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Organometallic Anticancer Compounds

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Introduction

The quest for alternative drugs to the well-known cisplatin and its derivatives, which are still used in more than 50% of the treatment regimes for patients suffering from cancer, is highly needed.[1,2] Despite their tremendous success, these platinum compounds suffer from two main disadvantages: they are inefficient against platinum-resistant tumors, and they have severe side effects such as nephrotoxicity. The latter drawback is the consequence of the fact that the ultimate target of these drugs is ubiquitous: It is generally accepted that Pt anticancer drugs target DNA, which is present in all cells.[3,4] Furthermore, as a consequence of its particular chemical structure, cisplatin in particular offers little possibility for rational improvements to increase its tumor specificity and thereby reduce undesired side effects.

In this context, organometallic compounds, which are defined as metal complexes containing at least one direct, covalent metal−carbon bond, have recently been found to be promising anticancer drug candidates. Organometallics have a great structural variety (ranging from linear to octahedral and even beyond), have far more diverse stereochemistry than organic compounds (for an octahedral complex with six different ligands, 30 stereoisomers exist!), and by rational ligand design, provide control over key kinetic properties (such as hydrolysis rate of ligands). Furthermore, they are kinetically stable, usually uncharged, and relatively lipophilic and their metal atom is in a low oxidation state. Because of these fundamental differences compared to "classical coordination metal complexes", organometallics offer ample opportunities in the design of novel classes of medicinal compounds, potentially with new metal-specific modes of action. Interestingly, all the typical classes of organometallics such as metallocenes, half-sandwich, carbene-, CO-, or π-ligands, which have been widely used for catalysis or biosensing purposes, have now also found application in medicinal chemistry (see Figure 1 for an overview of these typical classes of organometallics).

In this Perspective, we report on the recent advances in the discovery of organometallics with proven antiproliferative activity. We are emphasizing those compounds where efforts have been made to identify their molecular target and mode of action by biochemical or cell biology studies. This Perspective covers more classes of compounds and in more detail than a recent tutorial review by Hartinger and Dyson.[5] Furthermore, whereas recent reviews and book contributions attest to the rapid development of bioorganometallic chemistry in general,[6,7] this Perspective focuses on their potential application as anticancer chemotherapeutics. Another very recent review article categorizes inorganic anticancer drug candidates by their modes of action.[8] It should be mentioned that a full description of all currently investigated types of compounds is hardly possible anymore in a concise review. For example, a particularly promising class of organometallic anticancer compounds, namely, radiolabeled organometallics, has been omitted for space limitations. Recent developments of such compounds have been reviewed in detail by Alberto.[9]

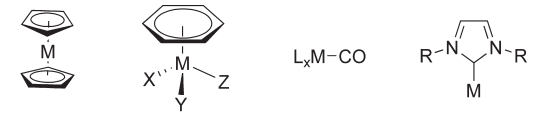

Figure 1. Summary of the typical classes of organometallic compounds used in medicinal chemistry.

Metallocenes

Metallocenes is the name for compounds with two π-bonded cyclopentadienyl (Cp[a]) ligands on a metal atom. Research into this class of compounds started in 1952 with the discovery of

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[a] Abbreviations: AAS, atomic absorption spectrometry; acac, acetylacetonato; bip, biphenyl; BNCT, boron neutron capture therapy; BSA, bovine serum albumin; Cat B, cathepsin B; CDK, cyclin-dependent kinase; cGMP, 3′,5′-cyclic monophosphate; CHO, Chinese hamster ovary; CMIA; carbonylmetallo immunoassay; CORM, carbon monoxide releasing molecule; Cp, cyclopentadienyl; Cp*, pentamethylcyclopentadienyl; CQ, chloroquine; CQDP, chloroquine diphosphate; dppf, 1,1′-bis(diphenylphosphino)ferrocene; EA, ethacrynic acid; en, ethylenediamine; FR, folate receptor; GR, glutathione reductase; GSH, glutathione; GST, glutathione transferase; ICP-MS, inductively coupled plasma mass spectrometry; MDR, multidrug resistance; MMR, mismatch repair; MudPIT, multidimensional protein identification technology; MS, mass spectrometry; NHC, N-heterocyclic carbene; NSAID, nonsteroidal antiinflammatory drug; *o*-bqdi, *o*-benzoquinonediimine; *o*-pda, *o*-phenylenediamine; PDT, photodynamic therapy; Pgp, P-glycoprotein; pta, 1,3,4-triaza-7-phosphatricyclo[3.3.1.1]decane; ROS, reactive oxygen species; SAR, structure−activity relationship; SERM, selective estrogen receptor modulator; TRAP, telomeric repeat amplification protocol; TrxR, thioredoxin reductase.

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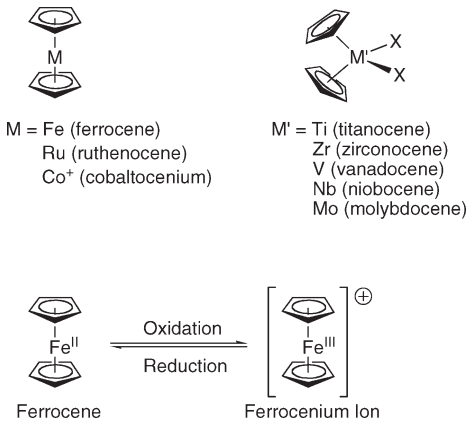
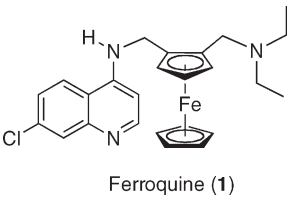
Scheme 1. (Top Row) Classical Metallocenes with Parallel Cp Rings (Left) and Bent Metallocenes (Right) with the Medicinally Relevant Metals Indicated and (Bottom Row) Reversible Oxidation Chemistry of Ferrocene



ferrocene (bis-cyclopentadienyl iron, Cp_2Fe) and the elucidation of its C_5-symmetric structure with two equivalent, π-bonded Cp rings. Because of their symmetrical structure, such compounds are also frequently referred to as "sandwich complexes". Today, other metal complexes with cyclic π-perimeters are also sometimes named metallocenes. Compounds with only one π-perimeter are classified as "half-sandwich metallocenes", such as the Ru(arene) complexes discussed below in some of the following sections of this Perspective. Structurally, the bis-cyclopentadienyl complexes can be classified into two classes, namely, the "classical" ones with parallel Cp rings and the "bent" metallocenes, which have other ligands bonded to the metal in addition to the Cp rings (Scheme 1). The sufficiently robust metallocenes used for medicinal applications contain metals from the iron and cobalt triad, with Fe, Ru, and Co being relevant to this article. The bent metallocenes typically comprise metals from the earlier transition metals, most importantly Ti, Zr, V, Nb, Mo in a medicinal context. Interestingly, all medicinally important bent metallocenes have a *cis*-dihalide motif as depicted in Scheme 1, which is similar to the *cis*-dichloro motif of the well-established anticancer drug cisplatin. This resemblance has spurred interest in metallocenes in the early days of medicinal inorganic chemistry, particularly through the work of Köpf and Köpf-Maier.[10−12]

Historically, however, the medicinal properties of ferrocene were previously investigated because it was the first organometallic compound for which antiproliferative properties were reported.[13] This report sparked the development of organometallic anticancer compounds.[14,15] Ferrocene by itself is not a particularly toxic compound. It can be injected, inhaled, or taken orally without causing major health problems. Like most xenobiotics, it is degraded in the liver by cytochromes. Because of its aromatic character, a metabolism related to benzene had been expected and was indeed found experimentally. As shown in experiments with rats that were orally given a single dose of ferrocene in sesame oil, ferrocene is enzymatically hydroxylated in the liver and urinally excreted in the form of conjugates to sulfate (minor product) and glucuronic acid as the main products.[16] In vitro, intact liver microsomes, NADPH, and molecular oxygen were found to be necessary for the hydroxylation of ferrocene. This process was inhibited in vitro by CO but significantly stimulated in vivo by pretreatment of the rats with phenobarbital. These findings give



