also inactive TiO_2 can form.[76] Indeed, Cp is readily displaced on interaction of [Cp_2TiCl_2] with transferrin,[79] the Fe^{III} transport protein present in blood at ca. 35 μM, so this protein may be responsible for delivering Ti^{IV} to cancer cells.

Since Ti^{IV} compounds appear to have a unique mechanism of anticancer activity, efforts are being made by various groups to improve their design. For example McGowan et al. have reported active, charged Ti^{IV} and Zr^{IV} complexes with functionalised Cp ligands.[80, 81] Tacke et al. have also synthesized a broad range of titanocene dichloride derivatives. Particularly promising is "Titanocene Y", bearing *p*-methoxybenzyl substituents on the Cp rings.[82] In this case, interactions with the serum protein albumin seem to be crucial for the cytotoxicity.[83] *In silico* docking experiments on human albumin using 16 different molecules of the Cp_2TiCl_2-family suggest that the binding sites are those of established drugs, for example the Ibuprofen binding site I.[83-86]

Interestingly, the vanadium and tin analogues of Titanocene Y are even more active towards cancer cells *in vitro* (Figure 10).

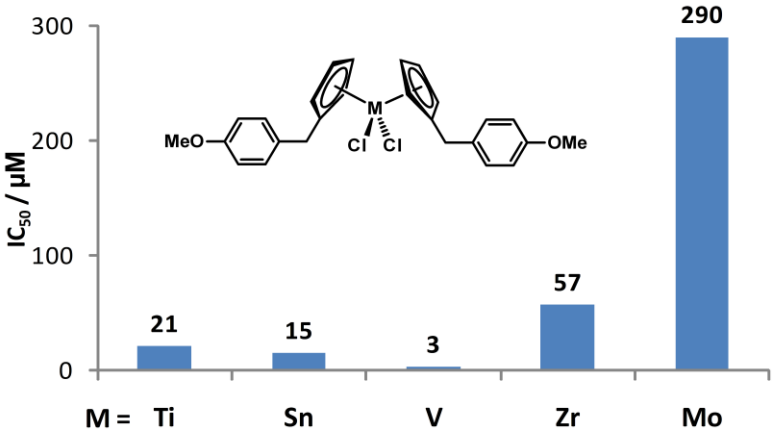


Figure 10 *In vitro* cytotoxicity (IC_{50} values) for Titanocene Y and other metallocene analogues towards renal epithelial LLC-PK cells.[84] The most active compounds have the lowest IC_{50} values.

Further derivatisation of the Cp ring with phenylpyrrole substitutents can give rise to even more active complexes (Figure 11).[87]

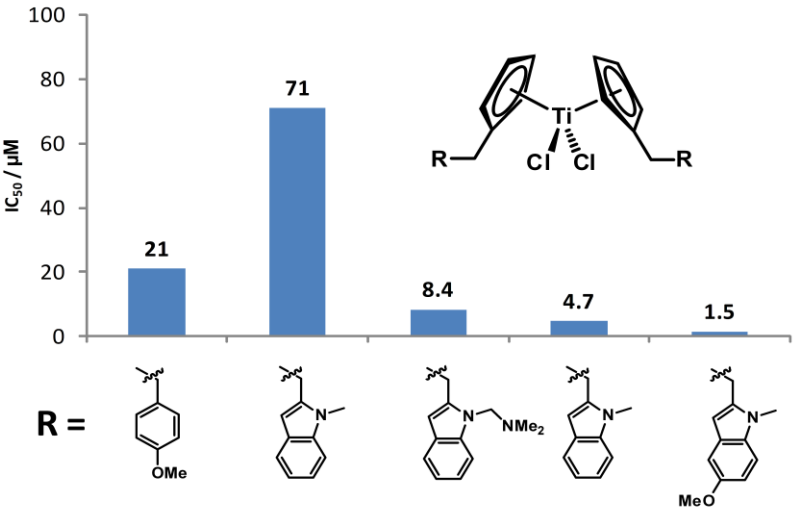


Figure 11 Activity of monosubstituted titanocenes towards LLC-PK cells (IC_{50}) in comparison with Titanocene Y (*p*-anisyl substituent, left).[84]

Bimetallic titanium-ruthenium complexes of the general formula $[(\eta^5\text{-}C_5H_5)(\mu\text{-}\eta^5\text{:}\kappa\text{-}C_5H_4(CR_2)(n)PR'R'')TiCl_2](\eta^6\text{-}p\text{-cymene})RuCl_2$ (**36**, Figure 12), consisting of a titanocene-dichloride component and a ruthenium-arene fragment, are markedly more active than their Ti or Ru monometallic components.[88] Furthermore, cathepsin B (cat B) inhibition seems to relate to the length of the alkyl linker, with the longer chain length facilitating inhibition. RAPTA-C (**15**, Figure 12) also inhibits cat B efficiently. Helicases/topoisomerases and HIST1H4 core histones may be the main targets for Titanocene C (Figure 12), and metallothioneins are upregulated as main effectors of drug resistance.

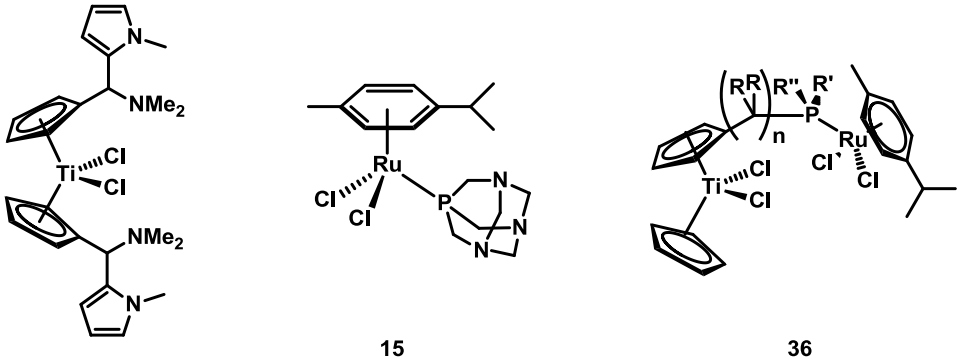


Figure 12 Titanocene C, RAPTA-C and the bimetallic mixed Ti/Ru organometallic complex **36** that combines both metallocene and metal arene structural features.

3.2 Antimalarial ferrocenes

The sandwich compound ferrocene, bis-cyclopentadienyl iron(II), is often compared to the classic organic molecule benzene due to its properties as an aromatic substrate. It behaves very similarly to benzene in many classic organic transformations (e.g. electrophilic aromatic substitution), and often outperforms the organic molecule in terms of reaction times.[89] Strikingly, regardless of its higher reactivity, ferrocene is much less toxic. In fact, the organometallic compound is relatively non-toxic, whereas benzene is carcinogenic and mutagenic to germ cells.[90]

Because of its similarities to the phenyl ring, ferrocene is much investigated as a substitute for it in medicinal compounds. One successful example is the derivatisation of the anti-malarial drug Chloroquine. The ferrocene derivative Ferroquine, designed by Biot et al., contains a ferrocenyl group covalently flanked by a 4-aminoquinoline and a basic alkylamine.[91, 92] This drug (currently being developed by Sanofi-Aventis) is in phase II clinical trials.[93] It shows activity in both Chloroquine-resistant and non-resistant parasites and has a multi-mode of action: a capacity to target lipids, an ability to inhibit the formation of hemozoin, and to generate reactive oxygen species.[92] Therefore, Ferroquine has become a promising new treatment for malaria. Its rhenium analogue, however, is much less active.[94]

3.3 Anticancer Ferrocifens

The growth of several types of breast and prostate cancer cells is dependent on the natural hormones estradiol and testosterone, respectively. These cancer cells over-express hormone receptors, and treatment usually relies on drugs that modulate the receptors in order to lower overall hormone levels. An example is the selective endocrine receptor-modulator (SERM) Tamoxifen, which is widely used for anti-oestrogen treatment of hormone-dependent breast cancer.[95]

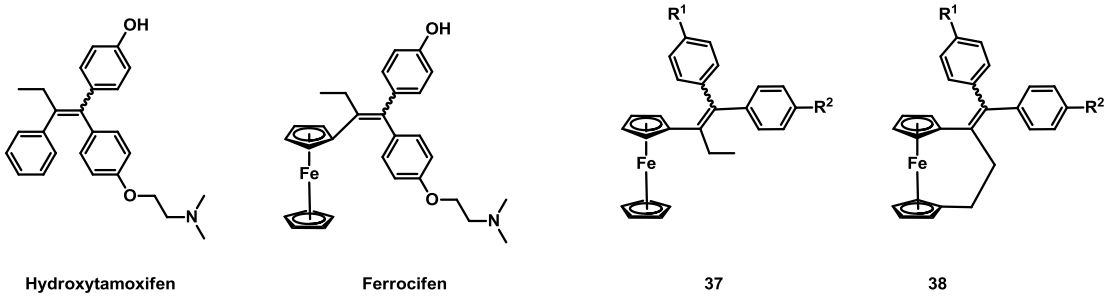


Figure 13 Hydroxytamoxifen, an active metabolite of the anti-oestrogenic drug Tamoxifen, and its ferrocenyl analog Ferrocifen. Derivatives **37** and **38** (with R^1, R^2 = OH) are the most active candidates to date.

Derivatives of Tamoxifen, like Hydroxytamoxifen (Figure 13), in which one phenyl ring is replaced with a ferrocenyl unit, exhibit improved activity. The drug-metabolite Hydroxytamoxifen is used as a vector to recognise the oestrogen receptor and to carry the redox active fragment ferrocene into the cells. The similarities between the structures are shown in Figure 13. After successful application of this idea by Jaouen et al. in 1996,[96, 97] the structures of "Ferrocifens" have been gradually refined,[98-104] and structure-activity relationships established.[100] Two promising compounds are shown in Figure 13 (**37** and **38**), with R^1 and R^2 both being hydroxyl groups.

These complexes have evolved from a number of systematic derivatisations, evaluated in cytotoxicity assays for hormone-dependent (MCF-7) as well as hormone independent (MDA-MB-231) breast cancer cell lines. The following principles result from this systematic drug design:

- The ferrocenyl (Fc) moiety is crucial for activity.
- Anti-oestrogenic behaviour occurs only when side chain R^2 = *N*,*N*-dimethylamino (Figure 13).
- Both oestrogenic (receptor-dependent) and cytotoxic (receptor-independent) behaviour occur (in hormone-dependent cancer cells) when the N,N-dimethylamino side chain is not present.
- High cytotoxicity arises with a phenol group positioned *para* to the ethylene bridge conjugated to the ferrocenyl group through an ethylene bridge.
- Phenol (only R^1 = OH) and diphenol (both R^1, R^2 = OH) derivatives are the most active.
- Activity increases when rigidity is enhanced by forcing the molecule into a cyclic, ferrocenophane shape.

In the National Cancer Institute (NCI) 60 cell line screen,[99] ferrocenophane (**38**) and ferrocenyl (**37**) derivatives (Figure 13, R^1 = R^2 = OH), show nanomolar activity

against e.g. leukaemia, CNS and renal cancer cells. The cyclic, rigid ferrocenophanes (**38**) are up to 15 times more potent than the more flexible ferrocenyl analogs (**37**). Most importantly, the diphenol-ferrocenyl derivative with both R^1 and R^2 = OH is 100x less toxic in normal cells than cancer cells. Further development of this compound may result in a highly selective new treatment for breast cancer.