Ferroquine (**1**)

Figure 2. Ferroquine (**1**) is presently the most advanced organometallic drug candidate and about to enter phase III clinical trials as an antimalarial drug.

conclusive evidence that the hydroxylation of ferrocene is carried out by cytochrome P_{450} enzymes, similarly to benzene and many other hydrocarbons. Although such studies have hardly been carried out on other organometallic complexes, it is not unreasonable to assume that a similar fate is experienced by several of the metal-based drugs discussed in this article, at least by those who possess π-bonded (quasi-aromatic) ligands. On the other hand, hydroxyferrocene is rather unstable and decomposes in aqueous solution, finally releasing solvated iron atoms. Indeed, ferrocene derivatives have been proposed as antianemics, and one such compound, ferrocerone, was clinically approved in the USSR. To the best of our knowledge, this compound was the first marketed transition organometal drug. In this context, it is worth mentioning that another ferrocene-containing compound, which is a close derivative of chloroquine, has successfully passed clinical phase II trials as an antimalarial drug candidate (ferroquine, **1**, Figure 2).[17,18] This compound is now undergoing field testing and may reach approval as a new antimalarial drug in the near future. Ferroquine has an activity similar to chloroquine on the malaria parasite *P. falciparum* but most notably is similarly active against chloroquine-resistant *P. falciparum* strains. It has been discussed that changes in lipophilicity, but possibly also some redox activation, could be responsible for the unexpected activity of this ferrocene antimalarial.[18] Following the success of ferroquine, many other organometallic antimalarials were synthesized and tested but as yet with lesser success.

The toxicity of ferrocene was also tested in beagle dogs that were fed up to 300 mg kg^{-1} per day for 6 months or even 1 g kg^{-1} for up to 3 months.[19] While no acute toxicity or even deaths were observed, massive Fe overload was diagnosed. However, all the dogs recovered afterward. The ferrocene-induced hepatic Fe overload could be reduced after the removal of large quantities of Fe by repeated venesection.[19]

Ferrocene can undergo a one-electron oxidation, yielding the ferrocenium cation (see Scheme 1, bottom). This cation is rather stable and the redox reaction is reversible for most ferrocene derivatives. Simple ferrocenium salts were the first iron compounds for which an antiproliferative effect on certain types of cancer cells was demonstrated.[13] The mechanism of action is still uncertain. Nuclear DNA, cell membrane, and the enzyme topoisomerase II[20,21] were proposed as possible targets. More precisely, Osella et al. showed that ferrocenium salts may generate hydroxyl radicals in physiological solutions.[22] An earlier report suggests that these radicals damage the DNA in a Fenton-type reaction.[23] The cytotoxic effect of decamethylferrocenium tetrafluoroborate ($Cp^*_2FeBF_4$, Cp^* = pentamethylcyclopentadienyl) was correlated to the production of 8-oxoguanine, the initial product of DNA oxidation. Direct evidence for hydroxyl and superoxide radicals stems from ESR and spin-trapping experiments. In one of the few studies of this kind, a synergistic effect between $Cp^*_2FeBF_4$

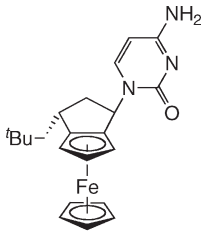


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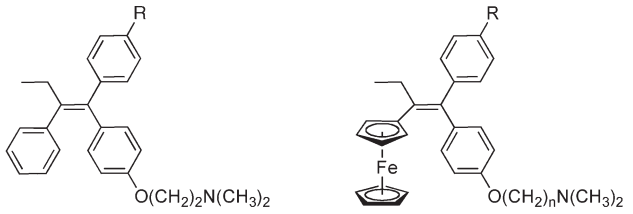
Figure 3. Nucleoside analogue of ferrocene.



Tamoxifen (**3**; R = H)
Hydroxy-tamoxifen (**4**; R = OH)

Ferrocifens **5** (R = H; n = 2, 3, 4, 5, 8)

Figure 4. Tamoxifens and ferrocifens. The most active derivative of **5** with $n = 4$ is referred to as *the* ferrocifen.

and the iron-dependent antitumor drug bleomycin was observed.[24] Finally, the cell membrane may be the cellular target similar to the peroxidation of membrane lipids which is the consequence of excess hepatic iron. A more detailed review on the physiological chemistry of ferrocene and the antiproliferative properties of ferrocene or ferrocenium alone has recently been given elsewhere.[25]

Neuse et al. used an interesting approach to enhance the cytotoxicity of ferrocene. They bound ferrocene to polymeric supports such as poly aspartamide.[26−30] The underlying idea is that enhanced water solubility may be a crucial factor for the activity of ferrocene. This assumption is confirmed by the fact that the cytotoxicity of ferricenium salts depends greatly on the nature of the counterion. Indeed, the poorly soluble heptamolybdate is inactive while ferricenium salts with good aqueous solubility such as the picrate and trichloroacetate display high antitumor activity.[13] It must be noted that smaller ferrocenyl polyamines were also tested by Brynes and co-workers at almost the same time but with limited success.[31] The work on polymer-bound ferrocene as anticancer drugs has recently been reviewed by Neuse.[32]

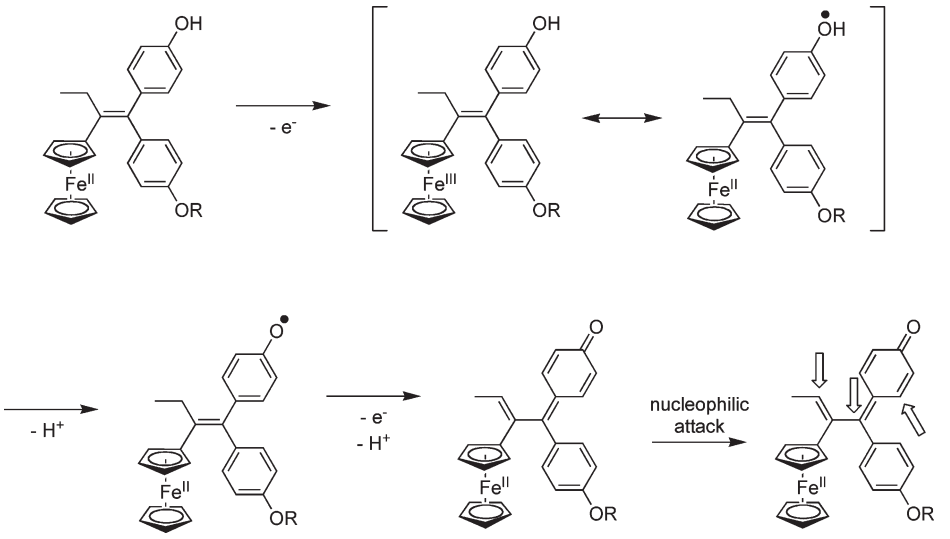
Numerous ferrocene derivatives have been tested for antiproliferative purposes.[33−38] Among those, a ferrocene−acridine conjugate was found to be highly cytotoxic. The acridine moiety served to bring the ferrocene close to DNA by intercalation.[36] Wagner and co-workers investigated the activity of borylated ferrocenes in boron neutron capture therapy (BNCT)[34] and found that their compounds exhibit an interesting organ distribution. One derivative in particular was found to penetrate the blood−brain barrier (BBB), which is of high importance for the treatment of brain tumors. Schmalz and co-workers synthesized several nucleoside analogues of ferrocene[39] (e.g., **2** in Figure 3) with IC_{50} values in the low micromolar range, although not as low as the iron tricarbonyl nucleoside analogues from the same group (see also the section Metal Carbonyl Complexes below).[40,41] No molecular target has been proposed so far for **2**, but the structure makes protein targets, maybe in RNA/DNA synthesis or repair pathways, likely candidates.

In more general terms, redox activity is a property that is not unique to metal compounds but frequently encountered with them. It is thus interesting to correlate the redox properties of metal compounds with electron transfer, oxidative stress, the formation of reactive oxygen species, and generally the redox status of cells.[33,42] While it is difficult to determine the exact "redox potential" or even "redox status" of a whole cell, the correlation between the redox activity of metal complexes and their antiproliferative properties has been only tentatively investigated. However, a mechanism whereby redox activation induces anticancer activity in ferrocene derivatives has recently been suggested by Jaouen and co-workers.[42] They substituted phenyl rings in established drugs and natural products with ferrocene groups.[43] Most significantly, this work has uncovered a group of derivatives of the anticancer drug tamoxifen, called ferrocifens by Jaouen's group.[42,44] Tamoxifen (**3** in Figure 4) is the front-line chemotherapeutic agent for patients with hormone-dependent breast cancer. Its active metabolite is hydroxytamoxifen (**4** in Figure 4). In general, breast tumors can be divided into two groups depending on the presence (ER(+)) or absence (ER(−)) of the estrogen receptor. About two-thirds of all cases belong to the ER(+) type, rendering them susceptible to hormone therapy by selective estrogen receptor modulators (SERMs) such as tamoxifen and giving the patients significantly improved chances for successful treatment compared to the ER(−) group of patients.[42] The antiproliferative action of tamoxifen arises from the competitive binding to ERα subtype, thus repressing estradiol-mediated DNA transcription in the tumor tissue. Unfortunately, expression of the ERα may become down-regulated under tamoxifen treatment, whereby the drug becomes ineffective.

It is believed that the same principle explains the activity of ferrocifens (**5**) against ER(+) cancer cell lines. Some SARs were derived from a group of ferrocene derivatives of tamoxifen as shown in Figure 4. Replacement of the phenyl group by ferrocene reduces receptor affinity by about 40%. Increasing the length of the dimethylaminoalkyl chain has an adverse effect on receptor binding. In addition, it also changes the bioavailability and determines whether estrogenic or antiestrogenic activity is observed in animal experiments. It appears that an optimum value is around $n = 4$. While the *Z* isomers bind more strongly to the ERα than the *E* isomers in in vitro tests, there is rapid isomerization under physiological conditions. Finally, ferrocifens were shown to be effective antiestrogens in MCF-7 breast cancer cell lines (ER(+)) and against estrogen-dependent tumor xenografts in nude mice.

Surprisingly, however, compound **5** with $n = 4$ was shown to be active against the ER(−) MDA-MB231 tumor cell line, which lacks the ERα and is hence not susceptible to treatment with tamoxifens. This indicates a new and different mode of action for **5**. Interestingly, the ruthenocene analogue of **5** also acts as an antiestrogen in ER(+) breast cancer cells but lacks the antiproliferative effect of ferrocifen against ER(−) cell lines.[45] Other organometallic fragments in place of the ferrocenyl group were also tested but were found to be inactive.[44,46] This suggests a dual mode of action for ferrocifen. In addition to tamoxifen-like binding to the ERα receptor, the second pathway must critically depend on the properties of ferrocene. In an elegant study, redox activation has been proposed as the second mode of action.[47] The active metabolite hydroxyferrocifen is readily oxidized, yielding a quinone methide intermediate. This intermediate is activated for nucleophilic attack by nucleophiles. Quinone methides of the metal-free 4-hydroxytamoxifen are known to be stable for hours under physiological

Scheme 2. Redox Activation of Ferrocifens as Proposed by Jaouen and Co-Workers[a]

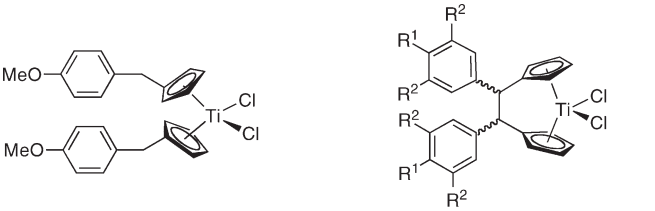


[a] The ferrocene serves as a "redox antenna". Following oxidation and proton abstraction a quinone methide is formed, which is readily attacked by nucleophiles at the positions indicated by arrows.

conditions. Adducts of such tamoxifen metabolites with glutathione and nucleobases are thought to be responsible for its general toxicity and mutagenic potential. It is now proposed that related chemistry applies to the activated ferrocifens. Extensive SAR studies[48−53] in correlation with electrochemical properties[52,54−56] support this hypothesis. Moreover, production of reactive oxygen species has been demonstrated in cell lines treated with ferrocifen and derivatives.[57] In this mode of action, which is summarized in Scheme 2, the metallocenes serve as a "redox antenna". It is particularly noteworthy that redox activity of the metallocene is the key for additional biological activity that exceeds that of a purely organic analogue. Once this redox-activation mode of action was established, it is clearly not dependent on the tamoxifen-related substructure. Recently, the same group has presented work on ferrocenyl diphenols and unconjugated phenol derivatives that also have good antiproliferative activity, presumably via a related mechanism of activation and formation of similar intermediates.[58,59]

In order to advance the use of ferrocifens toward clinical studies, several formulation studies were performed using nanoparticles,[60] lipid nanocapsules,[61,62] and cyclodextrins.[63] Further work from the same group includes, beyond synthesis, at least a preliminary testing of the antiproliferative activity of ferrocene derivatives of several classes of compounds, i.e., curcuminoids,[64] androgen derivatives,[65] and anti-androgens derived from the nilutamide lead structure,[66] indolones,[67] and ferrocenophane polyphenols.[68]

For the bent metallocene dihalides, SARs were established for the halides, and substitution of the Cp rings[11,14,15,69] and model studies of such compounds with amino acids, nucleic acids, proteins, and blood plasma were performed.[70] Titanium compounds were most active, and titanocene dichloride has even entered clinical trials.[71] Although very promising in animal models, the clinical response was not significant enough to justify continuing trials; i.e., titanocene dichloride clinical trials were recently abandoned. Furthermore, because of its decomposition and low solubility in water, there were also problems with the formulation of the drug. Earlier work investigated DNA interaction, induction of apoptosis, and topoisomerase inhibition as possible modes of action.[72−74]



Titanocene Y (**6**)

$R^1 = NMe_2$, R^2 = H: Titanocene X (**7**)
R^1 = H, R^2 = OMe: Titanocene Z (**8**)

Figure 5. Titanocene Y (**6**) and the ansa-bridged derivatives titanocenes X (**7**) and Z (**8**).

Despite the resemblance of titanocene dichloride with cisplatin, there has never been clear evidence of a similar mode of action, i.e., binding to DNA and eventually apoptosis of the cancer cell.[4] Instead, binding of the Ti^{4+} cation to transferrin following complete hydrolysis of Cp_2TiCl_2 was proposed,[75] and even a stimulatory effect of aqueous Ti species on hormone-dependent breast cancer cells was observed.[46] From an inorganic point of view, however, the existence of simple hydrated Ti^{4+} cations is highly unlikely in aqueous solution at pH 7, as oligomeric species and eventually insoluble titanium dioxide will form. A recent computational study on a benzyl-substituted titanocene (titanocene Y (**6**), Figure 5) suggests that the $Cp(R)_2Ti^{2+}$ dication binds to a DNA phosphate group, with additional interactions stabilizing the binding to DNA.[76] Although this result is in accord with chemical intuition, i.e., the hard Ti cation binding to anionic oxygen atoms, it is a single point computation assuming DNA as the molecular target. No protein targets were so far considered for the bent metallocenes.