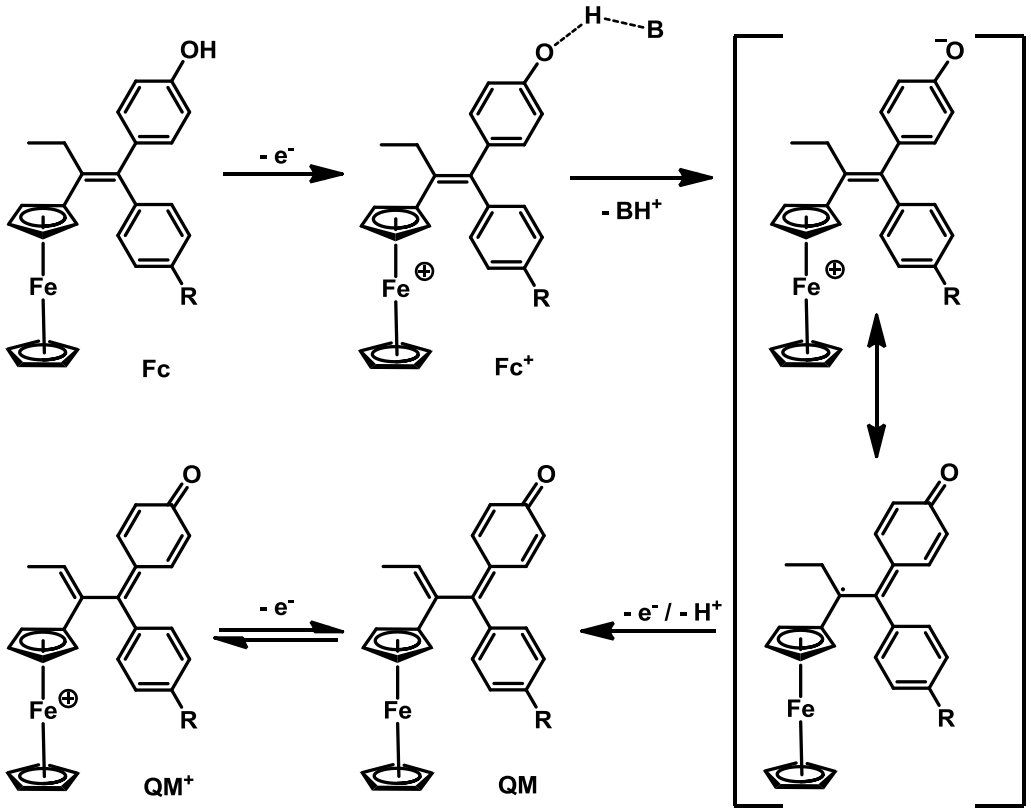


Figure 14 Activation of Ferrocifens (Fc) by oxidation to quinone methides (QM) via a PCET process.[100]

Inside cells, it is likely that Ferrocifens are activated by oxidation to quinone methides (QMs, Figure 14). These reactive species are formed only after oxidation of the ferrocene group to ferrocinium ions.[102] A pathway for the formation of quinone methides (QM) from ferrocenyl phenols (Fc), starting with a single-electron oxidation of the ferrocene is summarised in Figure 14. The residual, partly-delocalised unpaired electron acidifies the phenolic proton, which is subsequently abstracted by a base. A second single-electron oxidation, accompanied by deprotonation, stabilises the

quinone radical resulting in the QM structure. This quinone methide may then be reversibly oxidised again to the corresponding ferrocenium cation. The proposed QMs have recently been characterized after chemical oxidation of the pro-drug molecules.[102] These synthetic QMs have the same analytical data (HPLC retention times, MS spectra) as metabolites isolated after incubation of the Fc-complexes with liver microsomes, which contain the main enzymes responsible for xenobiotic metabolism. It can therefore be concluded that quinone methides are indeed active metabolites of Ferrocifens.

4. Ruthenium arene anticancer agents

In principle, low-spin d^6 half-sandwich complexes [(arene)M(X)(Y)(Z)] (arene = benzene or cyclopentadienyl derivative, M = Ru^{II}, Os^{II} or Ir^{III}) can be relatively inert toward substitution reactions and exert their anticancer activity by binding to target sites through outer sphere interactions. This inertness depends on the specific ligand sets. Examples of 'inert' but biologically active piano-stool complexes are the kinase inhibitors of Meggers et al. (e.g. complex **14** in Figure 1)[105-109] and the iodide azopyridine Ru^{II} and Os^{II} arene complexes (*vide infra*). In the kinase inhibitors the carbonyl ligand appears to increase the kinetic and thermodynamic stability of the complex, as the M-CO bond does not hydrolyse; the compound reaches its target without undergoing structural changes.

However, some half-sandwich complexes are more reactive, and can therefore be considered pro-drugs, which inside the body can be activated by ligand substitution or redox reactions. Ideally, these processes need to be controlled so that activation occurs only after the complex has reached its ultimate target, for example inside a diseased cell or tissue. The challenge for drug design is therefore to control ligand substitution reactions so that the metal complex reaches the biological target site intact, where, by different activation pathways, it can then be turned into a therapeutically active species.

It is notable that the transport as well as delivery (cell-uptake and -efflux) of natural (biologically-essential) metal ions in the body is carefully controlled by gene activation, protein production and chaperoning (homeostasis). Copper, iron and zinc, for example, are carefully passed from one protein to another so that they enter the cell when needed, pass through the cell membrane and reach the sites where they are required without becoming free, with some steps involving changes in oxidation state (Cu^{I}/Cu^{II}, Fe^{II}/Fe^{III}).[110] Thus, it is important to study protein interactions of potential metal-based drugs, for example with serum albumin or the transporter protein transferrin, in order to understand their biological activity.

4.1 Structural switches – activation by hydrolysis

There are three different ways in which the three legs of the half-sandwich 'piano stool' in either arene or Cp complexes of the d^6 metal ions Ru^{II}, Os^{II}, Ir^{III}, or Rh^{III}, can be occupied. As shown in Figure 15, occupation is possible by a tridentate ligand (X-Y-Z), or by one bidentate ligand (X-Y) and one monodentate ligand (Z), or by three moonodentate ligands (X, Y, and Z). The different ligand sets can give rise to relatively labile or rather inert complexes, depending on the nature of the X, Y, Z and the arene/Cp ligand. Such reactivity has for example been surveyed by Süss-Fink.[111] In addition, one or more of these positions can be tethered to the arene/Cp ring (e.g. arene-X). Complexes containing tridentate X-Y-Z ligands tend to be very stable towards substitution reactions, and are likely to act as scaffolds recognising targets by 'hand-and-glove' mechanisms. Complexes with only monodentate ligands are expected to be less stable towards substitution reactions, therefore compromising control over the speciation of the candidate drug once in aqueous/physiological solutions.

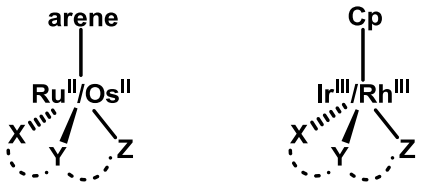
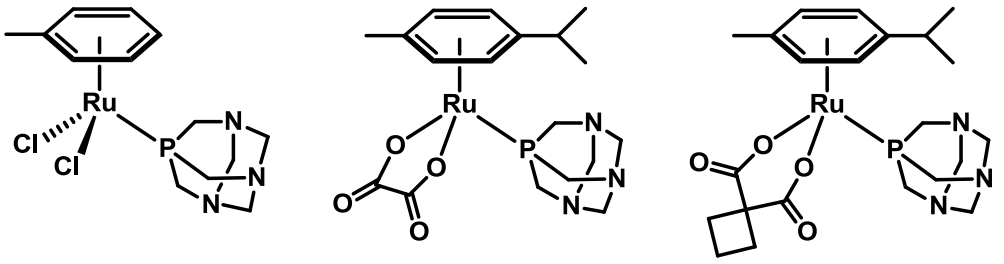


Figure 15 Coordination modes in 'piano-stool complexes.

One example for complex design with three monodentate ligands is that developed by Dyson and co-workers. The so-called RAPTA (*R*uthenium-*A*rene-*PTA*, with PTA = 1,3,5-triaza-7-phosphaadamantane, bear a phosphane ligand (PTA) that would be protonated in acidic environments. In certain diseases, changes in the cell metabolism trigger slight changes in the pH. Ideally, the coordinated phosphaamine ligand would be protonated and released at acidic pH, therefore trapping the charged Ru complex inside the diseased acidic tissue.[112] However, this complex was found to be prone to hydrolysis via cleavage of the Ru-Cl bonds[113], which has led to the synthesis of more stable chelate complexes, containing for example dicarboxylate ligands.[114] However, both RAPTA-C and the dicarboxylate complexes carbo-RAPTA (**40a**) and oxalo-RAPTA (**40b**) (Figure 16) have low cytotoxic potency towards cancer cell lines such as HT29 (colon), A549 (lung), and T47D (breast). They might be useful as antimetastatic agents, as investigated *in vivo* by Scolaro et al..[113, 115] Furthermore, the toluene derivative RAPTA-T (**39**) was found to inhibit important metastatic steps like cell detachment from the primary tumor *in vitro* and also to reduce lung metastases in vivo.[116]

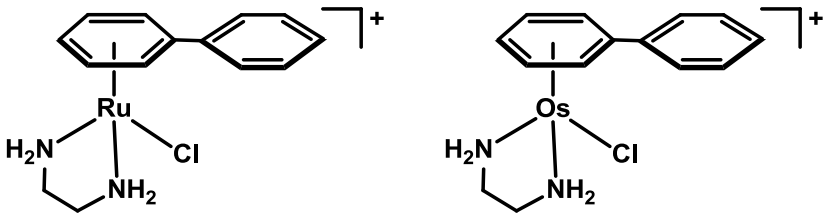


39 **40 a** **40 b**

RAPTA-T **carbo-RAPTA** **oxalo-RAPTA**

Figure 16 Examples of structures from the RAPTA complex family.

A study evaluating the biological potencies of two piano-stool complexes of the same overall ligand set that differ only in the metal (Figure 17) showed that ruthenium seems to play a key role in anti-metastatic activity.[117] Although the Os^{II} complex AFAP51 is more active against breast cancer cells *in vitro*, the Ru^{II} compound RM175 (*vide infra*) has *in vivo* activity against a mammary carcinoma and also reduced metastasis.[117] Although its *in vitro* toxicity against cancer cells is low, RM 175 is active against *in vivo* human ovarian and non-small cell lung cancer models.[118, 119]



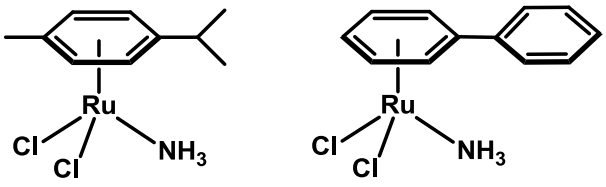
RM 175 **AFAP 51**

Figure 17 Two piano-stool complexes that differ only in the metal, the Ru^{II} complex RM175 and its Os^{II} analogue AFAP 51, exhibit significant differences in their anticancer activity in vitro and in vivo.

The major intracellular targets of RAPTA compounds appear to be proteins, although DNA binding has been observed.[112] These ruthenium complexes bind to metallothionein-2[120] and inhibit the enzymes thioredoxin reductase and cathepsin B.

RAPTA complexes may bind to the active site cysteine of cathepsin B,[121] perhaps by direct displacement of the Cl ligand. In addition, RAPTA-T (arene = toluene) partially inhibits PARP-1, a protein involved in DNA repair.[122] The inhibition is most likely caused by displacement of zinc from the PARP-1 zinc finger by Ru.[123] These ruthenium arene complexes with water soluble ligands seem to be promising enzyme inhibitors. Their osmium analogues appear to inhibit cathepsin B at least as efficiently. In general, the potency of the RAPTA-Os analogues *in vitro* towards cancer cell lines is low, but they appear to be active toward solid metastatic tumours *in vivo*.[116, 124, 125]

Recently, RAPTA-C, its osmium analogue as well as $CpRh^{III}$ and $CpIr^{III}$ derivatives have been compared to the Ru^{III} drug NAMI-A for their enzyme inhibition properties.[126] The Ru^{II} and Os^{II} complexes have similar IC_{50} values as NAMI-A (low μM range), but the Rh^{III} and Ir^{III} compounds are inactive. DFT calculations of adducts with N-acetyl-L-cysteine-N'-methylamide, which mimics the Cys residue in the cathepsin B active site, have provided more understanding of the binding behaviour.[126] Metal-sulfur binding is thermodynamically favoured only for the active compounds, while the Ir^{III} and Rh^{III} complexes form the weakest M–S bonds. These results might account for the differences in cat B inhibition properties.



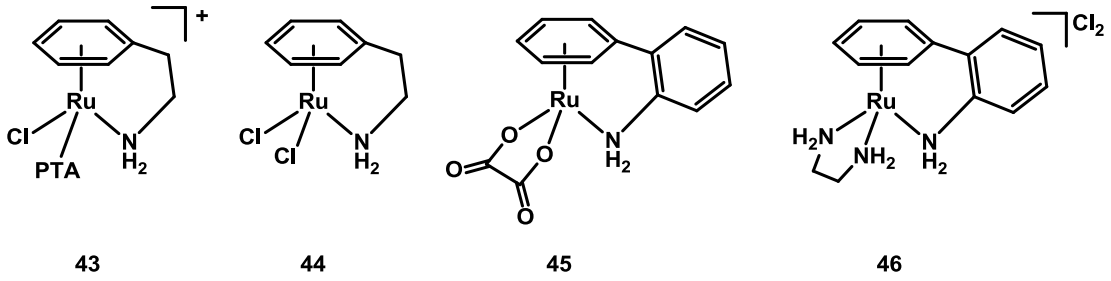
41 **42**

Figure 18 Examples of neutral piano-stool Ru^{II} complexes that have low cytotoxicity.