The two main problems of the titanocene dihalides, i.e., poor aqueous solubility and hydrolytic instability, were both addressed in recent years by chemical synthesis. To increase aqueous solubility, amino-substituted bent metallocenes were successfully prepared.[77] The two Cp rings are covalently linked together in *ansa*-titanocenes, and indeed, such compounds exhibit improved hydrolytic stability.[78,79] Both groups of compounds show promising biological activity. The group of Tacke has developed a versatile synthetic access to Cp-substituted

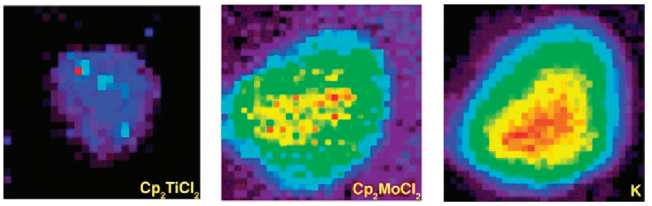


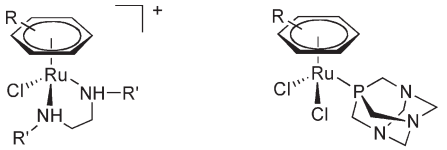
Figure 6. Intracellular distribution of Mo (from Cp_2MoCl_2), Ti (from Cp_2TiCl_2), and K as shown by X-ray fluorescence microscopy. Reproduced from ref 102, with kind permission of Springer Science + Business Media.

bent metallocenes via the fulvene route. This approach yields unbridged (via hydrido lithiation) as well as *ansa*-bridged metallocenes (via carbo lithiation).[80] In vitro cytotoxicity tests were performed for several derivatives. A screen against 36 human tumor cell lines of 14 different tumor types on the derivatives **6**−**8** (termed titanocenes Y, X, and Z, Figure 5) revealed the *p*-methoxybenzyl substituted titanocene Y (**6**) as the most active derivative. Even more interesting, this compound showed very good activity against renal cell cancer and pleura mesothelioma cell lines, for which no effective chemotherapeutic agents are currently available.[81] Further testing of this compound was performed, including tests against freshly explanted tumors[82,83] and in vivo tests against xenografted renal cancer (Caki-1),[84] prostate cancer (PC-3),[85] and breast adenocarcinoma (MCF-7) in mice.[82] Mechanistic studies particularly, but not exclusively, on titanocene Y revealed antiangiogenic effects but no myelosuppression,[86] activation of the immune system, and induction of apoptosis via caspases 3 and 7 but not caspase 8.[87,88] This is a desirable combination of properties for anticancer drugs. In an obvious extension of their work and inspired by second-generation platinum drugs, the Tacke group has recently replaced the two chloride ligands on titanocene Y with carboxylate groups to yield equally active compounds with possibly even more favorable pharmacokinetics.[89,90]

While Cp-substituted vanadocenes, zirconocenes, and even stannocenes were recently evaluated,[91−94] more in-depth research has concentrated on molybdocene derivatives as the most promising alternative to Cp_2TiCl_2. Again with relation to cisplatin, DNA was envisaged as the target, and in early work, several X-ray structures with the Cp_2Mo fragment coordinated to nucleobases were obtained.[95−97] Also early on, comparative hydrolysis studies of several bent metallocene dihalides were performed that identified Cp_2MoCl_2 as one of the most stable simple metallocenes. Unlike Cp_2TiCl_2, the Cp rings in the Mo derivative are less prone to hydrolysis. Furthermore, extensive spectroscopic studies, mainly by ^1H and ^{31}P NMR, were carried out in solution to assess the binding mode of molybdocene dichloride with DNA.[96,98−100] In more recent work, Harding and co-workers investigated cellular uptake and intracellular localization of several bent metallocenes dihalides by X-ray fluorescence.[101,102] Only low levels of Ti and V were detected inside cells, and only Mo seemed to accumulate in significant amounts in the cellular nuclei (Figure 6). Together, these findings agree well with the notion that all metallocenes have a different biological profile.

Organometallic Ruthenium Half-Sandwich Complexes

The idea of using ruthenium-containing organometallics as anticancer agents was first developed by Tochter et al.[103] before being intensively investigated in the Sadler and Dyson research groups. It was initially anticipated that the binding



Ru^{II} arene ethylenediamine derivatives RAPTA Derivatives

Figure 7. (η^6-Arene)ruthenium anticancer complexes.

of *all* ruthenium compounds to DNA was the main reason for their anticancer effect, similar to the platinum derivatives; i.e., the coordination of the metal center to DNA causes structural modifications, which would ultimately lead to the induction of apoptosis. Indeed, the ability of ruthenium complexes to bind to DNA or model compounds has been amply demonstrated,[104−114] although it was found that the actual DNA binding of certain ruthenium compounds was weaker than or/and different from the one observed for platinum derivatives.[106,115,116] But recent studies for a series of ruthenium anticancer compounds revealed that DNA is not always the primary target and that these species were actually binding more strongly to proteins than to DNA.[117−119] These findings clearly indicated the occurrence of significantly different modes of action, depending on the type of ruthenium complexes. However, the exact mechanism by which these metallodrugs exert their effects has not (yet) been fully understood. Nonetheless, in this section, we will highlight recent developments on the elucidation of the mechanism of action of anticancer ruthenium half-sandwich organometallic compounds, as well as the exact role of the metal center. A nonexhaustive catalogue of ruthenium organometallic antitumor agents can be found in recent reviews or book chapters.[5,120−123] We will use structure comparisons to explicit the mechanism differences/analogies of these compounds.

At a first glance, the structural similarity of the half-sandwich "piano stool" type organometallics presented in Figure 7 might suggest an analoguous mechanism of cytotoxic action. However, to the best of our current knowledge, they appear to be much different.

Salder et al. established that the mechanism of action of their compounds $[(\eta^6\text{-arene})Ru(en)(Cl)]^+$ (en = ethylenediamine) (Figure 7, left) has many of analogies to that of cisplatin. It first involves hydrolysis of the Ru−Cl bond of the prodrug to generate an active $[(\eta^6\text{-arene})Ru(en)(H_2O)]^{2+}$ species. Detailed kinetic studies showed that the Ru−Cl bond hydrolysis can be strongly influenced by the nature of the coligands as well as the nature of the metal ion (see also the section Organometallic Osmium Half-Sandwich Complexes below).[121,122] Importantly, this step is suppressed in the blood because of the high chloride concentrations enabling $[(\eta^6\text{-arene})Ru(en)(Cl)]^+$ to cross the cell and nuclear membranes. Once inside the cell, the hydrolysis of the chloro anion takes place because of the much lower chloride concentration (~25 times lower). It is then assumed that the aqua complex $[(\eta^6\text{-arene})Ru(en)(H_2O)]^{2+}$ binds to nuclear DNA with a high affinity for the N7 position of guanine bases as shown by NMR and X-ray crystallographic studies and transcription mapping experiments.[111−113] It must be pointed out that the analogy in the mode of action between $[(\eta^6\text{-arene})Ru(en)(Cl)]^+$ and cisplatin stops at this point. Indeed, the Ru arene compounds can only form monofunctional adducts compared to cisplatin which is known to form bifunctional adducts and DNA cross-links. Importantly also, $[(\eta^6\text{-arene})Ru(en)(Cl)]^+$ derivatives were found to be active against

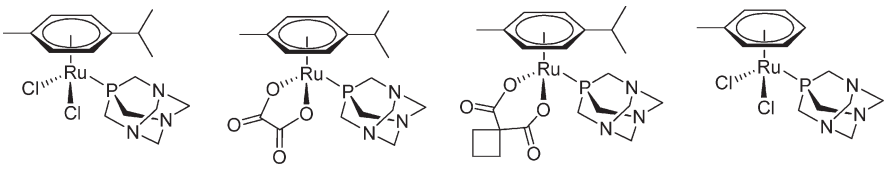


Figure 8. RAPTA derivatives.

cisplatin-resistant cell lines, indicating that the detoxification mechanism is different from the one of cisplatin.[124] However, in silico calculations undertaken by Deubel et al. to compare the difference in selectivity of cisplatin to organometallic ruthenium complexes toward biological targets show that organometallic ruthenium anticancer complexes are more similar to cisplatin than to inorganic Ru(II) complexes.[125]

Ru-RAPTA derivatives were originally designed to improve the aqueous solubility (pta = 1,3,4-triaza-7-phosphatricyclo-[3.3.1.1]decane, Figure 7). As for Ru(II) arene ethylenediamine compounds, RAPTA derivatives[126] containing two chloride ligands were also found to be susceptible to hydrolysis and it was first anticipated that DNA was a primary target.[127] Dyson et al. recently prepared RAPTA carboxylato derivatives (oxalo-RAPTA-C and carbo-RAPTA-C, Figure 8).[128] This work was evidently inspired by the structures of carboplatin and oxaliplatin. In analogy to the Pt compounds, it was assumed that the carboxylato ligands would hydrolyze more slowly and in a more controllable way than the chloride ligands in the original RAPTA-C compound. These RAPTA derivatives have an in vitro activity similar to that of RAPTA-C. All evidence taken together, RAPTA compounds seem to operate by a different mode of action compared to cisplatin, Ru(II) arene ethylenediamine compounds, and most of the known anticancer compounds in general. In vitro cytotoxicity studies showed that these compounds were much less cytotoxic than cisplatin. Indeed, many of the RAPTA compounds could not even be classified as cytotoxic and were also nontoxic to healthy cells. The extent of this nontoxicity was proven in an in vivo study when healthy mice were treated at quite high doses with RAPTA compounds without triggering toxic side effects.[129] But the most striking result observed was that both RAPTA-C and RAPTA-T inhibited lung metastasis in CBA mice bearing the MCa mammary carcinoma (the number and weight of the metastases were reduced) while having only mild effects on the primary tumor.[2] The only other metallo drug candidate displaying this outstanding behavior is imidazolium *trans*-[tetrachloro(dimethylsulfoxide)-(1*H*-imidazole)ruthenate(III) (NAMI-A).[2,118] This discovery is of high practical interest, as the removal of the primary tumor by surgery is frequently an efficient procedure while the treatment options for metastases are quite limited.[2]

Nonetheless, these very exciting findings engendered naturally a new and obvious question: If DNA is not the target for these RAPTA derivatives, then what is the target? The final answer has not yet been determined, but at this stage of the research, enzyme binding is the most probable explanation. It was shown by mass spectrometry that RAPTA compounds form adducts with proteins[130] and that the reactivity of RAPTA-C and cisplatin in the presence of proteins was much different.[131] To get more insight, Messori et al. studied the inhibition activity of a series of RAPTA compounds to two proteins, i.e., cathepsin B (Cat B) and thioredoxin reductase (TrxR), which are possible targets for anticancer metallodrugs.[132] They found that all tested Ru compounds were inhibitors of Cat B while none of them, with the exception of RAPTA-C, was inhibiting TrxR. Computer docking experiments validated this finding. Assuming that one of the two chloride ligands of the RAPTA derivatives was first replaced by a water molecule, it was then found that the Ru(II) center coordinates to the active site cysteine residue. Furthermore, other atoms of RAPTA (chloride, nitrogen of pta, etc.) bind other amino acids of Cat B, thereby stabilizing the metallodrug−enzyme complex.[132] Interestingly, a good correlation was observed between the inhibiting potency of the RAPTA derivatives and the calculated stability of the corresponding Cat B/RAPTA adducts.[132]

Other proteins have been proposed as the target for Ru organometallics. P-Glycoprotein (Pgp) is a plasma membrane protein that is responsible for drug efflux from cells and is involved in multidrug resistance (MDR).[120] Inhibitors of Pgp, namely, phenoxazine and anthracene derivatives, were synthetically modified and coordinated to Ru organometallics.[133] The aim was to obtain a synergistic effect by combining the selectivity of ruthenium complexes toward cancer cells and the ability of the phenoxazine and anthracene derivatives for Pgp inhibition. These newly formed complexes were found to be, in general, more cytotoxic and inhibited to a lesser extent the Pgp protein than the original Pgp inhibitor derivatives used as ligands. Interestingly, for one of these ruthenium derivatives (**13**, Figure 9), it was shown that the ruthenium coordination to the Pgp inhibitor derivative induced an even stronger protein inhibition. Furthermore, because of the presence of the fluorescent anthracene group, it was observed that **13** was accumulating in cell nuclei, suggesting a DNA synthesis inhibition as the mechanism of cytotoxic action. Nonetheless, because of the strong increase in cytotoxicity upon ruthenium coordination, Dyson et al. believe that their organometallic compound not only inhibits the enzyme but also induces cell death via a second mechanism, implying a bifunctionality of this compound.[133]

In a similar line of thought, namely, a dual cytotoxic mode of action, ethacrynic acid (EA) was coupled to two RAPTA derivatives (**15** and **16**, Figure 9)[134] as well as to other Ru arene organometallics.[135] EA is an effective glutathione transferase (GST) inhibitor, which has been investigated as a potential anticancer drug. EA is known to bind competitively to the hydrophobic cosubstrate (H-site) of GST, while the RAPTA compounds are recognized to react with soft nucleophilic centers such as thiol groups (see above).[134] **15** and **16** compounds were therefore thought to be able to bind not only to the enzyme at the H-site but also to interact with the reactive cysteine residues of GST P1-1 (this GST protein possesses two solvent-accessible cysteine residues that affect catalytic activity when modified). As assumed, these two new compounds were found to bind the catalytic H-site in a similar fashion as

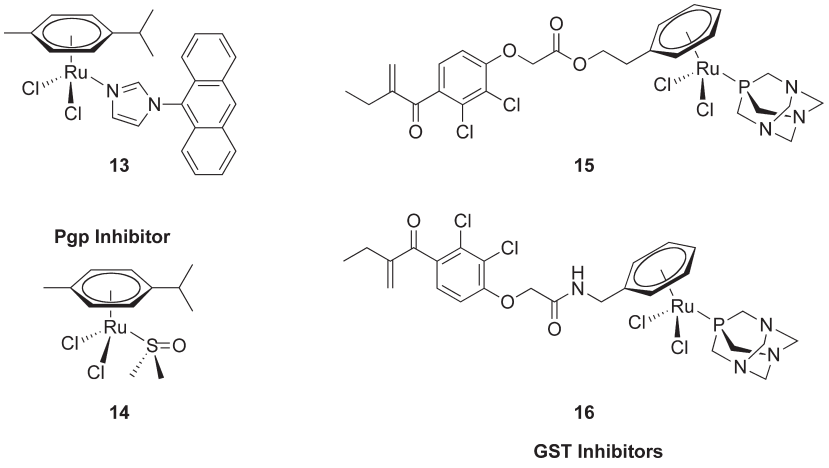


Figure 9. Ru arene enzyme inhibitors.