Neutral dichlorido complexes $[(\eta^6\text{-arene})Ru(NH_3)Cl_2]$ (arene is *para*-cymene, **41**, and biphenyl, **42,** Figure 18) also bearing three monodentate ligands are not active towards ovarian cancer cell lines up to a concentration of 100 μM.[127] They undergo rapid facile hydrolysis in two steps at 310 K, with the first aquation reaction ($t_{1/2}$ = ca.

1 min) being much faster than the second ($t_{1/2}$ = 45 min). Despite the lack of cancer cell cytotoxicity, both complexes bind to the model nucleobase 9-ethylguanine at 310 K. The complex bearing biphenyl as arene, however, undergoes decomposition via arene loss after 160 min.

Interestingly, arene-loss has also been documented for piano-stool Ru^{II} complexes in which one of the 'legs' is tethered to the 'seat', as for example [Ru(η^6:η^1-$C_6H_5(CH_2)_2NH_2$)Cl(PTA)] and [Ru(η^6:η^1-$C_6H_5(CH_2)_2NH_2$)Cl_2] (**43** and **44,** Figure 19).[128] For the latter, the two Ru-Cl bonds also hydrolyse very rapidly, yielding mainly mono-aqua mono-chlorido species. Although bifunctional adducts with 9-ethylguanine were formed, these complexes failed to cross-link calf thymus DNA. The second guanine is bound only weakly, which together with the rapid hydrolysis might explain their lack of cytotoxic activity.[128]

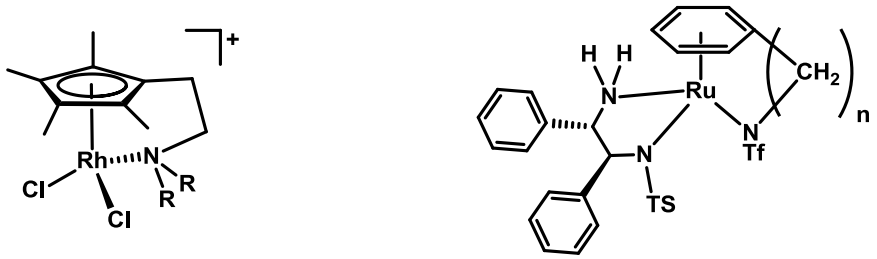


43 **44** **45** **46**

Figure 19 Piano-stool Ru^{II} complexes with tethered arene ligands.

An improvement in stability towards arene loss was observed for the chelating oxalato complex [Ru(η^6:η^1-$C_6H_5(CH_2)_2NH_2$)(oxalate)] (**45**). Only ca. 16% decomposed in 24 h in comparison to its dichlorido analogue **44** which fully decomposed within 8 h.[129] Changing the oxalato ligand for ethylenediamine, the resulting complex [Ru(η^6:η^1-$C_6H_5(CH_2)_2NH_2$)(en)]Cl_2 (**46**) did not decompose in aqueous or polar organic solvents such as methanol and dimethylsulfoxide.[130] Therefore, the N,N-ligand seems to have a greater stabilizing effect towards arene binding than the O,O-ligand. Interestingly however, **46** can undergo DMSO-induced opening of the Ru-arene-NH_2 tether ring. Most strikingly, equilibrium between the

open and the closed tether species is reached after 12 h in methanol. Both forms coexist in solution in a ratio of 2:1 (open/closed). In water (pH 7), complete ring closure of the tethered ligand occurs in less than 2 h at 298 K, opening again at basic pH. Finally, the closed tethered complex opens over time (18 h) in concentrated HCl. The opening-closing process is fully reversible over the pH range 2-12.[130] In conclusion, control of tether-ring-opening by pH variation provides a strategy for activation and cytotoxic selectivity of ruthenium arene anticancer drugs.



47 48

Figure 20 Tethered Rh^{I} catalysts for the reductive amination of carbonyls (47) or H_2 cleavage (48).

The concept of constraining the arene by a tether is also known to influence catalytic activity. For example, constraint of arene rotation in tosylated diamine Ru^{II} arene complexes by introduction of an arene-diamine tether can give more active catalysts for asymmetric transfer hydrogenation to ketones with higher enantioselectivity.[131] Similarly, arene-tethers significantly influence the catalytic performance of Rh^{III}-(η^5:η^1-Cp′-NHR) complexes (**47**) in the reductive amination of carbonyl compounds.[132] For catalysts such as [η^6:η^1-$C_6H_5(CH_2)_n$NTf]Ru[(S,S)-Tsdpen]$^{2+}$ (**48**) (Tsdpen = $TsNCH(C_6H_5)CH(C_6H_5)$-NH_2), tether-ring opening dramatically facilitates the heterolytic cleavage of molecular hydrogen (H_2), and activation by hydride binding can switch activity away from transfer hydrogenation towards asymmetric hydrogenation of aromatic ketones.[132] Thus, it can be concluded that reversible tether-ring opening can greatly influence the performance of a half-

sandwich complex and also provide a possible mechanism for activating a (potentially catalytic) drug so that it binds to a biological target site.

4.2 Activity-tuning *via* the chelating ligand

In complexes of the type $[Ru(\eta^6\text{-arene})(X\text{-}Y)Z]^+$, the three legs of the piano stool are occupied by a monodentate ligand (Z) and a bidentate chelating ligand (X-Y). Chelate complexes are generally more stable towards ligand substitution, and it is therefore possible to control aquation (substitution of the labile Z-ligand by water) by appropriate choice of the other building blocks in the structure. Thereby the toxic effects of this class of complexes towards cancer cells can be tuned.[118, 133]

A change in XY from en to acac not only significantly increases the pK_a of the aqua-complex,[134] but also influences recognition of the complex by biological targets. The selectivity towards G versus A nucleobases is changed by the choice of the donor atoms X and Y.[134] Primary and secondary amine N,N-ligands like en can act as H-bond donors and enhance the selectivity for G-binding by H-bond formation to C6O of the nucleobase. In contrast, O,O-ligands are H-bond acceptors and can H-bond with the $C6NH_2$ of adenine.[135] Therefore, H-bonding properties can be used to direct a metal complex towards specific targets. Selective recognition might be especially crucial for the activity of drugs targeting DNA.

Activation by hydrolysis of the Ru-Z bond may be important in the mechanism of action of this class of drugs, and therefore their chemical behaviour in aqueous media has been investigated.[134, 136] For example, the hydrolysis of $[(\eta^6\text{-arene})Ru(en)Cl]^+$ (**49**) is suppressed outside cells by the high concentration of chloride ions (103 mM). Chloride concentration is, however, significantly lower, ca. 4 and 23 mM, in the nucleus and cytoplasm, respectively.[137] Therefore, the chlorido form of the complex (Ru-Cl, **49**) can predominate in the extracellular medium, whereas hydrolysis products ($Ru\text{-}OH_2$/OH species, **50**/**51**) are likely to prevail inside cells.[135] Density functional calculations suggest that aquation occurs by a concerted

ligand exchange mechanism that is associative in character.[133] Thus, sterically-demanding ligands can significantly affect the rate of aquation.

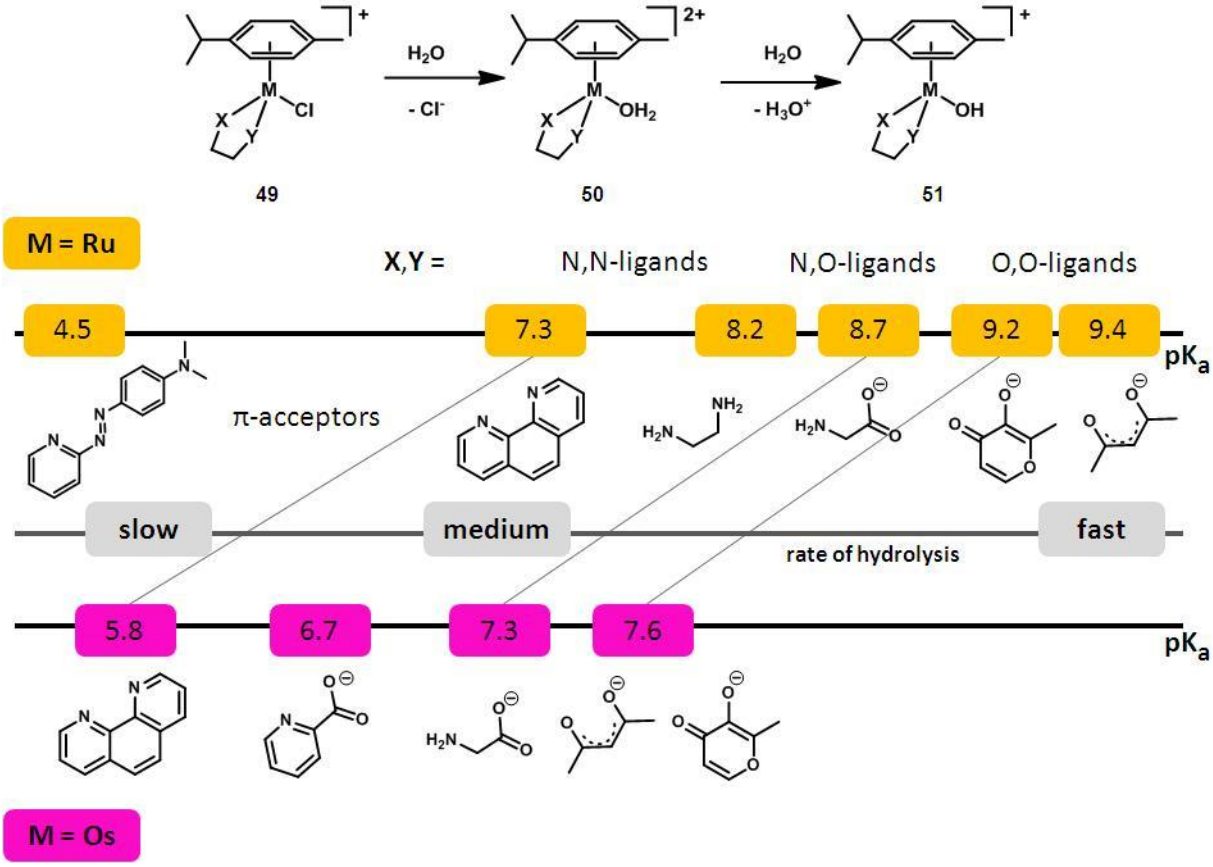


Figure 21 Comparison of hydrolysis of the M-Cl bond in arene Ru and Os diamine complexes, showing the dependcnce on the chelating ligand. Osmium arene complexes are more acidic and hydrolyse more slowly.

Ru–OH_2 (**50**) bonds tend to be more reactive than Ru–OH (**51**) bonds and the predominating species in solution at a given pH might therefore influence biological activity. Many diamine Ru^{II} arene aqua complexes have pK_a values of ca. 8. Therefore, at physiological pH ca. 7.4, the more active Ru-aqua adducts should prevail. In general, ruthenium arene complexes that hydrolyse readily exhibit cancer cell cytotoxicity, whereas those which do not undergo aquation exhibit little or no activity.

The rate and extent of hydrolysis of the Ru–Z bond are highly dependent on the nature of Z. For example, the difference in hydrolysis between Z = Cl and Z = Br in complexes $[(\eta^6\text{-arene})Ru(en)Z]^+$ is small, but hydrolysis of the iodido (Z = I)

complex is 3- to 7-fold slower. Ru-pyridine bonds (Z = py) in these complexes are even more inert and can completely block hydrolysis, at least on biologically-relevant timescales. Such complexes are not cytotoxic towards cancer cells within 24-h drug exposures. However, strategies have been developed to trigger hydrolysis of inert species. For example, $[(\eta^6\text{-}p\text{-cym})Ru(bpm)(py)](PF_6)_2$ (where bpm = 2,2'-bipyrimidine) can be activated by visible light to photo-dissociate the pyridine ligand selectively.[138] Controlled irradiation cleanly generates the reactive aqua species, able to bind to DNA nucleobases. Such behaviour creates a platform for photo-triggered binding of an anticancer pro-drug to biomolecules.

Analogous Os^{II} arene complexes $[(\eta^6\text{-arene})Os(XY)Z]$ can also be activated by hydrolysis of the Os-Z bond. There are two notable differences between Os^{II} and Ru^{II}. Firstly, aquation reactions can be up to 100x slower[139], and secondly, the resulting aqua adducts are ca. 1.5 pK_a units more acidic for Os^{II} (Figure 21). But for both metals changing the donor atoms in the chelating ligand from N to O increases the hydrolysis rates and decreases the acidity of the aqua adducts.[140, 141]

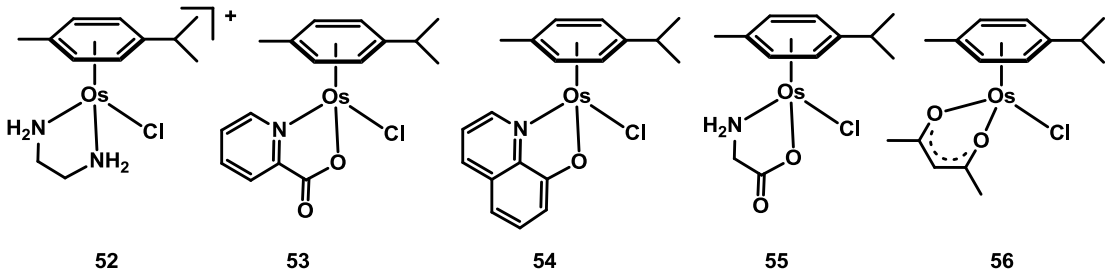


Figure 22 Systematic chelate variation in Os^{II} arene complexes.

For example, the hydrolysis of the O,O-bonded acac complexes $[(\eta^6\text{-arene})Os(acac)Cl]$ (arene = *p*-cymene (**56**), benzene or biphenyl) is too fast to be determined by NMR.[142] Also, the acac complexes are unstable in aqueous solution (and cell culture media) due to loss of acac and formation of inert trihydroxo-bridged dimers $[(\eta^6\text{-arene})Os(\mu\text{-}OH)_3Os(\eta^6\text{-arene})]^+$. Such reactions can explain why acac complexes are inactive towards human lung A549 and ovarian A2780 cancer cells.

The intermediate behaviour of N,O-chelated Os^{II} arene complexes compared to N,N- and O,O-chelates (**52** – **56**, Figure 22) is evident.[143, 144] However, within the N,O- series, the reactivity is highly dependent on the balance of the σ-donor/π-acceptor power of the ligand. For example, complexes with N,O-chelating amino acids (**55**) hydrolyse rapidly, whereas complexes with π-acceptor pyridine as N-donor and carboxylate as O-donor (**53**) hydrolyse much more slowly. The X-ray crystal structure of the adduct with 9-ethyladenine, $[(\eta^6\text{-p-cym})Os(pico)(9EtA\text{-}N7)]PF_6$, shows homo adenine base-pairing, and the 9-ethylguanine adduct in particular exhibits remarkable aqueous kinetic stability. These findings show how the rational control of chemical reactivity (i.e. hydrolysis, acidity of aqua adducts) can allow the design of cytotoxic anticancer Os^{II} arene complexes. Hydrolysis rates increase with chelating ligand donors in the order N,N < N,O < O,O. Furthermore, for activity a balance is necessary between the rate and extent of hydrolysis (anation by Cl^-), pK_a of the aqua adduct (lower reactivity of the hydroxide adduct), and formation of inert hydroxido-bridged dimers. The N,O-combination endows the picolinato complexes with potent cytotoxic activity towards A2780 human ovarian cancer cells that is similar to cisplatin.[144, 145]

Aquation-dependent cytotoxic activity supports the postulation that the cell killing effects relate directly or indirectly to coordinative binding to biomolecules. However, it does not exclude co-operative non-coordinative binding events that might contribute to the overall mechanisms of action of these complexes.

4.3 Activation by ligand-oxidation

Redox mechanisms may be involved in the activation of Ru-arene thiolato complexes. For example, reactions of the tripeptide GSH, which is present in most cells at millimolar concentrations, with $[(\eta^6\text{-bip})Ru(en)Cl]^+$ (**RM 175** (**57**), Figure 23) in buffered solutions (pH 7) yield the thiolato complex $[(\eta^6\text{-bip})Ru(en)(GS\text{-}S)]$ (**58**).[146] This subsequently undergoes oxidation to give the sulfenato complex $[(\eta^6$-

bip)Ru(en)(GS(O)-S)] (**59**).[147, 148] Whereas free sulfenates are relatively unstable, they are stabilized by coordination to Ru^{II}, and also by H-bonding. The sulfenate-Ru adduct can react further with N7 of guanosine-3',5'-cyclic monophosphate (cGMP), and the cGMP adduct $[(\eta^6\text{-bip})Ru(en)(cGMP\text{-}N7)]^+$ (**60**) is the major product even in the presence of a large molar excess of GSH. If this happens inside cells, the facile displacement of S-bound glutathione by N7 of guanine via the sulfenate intermediate may provide a potential route for RNA and DNA ruthenation. In other words, ligand oxidation activates the complex towards oligonucleotide binding.

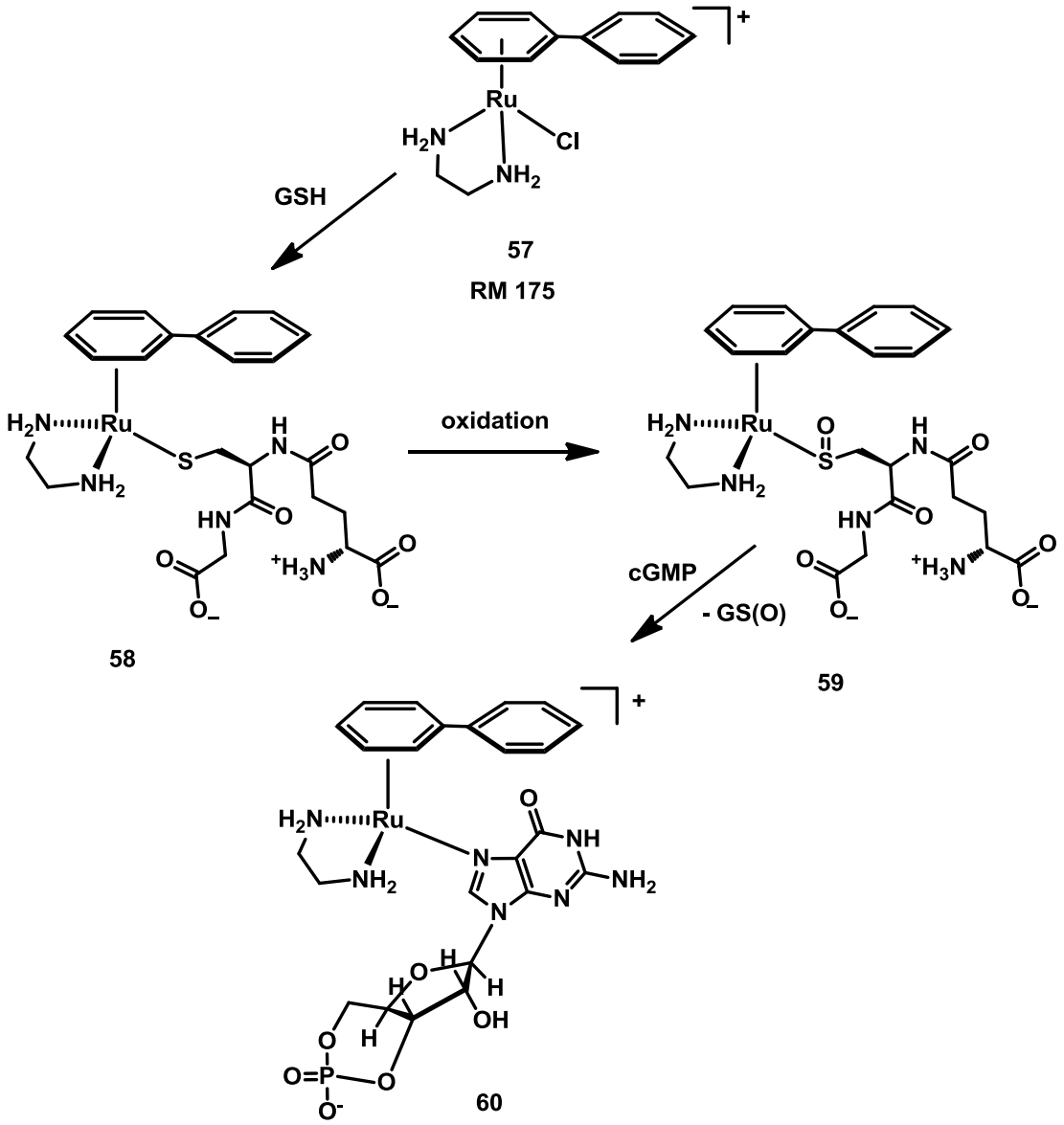


Figure 23 Reaction of an arene-osmium complex with glutathione and subsequent ligand oxidation that facilitates exchange for cGMP.

Unlike GSH adducts, $[(\eta^6\text{-arene})Ru(en)SR]^+$ complexes of thiols such as thiophenolate and isopropylthiolate are relatively stable, but are readily oxidized by H_2O_2 to give sulfenate adducts. These are stabilized by H-bonding involving the sulfenate oxygen and are formed in preference to sulfinates, even with excess of oxidant. Interestingly, the sulfenate complexes can be readily protonated (pK_a ca. 3.5), and protonation may result in a weakened Ru-SO bond.

Curiously, the antioxidant GSH can promote oxidation of the thiolato complex $[(\eta^6\text{-hmb})Ru(en)SR]^+$ (R = iPr) to give the sulfenate complex $[(\eta^6\text{-hmb})Ru(en)S(O)R]^+$, apparently mediated by the O_2/GSH couple under physiological conditions. XAS and DFT studies show that oxygenation of the thiolate sulfur has little effect on the strength of the Ru-S/Ru-SO/$RuSO_2$ bonds.[149, 150] In sulfenato complexes, the terminal oxo group contributes to charge donation, thereby making the ligand more susceptible to substitution. Protonation of the sulfenate oxo group is probably a key step in promoting its lability towards substitution by guanine (DNA).
Ruthenium arene complexes can also induce oxygenation of cysteine residues in proteins, for example Cys34 of human serum albumin (present in blood at a concentration of ca. 0.6 mM). The complex $[Ru(\eta^6\text{-}p\text{-cymene})(en)Cl]^+$ can induce oxidation of Cys34, the only thiol group in albumin, to the sulfinate, but when the arene is biphenyl (**57**, **RM 175**) no oxidation of Cys34 is observed, perhaps because entry into the protein cleft containing Cys34 is hindered by the bigger arene.[150]

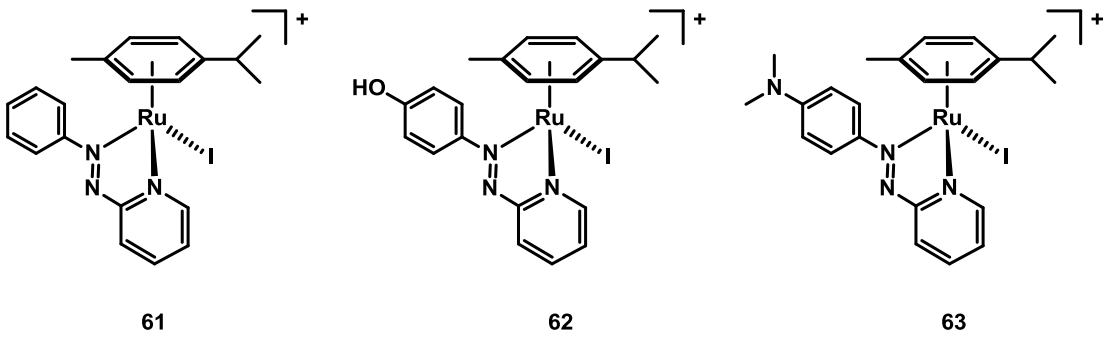
Recently, Süss-Fink et al. have reported relatively inert thiolate-bridged dinuclear Ru^{II} arene complexes which may be activated via reduction by glutathione with concomitant oxidation of GSH to GSSG and formation of H_2.[151]

4.4 Azopyridine complexes – the exeption that proves the rule?

Azopyridine Ru^{II} arene complexes $[Ru(\eta^6\text{-arene})(azpy)I]^+$ (e.g. arene = *p*-cymene or biphenyl, and azpy = N,N-dimethylphenyl- or hydroxyphenyl-azopyridine)

do not undergo activation by hydrolysis but are cytotoxic towards A2780 ovarian and A549 lung cancer cells.[152] The presence of the electron donor substituent on the phenyl ring (NMe_2 or OH) is critical for activity, since the parent phenylazopyridine complexes are inactive.