EA. Furthermore, the inhibition constants K_i of the complexes on GST P1-1 were 3 or 4 times lower than EA. The authors therefore concluded that the ruthenium centers were also involved in the inhibition of GST P1-1. Interestingly, it was demonstrated by X-ray crystallography and by ESI-MS that **16** decomposed, over a period of time, into a ruthenium derivative and EA. It is anticipated that the cleavage occurs, by virtue of a possible allosteric effect or simply over time, when the EA moiety of **16** is bound to the H-site. Importantly, this (selective?) release of the ruthenium moiety should enhance the toxic effect of the compound on cancer cells, which had already been sensitized by the EA moiety that inactivated GST.[134] This feature could be used to specifically deliver a cytotoxic payload for targeted chemotherapy.[134]

Still in a metal−drug synergism context, the group of Sánchez-Delgado coupled different Ru arene complexes to chloroquine (CQ), which is known to be an effective antimalarial compound as well as having anticancer properties (see the section Metallocenes above).[136] Contrary to the ferroquine mentioned above,[17,137] in which the ferrocenyl moiety is nontoxic (or at least commonly assumed to be), these compounds are made of two toxic moieties. The compounds had a consistently higher potency against CQ-resistant parasites than the standard drug chloroquine diphosphate (CQDP). In addition, two of their compounds (**17** and **18**, Figure 10) inhibit the growth of two HCT-116 colon cancer cell lines with IC_{50} values between 20 and 35 μM. They also observed that liposarcoma cell lines were especially sensitive to **17** with an IC_{50} value of 8 μM. This is of clinical interest, as this type of tumor does not respond to currently employed chemotherapies.[136]

Other proteins have been shown to be the target of cytotoxic ruthenium organometallics. For example, Sheldrick et al. have used an automated multidimensional protein identification technology (MudPIT), which combined biphasic liquid chromatography with electrospray ionization tandem mass spectrometry (MS/MS) to analyze tryptic peptides from *Escherichia coli* cells, which were first treated with [(η^6-*p*-cymene)$RuCl_2$-(DMSO)] (**14**, Figure 9).[138] They showed that five proteins, namely, the cold-shock protein CspC, the three stress-response proteins ppiD, osmY, and SucC, and the DNA damage-inducible helicase dinG were the target of their Ru arene compounds.[138] Using electrophoretic mobility shift assays, Brabec, Sadler and co-workers also examined the binding properties of the mismatch repair (MMR) protein MutS in *Escherichia coli* with various DNA duplexes (homoduplexes or mismatched

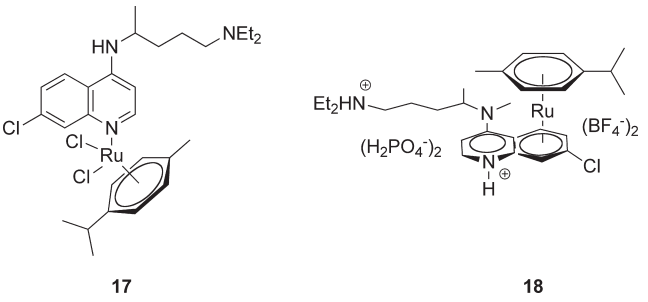


Figure 10. Ru arene chloroquinone antimalarial and antitumor agents.[136]

duplexes) containing a single centrally located adduct of Ru(II) arene compounds.[139] They showed that the presence of the Ru(II) arene adducts decreased the affinity of MutS for ruthenated DNA duplexes, which either had a regular sequence or contained a mismatch, and that intercalation of the arene contributed considerably to this inhibitory effect.[139]

Interestingly, it was recently demonstrated that iodo-containing Ru(II) arene organometallic derivatives **19** and **20** (Figure 11) were highly cytotoxic to human ovarian A2780 and human lung A549 cancer cells. Although these complexes are remarkably inert toward ligand substitution. No hydrolysis was observed by NMR and ESI-MS.[140] Fluorescence-trapping experiments in A549 cells suggested that this potency arose from an increase in reactive oxygen species (ROS). Surprisingly, these Ru complexes act as catalysts for the oxidation of the tripeptide glutathione (GSH), which is a strong reducing agent present in millimolar concentrations in cells. Indeed, millimolar amounts of GSH were oxidized to glutathione disulfide in the presence of micromolar ruthenium concentrations! The same group showed that the anticancer complex [η^6-bip)Ru(en)Cl]$^+$ **21** (bip = biphenyl) (Figure 11) readily reacts with GSH, at pH 7, in a typical cytoplasmic concentration of chloride and at Ru concentrations relevant to cytotoxicity to give a thiolato complex [η^6-bip)Ru(en)(GS-*S*)] as the major product.[113] Unexpectedly, this complex is very sensitive to air and is oxidized to the sulfenato complex [η^6-bip)Ru(en)(GS(O)-S)]. However, under physiologically relevant conditions, competitive reaction of complex **21** with GSH and guanosine 3′,5′-cyclic monophosphate (cGMP) gave rise to a cGMP ruthenium adduct, accounting for 62% of total Ru, even in the presence of a 250-fold molar excess of GSH.[113] This suggests that oxidation of

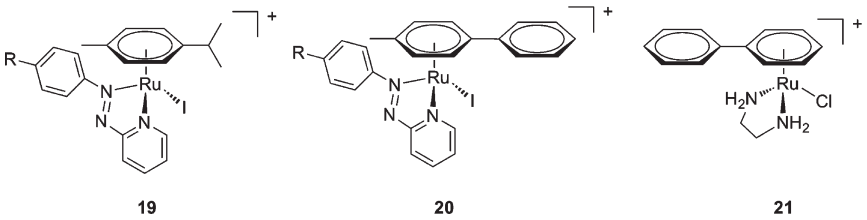


Figure 11. Structures of catalytically active organometallic anticancer complexes.

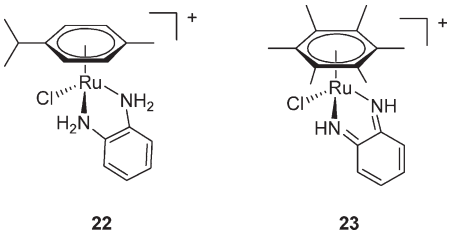


Figure 12. Ru arene anticancer complexes with redox-active diamine/diimmine ligands.

coordinated glutathione in the thiolato complex to the sulfenate provides a facile route for displacement of S-bound glutathione by the guanine N7 atom, a route for RNA and DNA ruthenation even in the presence of a large excess of GSH.[113]

It must be pointed out that Sadler et al. recently unambiguously identified diruthenium and tetraruthenium clusters when complex **21** was reacted with GSH, using nanoscale liquid chromatography Fourier transform ion cyclotron mass spectrometry combined with ^{18}O-labeling.[141] The same group also recently observed the binding of Ru arene compounds with human serum albumin by means of mass spectrometry combined with trypsin digestion, specific side chain modifications, and computational modeling.[142] Finally, a loss of cytotoxic activity of Ru arene organometallics upon oxidation of their amine ligand such as *o*-phenylenediamine (*o*-pda) to the corresponding imine ligand *o*-benzoquinonediimine (*o*-bqdi) (Figure 12) was observed.[143] For example, the IC_{50} values against A2780 ovarian cancer cells of **22** is 11 μM while compound **23** displays a value above 100 μM. Interestingly, the *o*-bqdi complexes can be reduced by GSH but readily undergo reoxidation in air.

Dyson et al. recently described the preparation of a series of RAPTA-type complexes with fluoro-substituted η^6-arene ligands.[144] Electron-withdrawing fluoro or CF_3 units were added to the arene to modulate the pK_a values of the complexes. The activity of these organometallics was found to be strongly influenced by the presence of the substituents. IC_{50} values of the fluoro compounds were in general much lower than those of the nonfluorinated analogues.

Organometallic Osmium Half-Sandwich Complexes

Probably because of its reputation of being highly toxic (see OsO_4) and relatively inert toward substitution, osmium organometallics have been neglected as therapeutic agents in comparison to its lighter congener ruthenium.[121,145] Recently however, several research groups[146−154] investigated the anticancer activity of osmium(II) arene half-sandwich complexes. Their results indicate that Os(II) organometallics might be promising candidates as antitumor drugs.