Phenylazopyridine and arene ligands act as competitive π-acceptors towards Ru^{II} $4d^6$ electrons. The pK_a* values of the pyridine nitrogen (NH proton) of the ligands are low (azpy (**61**) 2.47, azpy-OH (**62**) 3.06 and azpy-NMe_2 (**63**) 4.60), suggesting that they are weak σ-donors. This, together with their π-acceptor behaviour, serves to increase the positive charge on ruthenium, and, together with the π-acidic η^6-arene, partially accounts for the lack of hydrolysis of the azpy-NMe_2 and -OH complexes and the slow arene loss for the azpy complexes (half life 9 - 21 h at 310 K). The pK_a^* of the coordinated water in $[(\eta^6\text{-}p\text{-cym})Ru(azpyz\text{-}NMe_2)OH_2]^{2+}$ (aqua-**63)** is low, 4.60, consistent with the increased acidity of the ruthenium centre upon coordination to the azpy ligand.



61 62 63

Figure 24 Variation of substituents of azopyridine (azpy) ligands in Ru^{II} *p*-cymene complexes.

In contrast, the chlorido complexes are an order of magnitude less potent and are less inert, tending to undergo arene loss in aqueous solution. The combination of the π-bonded arene ligand, a strongly chelating σ-donor/(strong) π-acceptor azopyridine ligand and iodide as the monodentate ligand is special. The mechanism of action appears to involve modulation of the redox balance in cancer cells, as detected

by an increase in reactive oxygen species (fluorescence trapping experiments) in A549 human lung cancer cells.[153] These azopyridine complexes can undergo activation by reduction of the ligand by glutathione. While azopyridine ligands alone are difficult to reduce, the reduction potentials are biologically accessible when the azopyridine is coordinated to Ru^{II}.

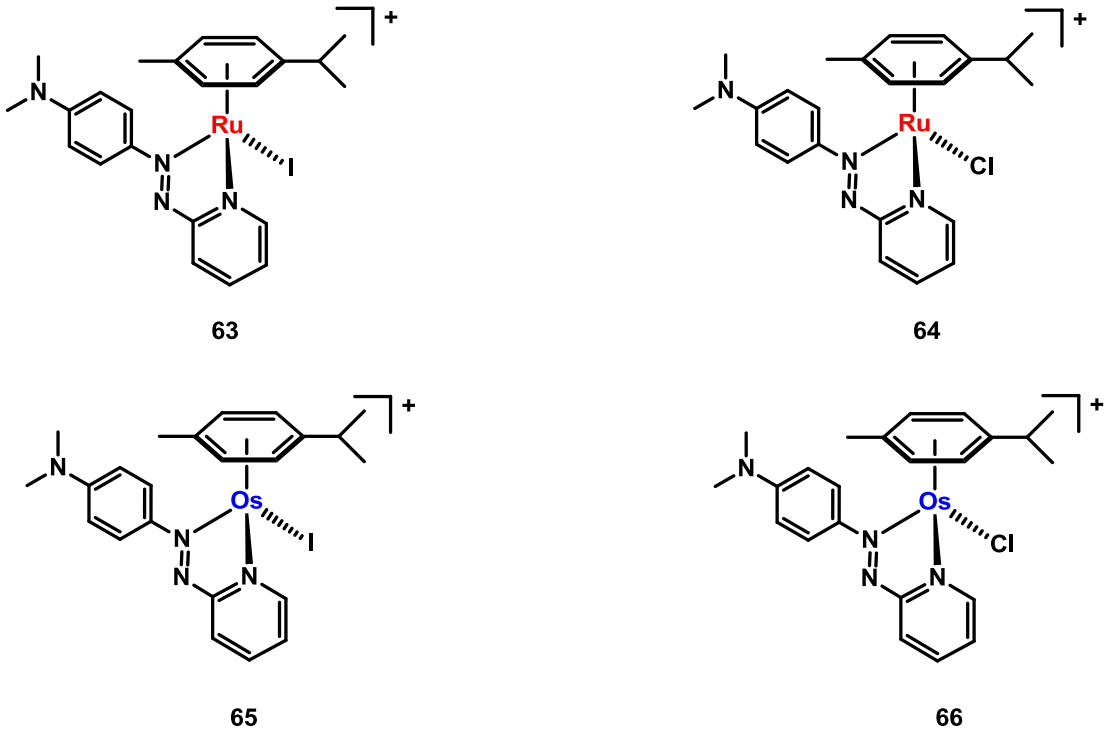


Figure 25 Systematic structural development of anticancer complexes: change of leaving group from I in **63/65** to Cl in **64/66**; chane of metal centre from Ru^{II} in **63/64** to Os^{II} in **65/66**.

Likewise, iodido Os^{II} complexes $[Os(\eta^6\text{-arene})(XY)I]^+$, XY = *p*-hydroxy or *p*-dimethylaminophenylazopyridine, arene = *p*-cymene or biphenyl, which are potently cytotoxic at sub-micromolar concentrations towards a panel of human cancer cell lines, are inert towards hydrolysis. Substituents on both the phenyl and pyridine rings can have a major effect on activity.[154, 155] Encouragingly, they exhibit low toxicity and negligible deleterious effects in a colon cancer xenograft model, giving rise to the possibility of a broad therapeutic window.[154] In particular, a single iv dose of the

iodido complex [Os(η^6-p-cym)(azpy-NMe_2)I]PF_6 (**65**) significantly delays the growth of such a colorectal cancer xenograft.

Ligand oxidation can inactivate Ru^{II} arene complexes, when the chelating ligand is diaminobenzene. In the oxidised form, the diimine complexes (**68**) are inactive (non-cytotoxic), whereas in the reduced diamine form (**67**), the compounds are active.[156]

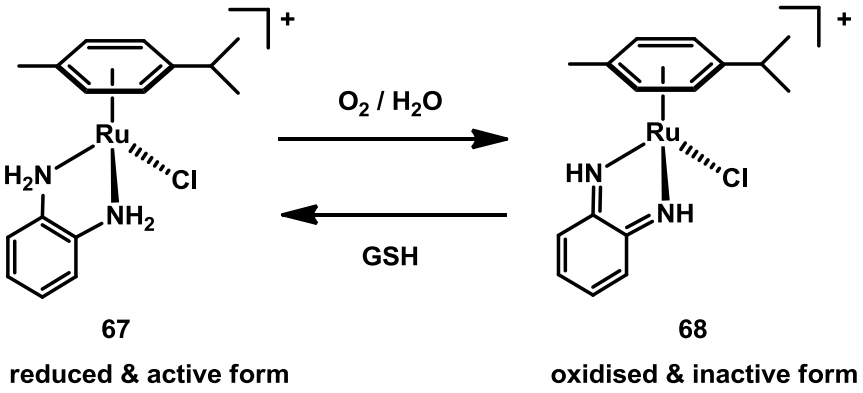


Figure 26 Ligand oxidation as activity switch.

Upon oxidation, for example by dioxygen in methanol or water, the ligand becomes a stronger π-acceptor, which results in lower hydrolysis rates. Although the oxidised form of the complex can be reduced by glutathione, intracellular reduction appears to be too slow to (re)activate the complex *in vitro*. Thus, ligand tuning might be a possible way to hasten the reduction and afford pro-drugs that can undergo redox-activation inside tumour cells.

4.5 From non-hydrolysable complexes to stable multi-core assemblies

Therrien and co-workers have created so-called "complex-in-a-complex" cations as “trojan horses” for the delivery of drugs to cancer cells.[157] These involve the encapsulation of a relatively hydrophobic complex (e.g. $Pd(acac)_2$) within a hydrophobic pocket of a metal-containing host (**69**). These metalla-cages (Figure 27) can release hydrophobic drugs like pyrene-R (**70**) efficiently following uptake into a

cell.[158] Combined microscopy and flow cytometry experiments show that the fluorescent cargo molecule is successfully delivered into the cells. An assisted diffusion pathway is involved in internalisation of the cage compound.

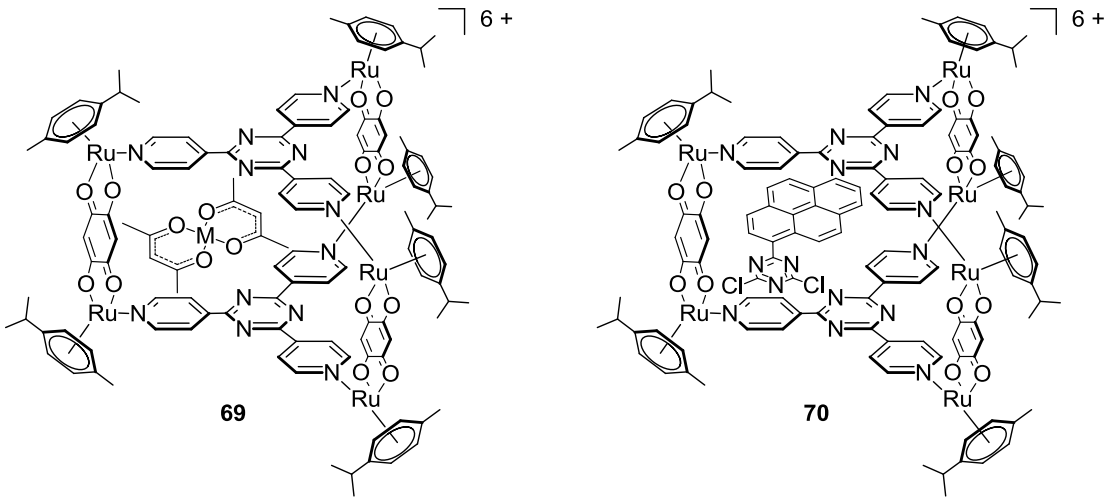


Figure 27 Examples of organometallic cage assemblies containing piano-stool building blocks.

More elaborate cargos such as lipophilic pyrenyl functionalized poly(benzyl ether) dendrimers, have also been encapsulated in these triangular prismatic hosts, to give assemblies similar to **69**.[159] These hexacationic water-soluble systems can transport pyrenyl-containing dendrimers (Figure 28) into cancer cells, and proved to be more cytotoxic than the empty cage structures. The assemblies are stable in aqueous media for 24 hours, a period relevant for drug delivery purposes. Therefore, these systems are promising candidates for delivery of treatments using the enhanced permeability and retention (EPR) effect.

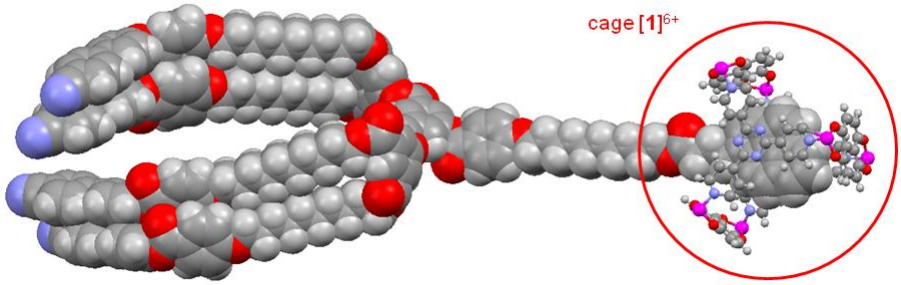
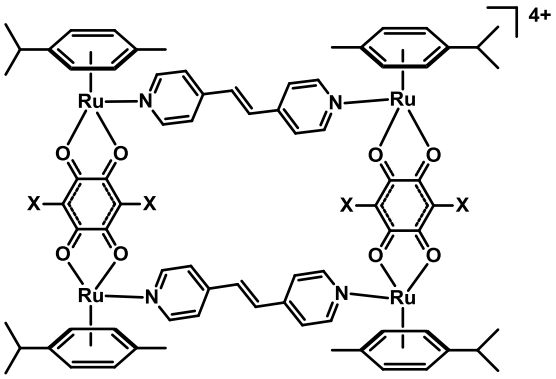


Figure 28 Simulation of the structure (obtained with HyperChem software) of a pyrenyl containing dendrimer (***1***) into the hexacationic cage assembly (***cage***$^{6+}$). Original image used with the kind permission of Wiley VCH**.**[160]

Cationic osmium and ruthenium metalla-rectangles (**71**) are as active towards A2780 human ovarian cancer cells as cisplatin, with the advantage of being nearly as active even towards cisplatin-resistant cells.[161-163]



71

Figure 29 Example of a metalla rectangle that shows activity towards A2780 human ovarian cancer cells.