The kinetic and thermodynamic properties found for the first Os complexes were unsatisfactory despite the fact that they were isostructural to the active Ru complexes.[121] Concomitantly, the biological activity was low. SARs were then established and then used to improve their activity. The ligand exchange rate (chloride against water, see discussion above in the section Organometallic Ruthenium Half-Sandwich Complexes) was too slow (40−100 times slower than the Ru analogues, depending on the pH value). The water bound to the osmium is indeed more acidic by 1−2 pK_a units than when bound to analogous ruthenium complexes. In order to restore activity, a new series of Os complexes were prepared in which the neutral N,N-chelate ethylenediamine was replaced by O,O and N,O anionic chelating ligands with a stronger *trans* effect. Following this thought, picolinate was identified as an ideal ligand candidate and the respective complexes [(η^6-arene)Os(pico)Cl] (**24**, Figure 13) had faster hydrolysis rates and potent anticancer activity comparable to that of carboplatin.[148] Furthermore, their mechanism of action is thought to be similar to that of their Ru organometallics, nuclear DNA being the biological target as shown by studies demonstrating the binding of such complexes to DNA.[150]

Arion, Keppler, and co-workers investigated the binding of ruthenium and osmium to paullone derivatives, which are known to be potent inhibitors of cyclin-dependent kinases (CDKs) (**25**, Figure 13).[152] This "metalation" was thought to increase the solubility and bioavailability of the paullone ligands. They showed that complexes such as **25** had respectable antiproliferative activity in submicromolar to very low micromolar concentrations in three cell lines, with no significant differences between the Os and Ru complexes. No CDK inhibition was published so far on those compounds, and their binding to 5′-GMP was found to be significantly different depending on the complexes. This indicates that they exert their anticancer activity either by binding to crucial proteins or by noncovalent DNA interactions.[152]

Dyson et al. have also evaluated the activity of Os(II) and Rh(III) analogues of RAPTA-C (**26**, **27**, Figure 13).[153,154] Depending on the cell lines, significantly different IC_{50} values were determined for **26**, **27**, and RAPTA-C, with **27** being more cytotoxic than the two other compounds and **26** exhibiting essentially similar cytotoxicity as RAPTA-C.[154] Furthermore, using a combined experimental and theoretical approach, it was reported that the binding of RAPTA-C and **26** to a 14-mer oligonucleotide was nonspecific contrary to cisplatin, indicating a different mechanism of action and/or a different biological target.[153]

Telomerase is a ribonucleoprotein with DNA polymerase activity that maintains the length of telomeric DNA by adding hexameric units to the 3′ single strand terminus. It is therefore a crucial enzyme for cancer progression. Rosenberg, Osella, and co-workers investigated telomerase inhibition by a series of water-soluble cyclometalated benzoheterocycle triosmium clusters (**28**−**31**, Figure 14).[155] Their motivation was that quinoline derivatives had shown interesting biological properties, especially in inhibiting enzymes.[156] Among all compounds, only the negatively charged clusters (by virtue of the

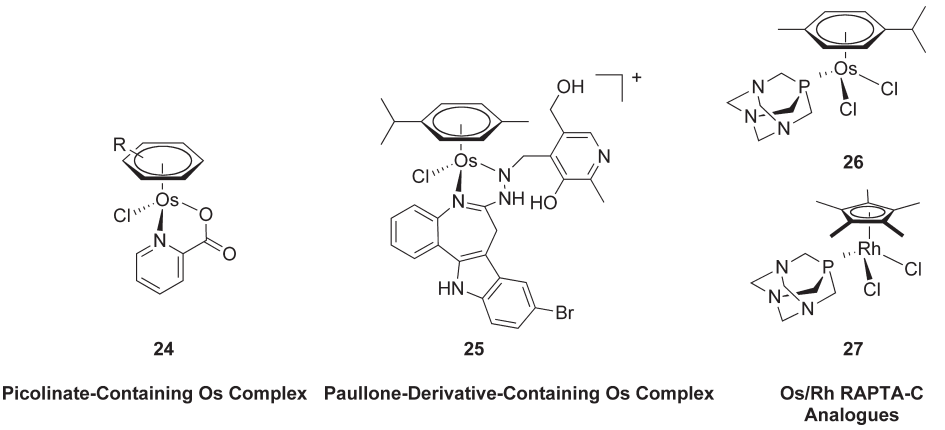


Figure 13. Os(II) arene anticancer compounds.

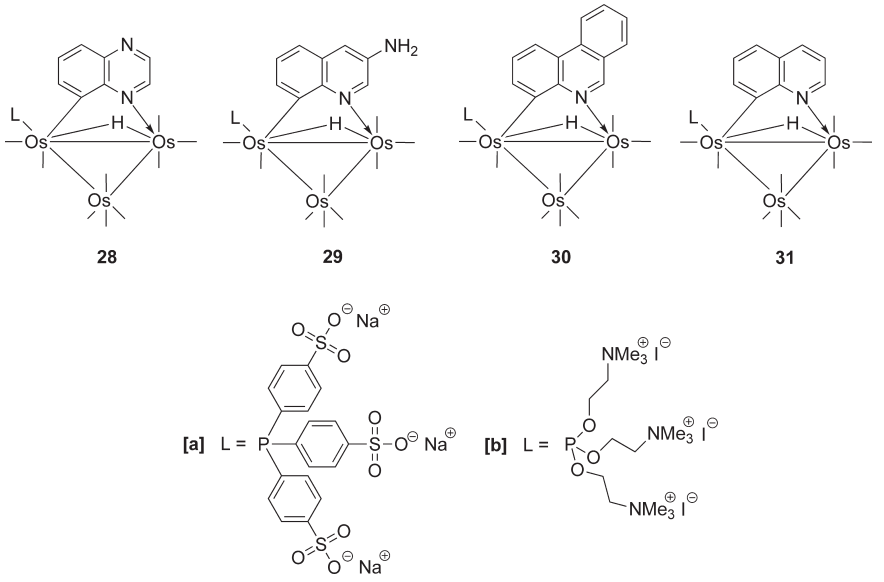


Figure 14. Triosmium clusters as potential inhibitors of telomerase enzyme. Ligand sites on Os denoted by (−) indicate CO ligands.

sulfonated phosphines) exhibited good activity as telomerase inhibitors when tested on semipurified enzymes in a cell-free assay. However, they were ineffective in vitro on Taq, a different DNA polymerase. Furthermore, none of the osmium clusters decreased the telomerase activity in the MCF-7 breast cancer cell line, as observed by the telomeric repeat amplification protocol (TRAP assay). This may well be due to the low aptitude of these organometallics to cross the cell membrane. However, all compounds were acutely cytotoxic, probably because of their accumulation on cell membranes, as shown for compound **29a** by inductively coupled plasma mass spectrometry (ICP-MS). It was hypothesized that **29a** interfered with the normal trafficking and functions of the membrane. Gobetto, Rosenberg, and co-workers also investigated the interaction of other positively and negatively charged triosmium carbonyl clusters with albumin, using the transverse and longitudinal relaxation times of the hydride resonances as ^{1}H NMR probes of binding to the protein.[157] Evidence of binding was observed for both the positively and negatively charged clusters. However, they exhibit distinctly different rotational correlation times.[157] It was anticipated that the negatively charged clusters bind more tightly than their positive analogues, as albumin is rich in positively charged amino acids.[157] The same researchers also established guanines as the binding sites for another positively charged water-soluble benzoheterocycle triosmium cluster to single- and double-stranded DNA by using a range of different biochemical methods.[158]

It is worth mentioning that $Os_3(CO)_9$ type clusters and dicobalt carbonyl fragments were also reacted with derivatives of tamoxifen, a widely used drug in the treatment of hormone-dependent breast cancer (see also the section Metallocenes above).[159] The organometallic moiety was found to increase the lipophilicity and reduced the affinity, via steric hindrance, for the estrogen receptor, but no cytotoxicity studies were carried out on the compounds.