Furthermore, FID and SPR studies show that four octacationic ruthenium coordination cubes bind strongly to duplex and quadruplex (telomeric and *c-myc*) DNA.[164]

4.6 Biologically active XY ligands

If the chelating ligand XY is itself biologically active, then its release inside tumour cells might enhance the activity of the complexes. This strategy has been used by several groups and examples are displayed in Figure 30. For example, Keppler et

al. have studied the cytotoxicity of *p*-cymene Ru^{II} and Os^{II} complexes with indoloquinolines as N,N-ligands (**72**),[165] because indolo[3,2-*d*]benzazepines (paullones) are potential kinase inhibitors. The Os derivatives seem to be more active than their Ru analogs, with electron-rich ligands enhancing the activity, but their mechanism of action was not further investigated. Another example is that of Turel et al.,a *p*-cymene-ruthenium complex of the antibiotic ofloxacin as O,O-bidentate ligand (**73**) and Cl as leaving group.[166] However, the complex was non-toxic towards cancer cell-lines. Ru(arene) adducts with antibacterial quinolones nalidixic acid (**74**) and cinoxacin (**75**) prepared by Kljun et al. are potential antibacterial agents,[167] and Grguric-Sipka et al. have studied a series of *p*-cymene-ruthenium complexes with pyridine dicarboxylic acids as chelating ligands.[168]

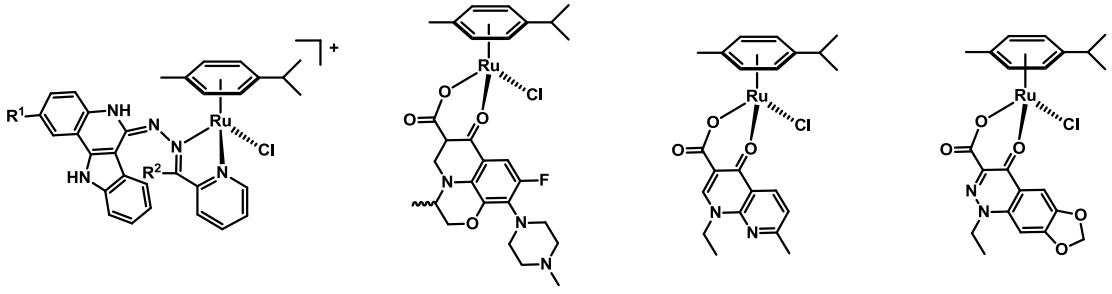
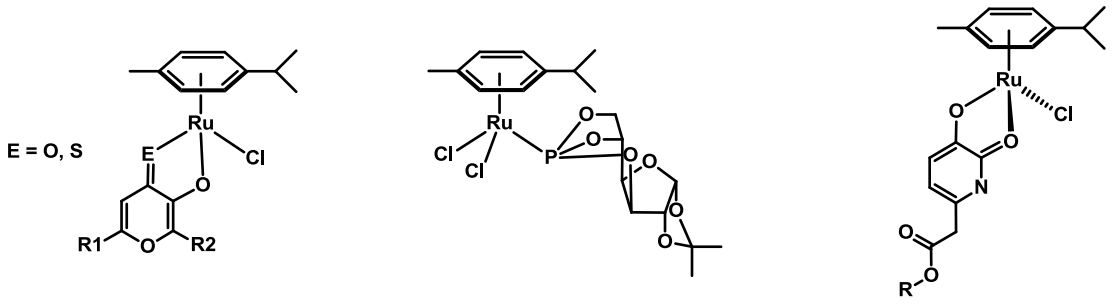


Figure 30 Ru^{II} *p*-cymene complexes bearing biologically active molecules as chelating ligands.

A remarkable change that switches on cancer cell cytotoxicity occurs for *p*-cymene-Ru complexes when the O,O-donor maltol (**76O**) is replaced by S,O-donor thiomaltol derivatives (**76S**).[169] This activation can be correlated with the higher stability of the S,O-chelates in aqueous solution.



76 77 78

Figure 31 Further examples of chelatin ligands that influence the stability of the complexes towards hydrolysis.

O,O-ligands have been used to prevent arene-Ru complexes from hydrolysing in RAPTA analogs derivatised with a sugar-bearing phosphate ligand and a biscarboxylate as chelator (**77**).[170] The Ru-P bond appears to stabilise the O,O-complex by accepting electron density. These compounds do not hydrolyse and do not exhibit significant reactivity towards biomolecules. However, they are not active towards cancer cells either, which leads to the conclusion that a window exists in which hydrolysis of at least one M-L bond triggers cytotoxicity.[170]

With hydroxypyr(id)ones as O,O ligands (**78**) for example, hydrolysis of the Ru-Cl bond and subsequent binding to amino acids proceeds quickly for Ru^{II} and Os^{II}-arene complexes.[171] This means that amino acids, e.g. in cell culture media, can potentially displace the chelating ligand from the metal centre. Interestingly, the Os complexes are slightly more active in *in vitro* cytotoxicity studies, perhaps related to the slower hydrolysis of the Os-Cl bond. Adducts with N7 of 5'-GMP have been characterised. Furthermore, both the Ru and Os complexes show promising activity in protein kinase inhibition studies, comparable to other CDK2/cyclin A inhibitors.[171]

Phenyl picolinamide derivatives can act as N,N- or N,O-donors in Os^{II} and Ru^{II} complexes $[(\eta^6\text{-arene})(Os/Ru)(XY)Cl]^{n+}$ (Figure 32). Electron-withdrawing substituents on the phenyl ring result in N,N-coordination (**80**), whereas electron-donating substituents lead to N,O-coordination (**79**).[172] Dynamic interconversion between N,O- and N,N-configurations occurs in solution and is temperature- as well

as pH-dependent. Interestingly, the interconversion with varying pH is reversible, while the temperature-induced changes are not.

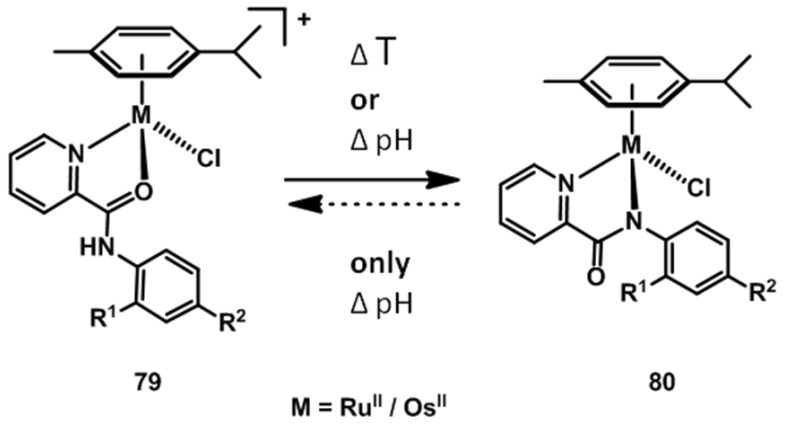


Figure 32 Parameters that can lead to a change in coordination mode and thereby switch on cytotoxicity for picolin amide complexes: Increase in temperature switches non-reversibly, pH changes switch reversibly.

The neutral N,N-coordinated compounds hydrolyse rapidly (< 1 min at 298 K), and exhibit significant (32-70%) binding to guanine, but no binding to adenine. Some N,N-coordinated compounds also show significant activity against colon, ovarian and cisplatin-resistant human ovarian cancer cell lines. In contrast, N,O-coordinated complexes hydrolyse slowly, do not bind to guanine or adenine, and are non-toxic. This, again, shows that the choice of chelating ligand can finely tune the properties of anti-cancer complexes and also that a variety of specific switches exist for their cytotoxicity.

The effect of substituents on the pyridyl ring has been explored for Os^{II} arene anticancer complexes containing picolinate derivatives (R-pico) as chelating ligand and has led to the identification of structure – activity relationships.[145] The rates of hydrolysis at 288 K of derivatives of $[(\eta^6\text{-biphenyl})Os^{II}(\text{R-pico})Cl]$ (**81**) decrease with decreasing electron-donor strength of the substituent OH > Me > Br > COO^- (Figure 33). In this series, a *para* carboxylate proved to be the most deactivating substituent, slowing the hydrolysis down the most. This complex was also the least active in

cancer cell tests, probably because the negative charge on the chelating ligand limits cellular uptake.

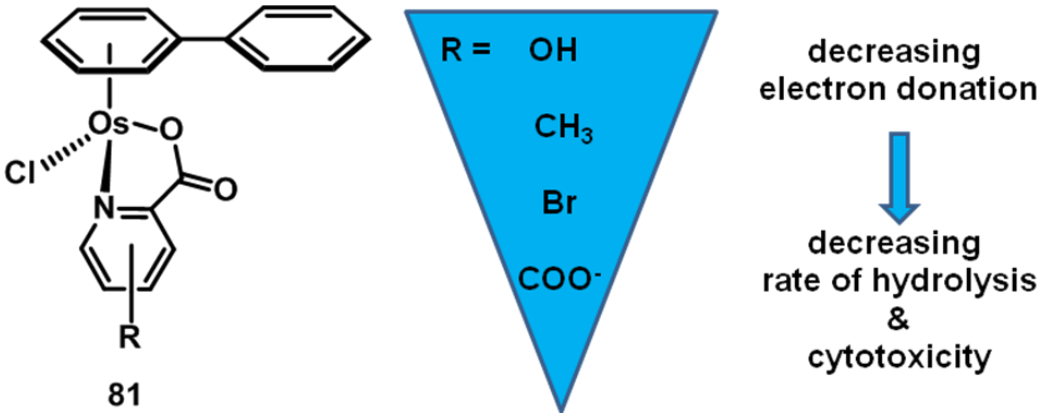


Figure 33 Effect of substituents on rate of hydrolysis and cytotoxicity of Os-pico complexes.

All the *para*-substituted pico complexes readily form adducts with both 9-ethylguanine (9EtG) and 9-ethyladenine (9EtA), and therefore may attack DNA (or RNA) inside cells. These adducts are, however, less favoured for ortho-substituted complexes. In cytotoxicity assays with A2780 human ovarian and cisplatin-resistant A2780cis human ovarian, A549 human lung and HCT116 human colon cancer cells, only the *para*-substituted complexes bearing a methyl substituent (*p*-Me) exhibited significant activity, with IC_{50} values < 25 μM, and are as active as cisplatin in A2780 and HCT116 cell lines. The most promising Os^{II} complex in this series contains a *para*-Me(pico) ligand.[173] Hence, substitution on the XY ligand can tune the properties and therefore activities of these metal complexes.

5 DNA as a target

Although proteins may be significant targets for the anticancer activity of arene complexes, DNA may also be a target, depending on the complex. As with other bio molecules, interactions of complexes of the type [(η^6-arene)M(XY)Z] with DNA are determined by the nature of the arene, the leaving group (Z) and chelating ligand XY.

5.1 Arene complexes

The arene-ligand in half-sandwich complexes is not static but able to move in various ways depending on its nature (Figure 34). When the arene is, for example, benzene or the substituted analogue hexamethylbenzene (hmb) rapid rotation around the perpendicular axis occurs. However, there appear to be no experimental determinations of rotation rates, perhaps because they are too rapid to measure. Computational studies suggest that the arene ligand in **[(**η^6 benzene)Ru(en)(OH_2)]$^{2+}$ completes a full 360° rotation within 2 ps.[174] Such rotation may allow the arene to optimise interactions with DNA.

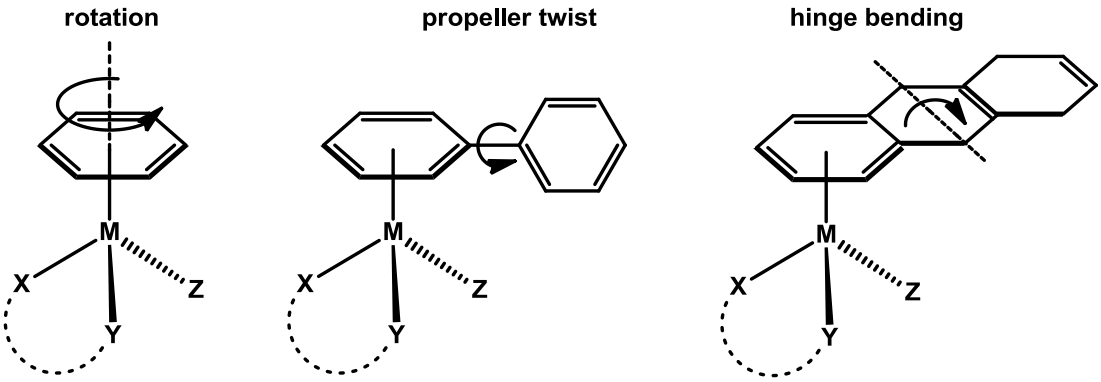


Figure 34 Possibilities of arene motion and bending in half-sandwich complexes**.**

Hydrophobic stacking interactions between extended arene ring systems and DNA bases, especially involving the purine ring system of guanine, can have a major influence on DNA binding reactions. The rates of reaction of cyclic guanosine monophosphate with [(η^6-arene)Ru(en)X]$^{n+}$ where X = Cl^- (**49**) or H_2O (**50**) decrease in the order: tetrahydroanthracene (tha) > biphenyl (bip) > dihydroanthracene (dha) >> *p*-cymene (*p*-cym) > benzene (bz), suggesting that N7-binding is promoted by favourable arene-purine hydrophobic interactions in the associative transition state.[175]

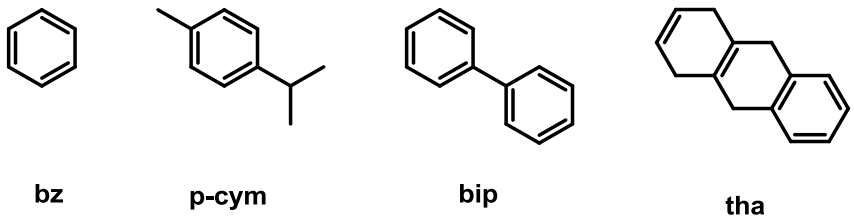


Figure 35 Different arene ligands: extended arenes can act as DNA intercalators.

In the 9-ethylguanine (9EtG) adduct $[(\eta^6\text{-arene})Ru(en)(9EtG\text{-}N7)][PF_6]_2$ strong π-π arene-nucleobase stacking is present. The outer ring of the anthracene dha or tha is stacked over the purine base at distances of 3.45 Å and 3.31 Å, respectively.[175] These extended arene ligands are flexible through rotation around the arene-Ru bonds, through propeller twisting for bip, and hinge-bending for tha and dha (Figure 34). Propeller twisting of bip decreases by ca. 10^o so as to maximise intra- or inter-molecular stacking with the purine ring, and stacking of tha and dha with the purine ring is optimised when their tricyclic ring systems are bent by ca. 30^o. Such arene-base interactions on DNA give rise to an unusual dual mode of binding: coordination to N7 of G accompanied by arene intercalation.[176]

For four complexes $[(\eta^6\text{-arene})Os(4\text{-methyl-picolinate})Cl]$ that differ only in the arene, there is a correlation between hydrophobicity (log P), cellular uptake and cytotoxicity which increases in the order arene = bz < *p*-cym < bip < tha.[173] This suggests that log P is a useful tool for predicting the cytotoxicity of this class of compounds. Cell distribution studies using fractionation and TEM imaging showed that all four compounds distribute similarly within cells, with 64–76% accumulating in the cytosolic fractions and 11–17% in the nuclei. For the bip complex the contrast and morphological changes in TEM images suggest that the induced apoptosis involves mitochondria. These Os complexes may also find use as stains for EM cell sections.

Planar organic polycyclic aromatic intercalators, such as ethidium bromide and acridine and its derivatives can inhibit nucleic acid synthesis *in vivo* and are known to cause frameshift mutations in bacteria like *E. coli*.[177]. Metallo-intercalators

can unwind DNA and insert their planar ligands between intact base-pairs, while metallo-insertors eject bases from a single base-pair, and their planar ligands act as π-stacking replacements.[176]

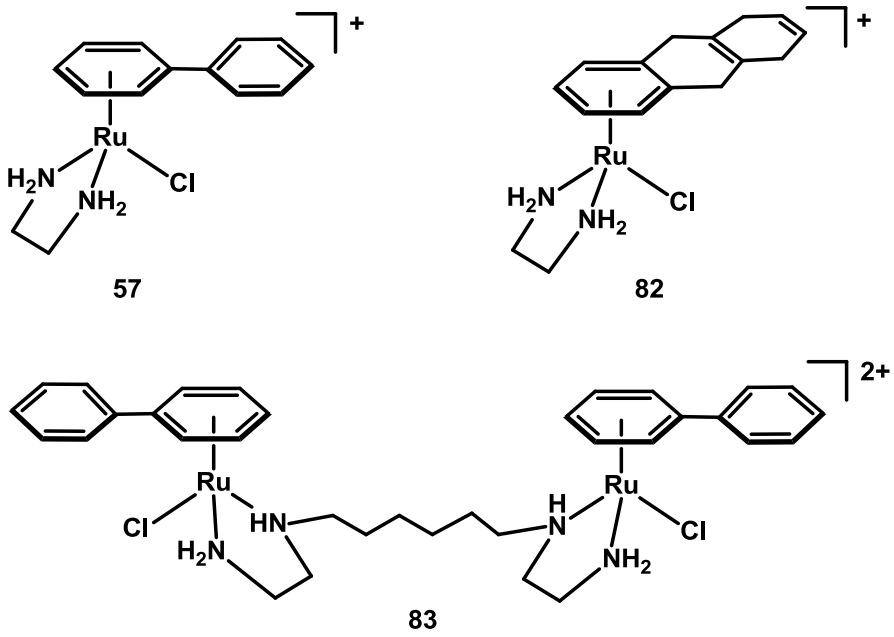


Figure 36 Ru^{II} arene complexes that interact with DNA.

The anticancer complex $[(\eta^6\text{-biphenyl})Ru(en)Cl]^+$ (**57**) binds specifically to the G bases in the 14-mer DNA duplex d(ATACATGGTACATA)·d(TATGTACCATGTAT) with the fragment $\{(\eta^6\text{-biphenyl})Ru(en)\}^{2+}$ binding to N7 of G7 or G18 and the uncoordinated phenyl ring of the arene intercalated between G7 and T6 or G18 and T17, respectively, or stacked on flipped-out base T17 (Figure 37).[178]

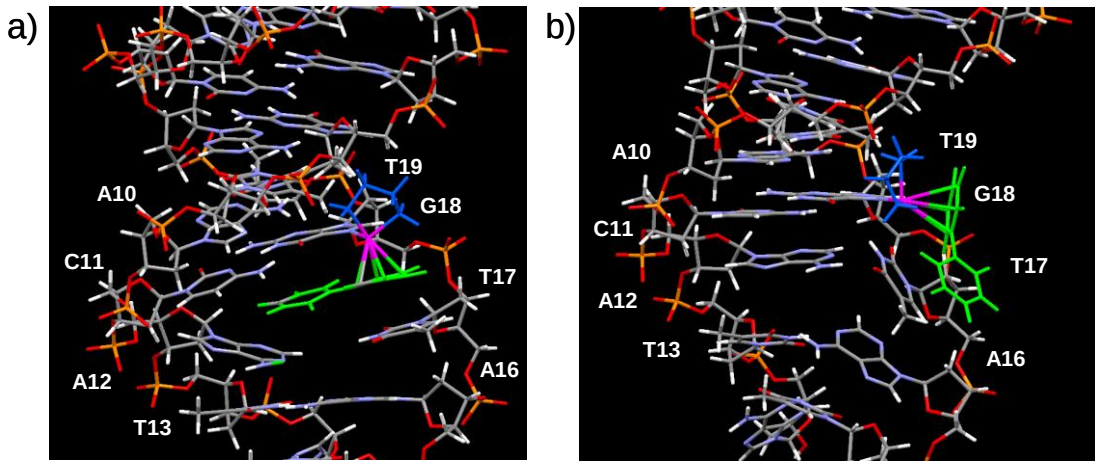


Figure 37 Structures of adducts of $\{(bip)Ru(en)\}^{2+}$ with a 14-mer duplex oligonucleotide based on solution NMR data, showing **(a)** intercalation of the arene between DNA bases, and **(b)** stacking of the arene on a flipped-out T base. Figure adapted from ref 159.

The dinuclear complex $[((\eta^6\text{-biphenyl})RuCl(en))_2\text{-}(CH_2)_6]^{2+}$ (**83**) induces a large unwinding (31°) of plasmid DNA, and effectively inhibits DNA-directed RNA synthesis *in vitro*. This unwinding angle is more than twice that induced by the mononuclear complex **57** and is attributable to cross-linking of the DNA and perturbation of the DNA structure by a double intercalation by the two pendent phenyl rings.[179]

A similar study was conducted for dinuclear Ru^{II} p-cymene complexes. These are able to unwind DNA and also to cross-link not only two DNA strands but also DNA to proteins.[180]

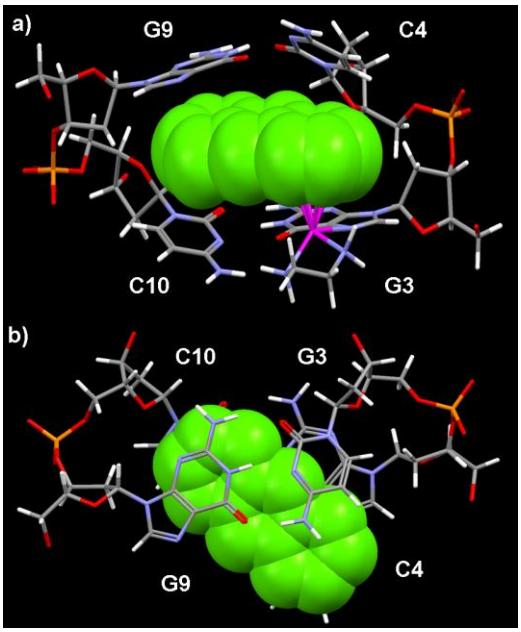


Figure 38 Model of duplex DNA ruthenated at N7 of G3 (a, side and b, top view) with $\{(\eta^6\text{-tha})Ru(en)\}^{2+}$ (**82**) showing penetrative intercalation. Ru pink, tetrahydroanthracene ligand (tha) green space-filling.[181]

The tetrahydroanthracene complex $[(\eta^6\text{-tha})RuCl(en)Cl]^+$ (**82**) is 10x more cytotoxic to cancer cells than the biphenyl complex, perhaps due to the penetrating intercalation of the two uncoordinated rings of the Ru-bound arene between DNA bases.[181] Such binding is found for the DNA hexamer $d(CGGCCG)_2$, with selective intercalation between two base pairs, G^3/C^{10}:C^4/G^9 or G^6/C^7:C^5/G^8 (Figure 38).

The *o*-, *m*- and *p*-isomers of the terphenyl arene complex $[(\eta^6\text{-terp})Ru(en)Cl]^+$ can be separated by differences in their mobility on travelling through N_2 under the influence of a continuous train of transient voltage pulses (ion mobility mass spectrometry); the *para* isomer has the biggest collision cross section and travels the slowest.[182] Circular and linear dichroism, competitive binding experiments with ethidium bromide, DNA melting and surface-enhanced Raman spectroscopic data are consistent with combined intercalative and monofunctional (coordination) binding of the *p*-isomer $[(\eta^6\text{-}p\text{-terp})Ru(en)Cl]^+$ which probably contributes to its higher cytotoxic potency compared to the *o*- and *m*-isomers.[183] $[(\eta^6\text{-}o\text{-terp})Ru(en)Cl]^+$ inhibits growth of the cancer cells through induction of apoptotic cell death by regulating the expression of Bcl-2 family proteins and G_0/G_1 cell cycle arrest. This complex has a

very low mutagenicity and a different mode of action compared to platinum antitumour drugs in clinical use.[184] Ion mobility mass spectrometry can also detect shape changes in DNA on ruthenation. For example $[(\eta^6\text{-bip})Ru(en)]^{2+}$ (**57**) adducts of d(CACGTG) are more compact than the free oligonucleotide.[185]

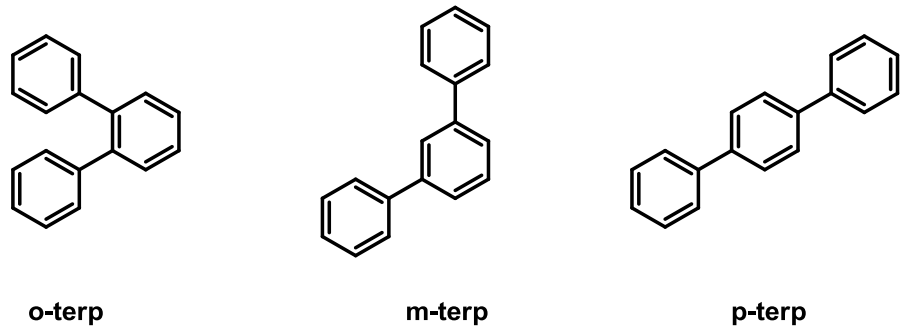


Figure 39 Variation of terphenyl ligands: substitution pattern influences DNA interactions and cytotoxicity of their Ru^{II} piano-stool complexes $[(terp)Ru(en)Cl]^+$.

The conformation of DNA modified by these monofunctional Ru^{II} arene complexes as well as their recognition by DNA-binding proteins and repair have been investigated by Novakova et al.[186] The thermodynamic properties, conformation, and recognition of DNA duplexes uniquely and site-specifically modified by monofunctional adducts of Ru^{II} complexes of the type $[(\eta^6\text{-arene})Ru^{II}(en)Cl]^+$, where the arene is *para*-, *meta*-, or *ortho*-terphenyl have been explored.[187] The *para* complex exhibits promising cytotoxic effects in human tumour cells whereas the *meta* and *ortho* isomers are much less cytotoxic.[183] Concomitantly with the high cytotoxicity of the *para* complex, its DNA binding mode involves combined intercalative and monofunctional (coordination) binding modes whereas less cytotoxic isomer of the metal complex binds to DNA via only a monofunctional coordination to DNA bases. The affinity of replication protein A for a DNA duplex containing an adduct with the *para* isomer is markedly lower than to that of the *meta* isomer. Also importantly, when the *para* complex is bound to DNA, it induces a considerably lower level of repair synthesis than the *meta* isomer. This suggests a less efficient removal from

DNA and enhanced persistence of the more potent and intercalating complex. These results support the view that these monodentate Ru^{II} arene complexes belong to a class of anticancer agents for which structure-pharmacological activity relationships may be correlated with their DNA binding modes.

5.2 Cp complexes

In an analogous way to the increase of activity seen for half sandwich arene complexes of Ru^{II} and Os^{II}, activity can also be switched on by extending the Cp* ring in Ir^{III} complexes with intercalating phenyl substituents.[188]

The organometallic half-sandwich Ir^{III} complexes $[(\eta^5\text{-}Cp^x)Ir(XY)Cl]^{0/+}$ contain not only the classical Cp* ligand but also the new Cp ligands Cp^x = tetramethyl(phenyl)cyclopentadienyl (Cp^{xph}) or tetramethyl(biphenyl)-cyclopentadienyl (Cp^{xbiph}), as well as different N,N- and N,O-chelating ligands, N,N = 1,10-phenanthroline, 2,2'-bipyridine, ethylenediamine, and N,O = picolinate. All complexes hydrolyse rapidly, perhaps surprisingly so for a low-spin $5d^6$ metal ion. Complexes with N,N-chelating ligands (e.g. **84** – **86**) also readily form adducts with 9-ethylguanine but not 9-ethyladenine. The N,O-chelating picolinate complexes, however, bind to both purine bases and show lower cytotoxic activity. Their potency towards A2780 human ovarian cancer cells increases with phenyl substitution on the cyclopentadienyl ligand: $Cp^{xbiph} > Cp^{xph} > Cp^*$; with some Cp^{xbiph} complexes even exhibiting sub-micromolar activity. Hydrophobicity (log P) as well as cell and nucleus accumulation of iridium also correlate with their cytotoxicity (IC_{50}) (Figure 40). They distribute similarly within cells. Ability to displace DNA intercalator ethidium bromide from DNA correlates with cytotoxicity and viscosity of Ir-DNA adducts. Hydrophobicity and intercalative ability of Cp^{xph} and Cp^{xbiph} therefore make a major contribution to the anticancer potency of these Ir^{III} complexes. [188]

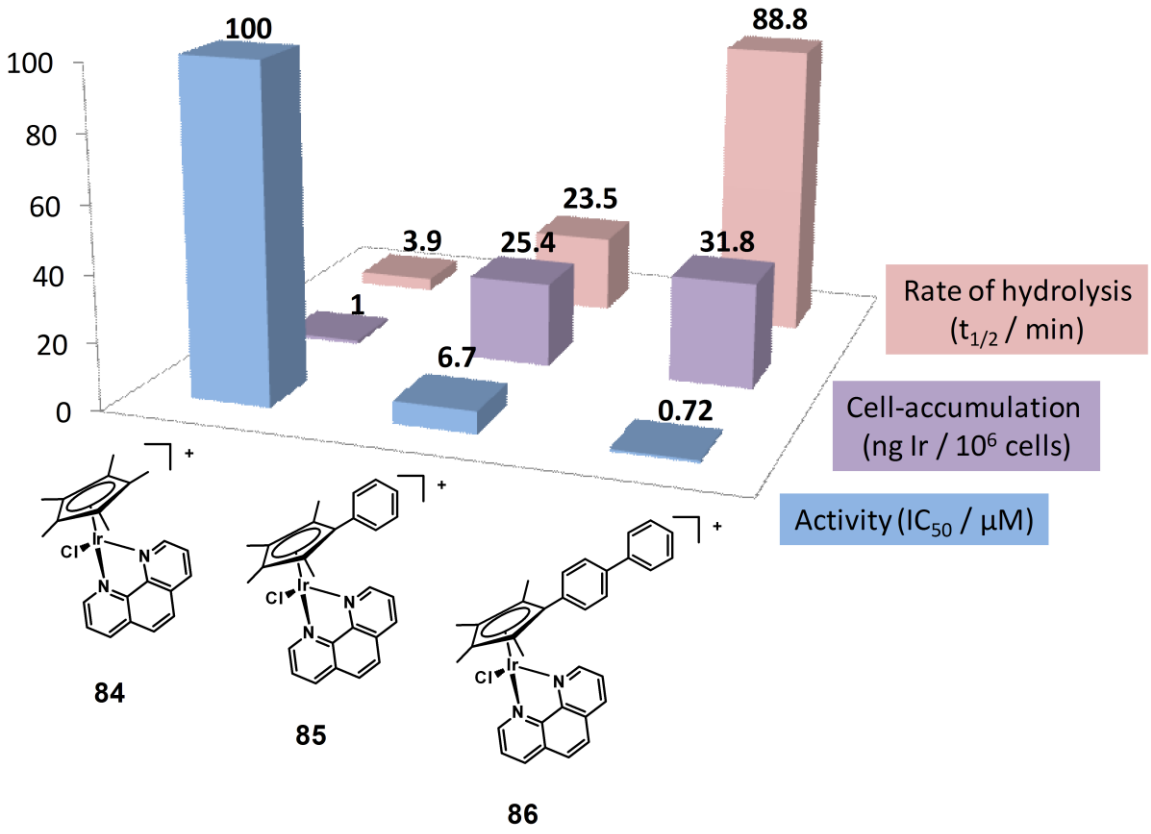


Figure 40 Relationship between cytotoxicity (IC_{50} in A2780), intercalative ability, cellular accumulation, hydrophobicity, and rates (288 K) and equilibrium constants (278 K) for hydrolysis of iridium complexes $[(\eta5\text{-}Cp^x)Ir(phen)Cl]^+$ [188]

5.3 Classical intercalators

More classical intercalators can also be incorporated into the chelating ligand, sometimes with dramatic effects on biological activity. For example, the replacement of phen in $[(\eta^5\text{-}C_5Me_5)Ir(phen)Cl]^+$ (**87**) by strongly intercalating dipyridoquinoxaline (dpq, **88**) and dipyridophenazine (dppz, **89**) ligands switches on cancer cell cytotoxicity.[189]

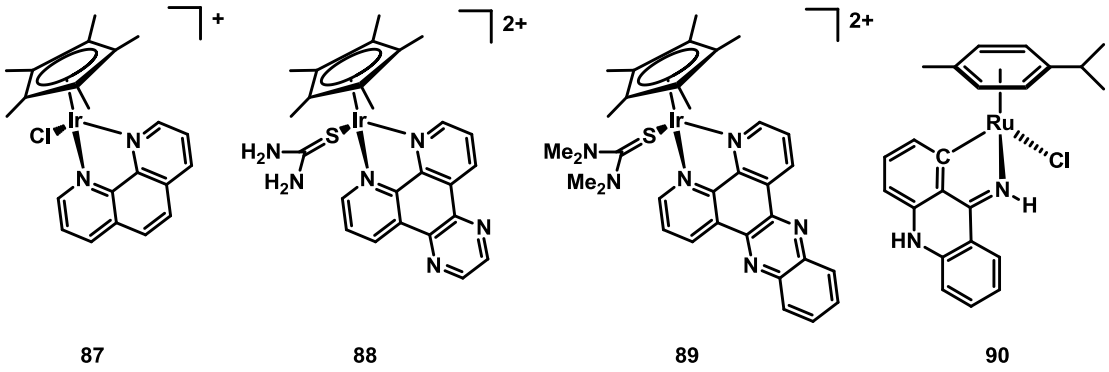


Figure 41 Cp*Ir complexes with intercalating ligands.

These dipyridyl ligands can promote initial DNA recognition by intercalation followed by a switch to nucleobase-metal binding. The complex [(η^6-*p*-cymene)Ru(NC)Cl] (**90**) (HNC = 9-aminoacridine, 9-AA) containing the C,N-chelating intercalator 9-AA exhibits good activity towards a variety to cell lines and is luminescent.[190]

6. Conclusions

Organometallic complexes are showing promise as novel therapeutic agents. In this article we have focused on pseudo-octahedral transition metal complexes containing cyclopentadienyl or arene ligands, especially those with anticancer activity.

In modern pharmacology, drugs need to be targeted to specific sites in tissues and cells, preferably with no or few side-effects. Their mechanisms of action need to be at least partially understood if drugs are to progress to the clinic (i.e. to attract the large amount of investment needed for preclinical development). By their very nature, metallodrugs are likely to be 'prodrugs'; most complexes will undergo ligand substitution and/or redox reactions on the way to or at the biological target site, although some highly inert metal complexes can be recognized by enzymes and exert their biological effects as intact complexes.

It is important to establish that the biological activity of non-inert complexes can be controlled by appropriate choices of the metal, its oxidation state, and the types of bound ligands. Furthermore, the presence of metal-carbon bonds endows complexes with special features - both steric and electronic, and the rational design of organometallic therapeutically active complexes was shown to employ them.

Features which provide an appropriate balance between stability in the required solvent, activation so that a specific substrate can bind, followed by conversion into a product have become well known in the field of catalysis in recent years with some notable successes, e.g. Grubbs' catalysts. Hence we have asked whether knowledge from catalyst design can be profitably transferred to drug design. Of course, the parallel has limitations. For example, the solvents are often (but not always) very different. Biological systems are largely aqueous and also contain oxygen. Another major difference is the environment of the complex in solution, which can be well defined for catalysis, but complicated and rich in substances which can inactivate (poison) the metal centre or destroy the ligands in a biological system. If these can be addressed, there is also the possibility of designing catalytic organometallic drugs.

A variety of specific switches can already be identified for the cytotoxicity of arene anticancer complexes: The choice of the chelating ligand can finely tune the properties of anti-cancer complexes, for example their hydrolysis. The discussion above shows that a window exists in which hydrolysis of at least one M-L bond can trigger cytotoxicity. Furthermore, aquation-dependent cytotoxic activity supports the postulation that cell-killing effects relate in a direct or indirect manner to coordinative binding to biomolecules. Within these, ligand oxidation can also activate some complexes towards DNA binding. Therefore, specific ligand tuning might be a possible way to influence the reduction potential and afford pro-drugs that can undergo redox-activation inside tumour cells. For example, changing the different building blocks of arene ruthenium(II) or osmium(II) as well as iridium(III)

cyclopentadienyl complexes can lead to engineered therapeutics and even to the possibility of catalytic drugs.

However, some examples do already break these rules. As we have shown above, some Ru^{II} or Os^{II} complexes do not hydrolyse and yet are extremely active *in vitro* and *in vivo*. Thus, establishing trends and deriving guidelines for structural design is helpful, but must always be aided by the 'exception that proves the rule'.[191] Moreover, "rule-breakers" can lead to new starting points for structural design.

It will be intriguing now to follow the progress of, for example, the antimalarial drug ferroquine, the anticancer ferrocifen derivatives, and also ruthenium, osmium, rhodium and iridium cyclopentadienyl and arene anticancer complexes. Will they indeed prove to have unique mechanisms of action not shared by exisiting organic drugs, or the platinum complexes already used in the clinic? Moreover, can proteomic and genomic approaches be used to pinpoint target sites and specific effects on cellular signaling pathways of organometallic drugs? We can predict that such studies are very likely to show that bioorganometallic chemistry can make a major new contribution to medicine, just as it has to catalysis.

Acknowledgements

We thank the European Research Council (Advanced Investigator grant 247450 for PJS), and WPRS (Studentship for LN), EPSRC and Science City (AWM/ERDF) for support. We also thank colleagues in EC COST Action D39, Professor Martin Wills and David Morris (Warwick) for stimulating discussions, and Dr Nicolas Barry and Anaïs Pitto-Barry for supplying illustrations of the cage compounds.

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