Organometallic Iridium and Rhodium Complexes

In contrast to their Ru(II) congeners, the isoelectronic Rh(III) and Ir(III) half-sandwich compounds have attracted much less attention as potential anticancer agents.[154,160−162] But interestingly, among the few examples reported in the literature, different biomolecules were reported as (potential) targets. Nevertheless, even though it was shown that these compounds were indeed targeting the desired biomolecules, their exact mode of cytotoxic action is still unknown. Hence, Sheldrick et al. showed that Ir(III) and Rh(III) complexes such as **32** (Figure 15) bind DNA through intercalation of

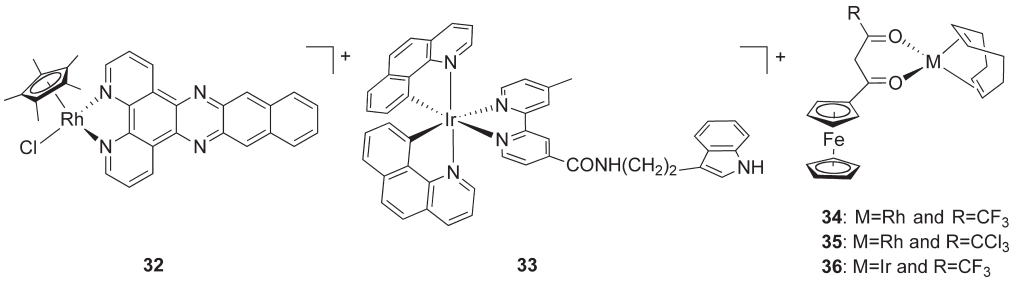


Figure 15. Examples of Rh(III), Ir(III), Rh(I), and Ir(I) cytotoxic organometallic compounds.

their polypyridyl ligands.[160,161] Polypyridyl-containing half-sandwich complexes with Ru(II) and Rh(III) central atoms and hexamethylbenzene or pentamethylcyclopentadienyl ligands showed stable intercalative binding with DNA and exhibited excellent cytotoxic activities.[114,161] Cellular uptake studies by atomic absorption spectroscopy (AAS) revealed that the antiproliferative effects of the complexes were mainly correlated to the size of the polypyridyl ligands, thereby highlighting the special role of ligand lipophilicity on the bioactivity of this class of organometallic antitumor drug candidates. Whereas an interaction with the DNA might significantly contribute to the cytotoxic activity of the agents, the presence of additional cellular targets or alternative modes of action is very likely to contribute to this activity and is therefore the subject of ongoing research projects.

Another example of anticancer Ir(III) compounds has been reported by Lo and co-workers. In order to design new biological probes for bovine serum albumin (BSA), Lo et al. prepared a series of luminescent Ir(III) complexes (**33**, Figure 15) containing an indole derivative (indole is known to bind to BSA) which were found to be highly cytotoxic toward HeLa cells.[162] It is also interesting that other cationic Ir(III) complexes have been recently reported for phosphorescence staining in the cytoplasm of living cells and were shown to be noncytotoxic.[163]

Interestingly, the heterobiorganometallic ferrocene-containing Rh(I) derivative **34** (Figure 15) had a similar cytotoxicity in prostate cancer cell lines to cisplatin but a significantly different pathway for activation of cell death.[164] While cisplatin predominantly induces apoptosis, **34** induces late necrosis and abnormal nuclear morphology.[164] This latter finding is of interest because apoptosis-resistant cells might be better killed with drugs inducing the necrotic pathway.[164] Furthermore, the same complex **34** and other related Rh(I) and Ir(I) analogues (**35** and **36**, Figure 15) were also found to be cytotoxic toward Chinese hamster ovary (CHO) cells with the Rh(I) complexes having IC_{50} values close to that of cisplatin, while the Ir(I) complex had a slightly higher IC_{50} value.[165] These compounds were also tested for their capacity to sensitize hypoxic CHO cells against irradiation. Indeed, tumors are notoriously hypoxic and radioresistant. Both factors limit the success of radiotherapy. Modulation of the radiosensitivity by drugs such as cisplatin is in routine clinical application.[165] Indeed, the Rh(I) complex **34** proved to be an excellent radiosensitizer with properties similar to those of cisplatin.[165]

Rhenium Organometallics

Re organometallics are another very new class of promising antiproliferative compounds. Until recently, only few examples of cytotoxic Re complexes were described in the literature.[166−168] However, over the past years, several compounds with interesting cytotoxicity were reported and their possible mode of action was explored.[169−174] A non-exhaustive list of toxic Re compounds is presented in Figure 16. It is still premature to draw any definite conclusions on a molecular basis for the activity of the Re organometallics presented in this figure. However, a few targets are now envisaged. Hor and co-workers assumed that complexes such as **40** and **41** (Figure 16) were likely to bind to DNA bases or side chains of amino acid residues in peptides and proteins after displacement of the labile ligands.[166,167] Other related Re compounds such as $[Re_2(\mu\text{-}OH)_3(CO)_6]^-$, $[Re_2(\mu\text{-}OH)(\mu\text{-}OPh)_2(CO)_6]^-$, $[Re_2(\mu\text{-}OMe)_2(\mu\text{-}dppf)_2(CO)_6]^-$, and $[Re_2(\mu\text{-}OPh)_2(\mu\text{-}dppf)_2(CO)_6]^-$ (dppf = 1,1′-bis(diphenylphosphino)-ferrocene) have been shown to interfere with nucleic acid metabolism at multiple enzyme sites in L1210 lymphoid leukemia cells, causing DNA strand scission after 60 min of incubation.[168] Ma et al. observed by spectroscopic titrations and viscosity experiments that complex **39** (Figure 16) had a modest DNA binding constant and was interacting with DNA via groove binding.[169] Modeling studies suggested that the minor groove was the favored binding site.[169] Lo et al. prepared and carefully characterized a series of luminescent Re complexes such as **37**, **38**, and **44**, which were generally highly cytotoxic.[172−174] However, the exact target or mechanism of action of these compounds is unknown at this stage of the research. Recently, Doyle, Zubieta, and co-workers prepared two new fluorescent Re tricarbonyl bioconjugates, namely, a folate (**43**)[170] and a vitamin B_{12} (**45**)[171] conjugate (Figure 15). **43** was screened against a doxorubicin- and cisplatin-resistant human ovarian cancer cell line (A2780/AD) which overexpresses the folate receptor (FR). As expected, **43** was internalized by a folate receptor-mediated endocytotic pathway in this cell line. In contrast, no internalization of **43** was observed with a FR-negative Chinese hamster ovary (CHO) cell line. **43** was more cytotoxic than cisplatin toward the FR-positive cell line. The toxicity of **43** was attributed to intercalation into DNA. The structure of the DNA complex with **43** is consistent with the criteria for minor-groove binding with the quinoline rings preferring the A·T sites of the helix and with the positive charge contributed by the metal ion.[170] Interestingly, no inhibition of topoisomerase I activity was observed with the Re complex of **43**.[170]

The fluorescent Re bioconjugate **45** (Figure 16) was prepared to target the cubilin receptor through the vitamin B_{12} uptake pathway.[171] Vitamin B_{12} is important for rapidly growing cancer cells and is therefore an interesting carrier for drug delivery assuming that receptors involved in its uptake can be targeted. In vitro antiproliferative cell assays against cubilin-expressing placental choriocarcinoma BeWo and CHO cell lines were undertaken, and **45** was found to be only moderately cytotoxic toward BeWo cells (IC_{50} = 376 μM). The parent Re compound **42** was found to be 10 times more

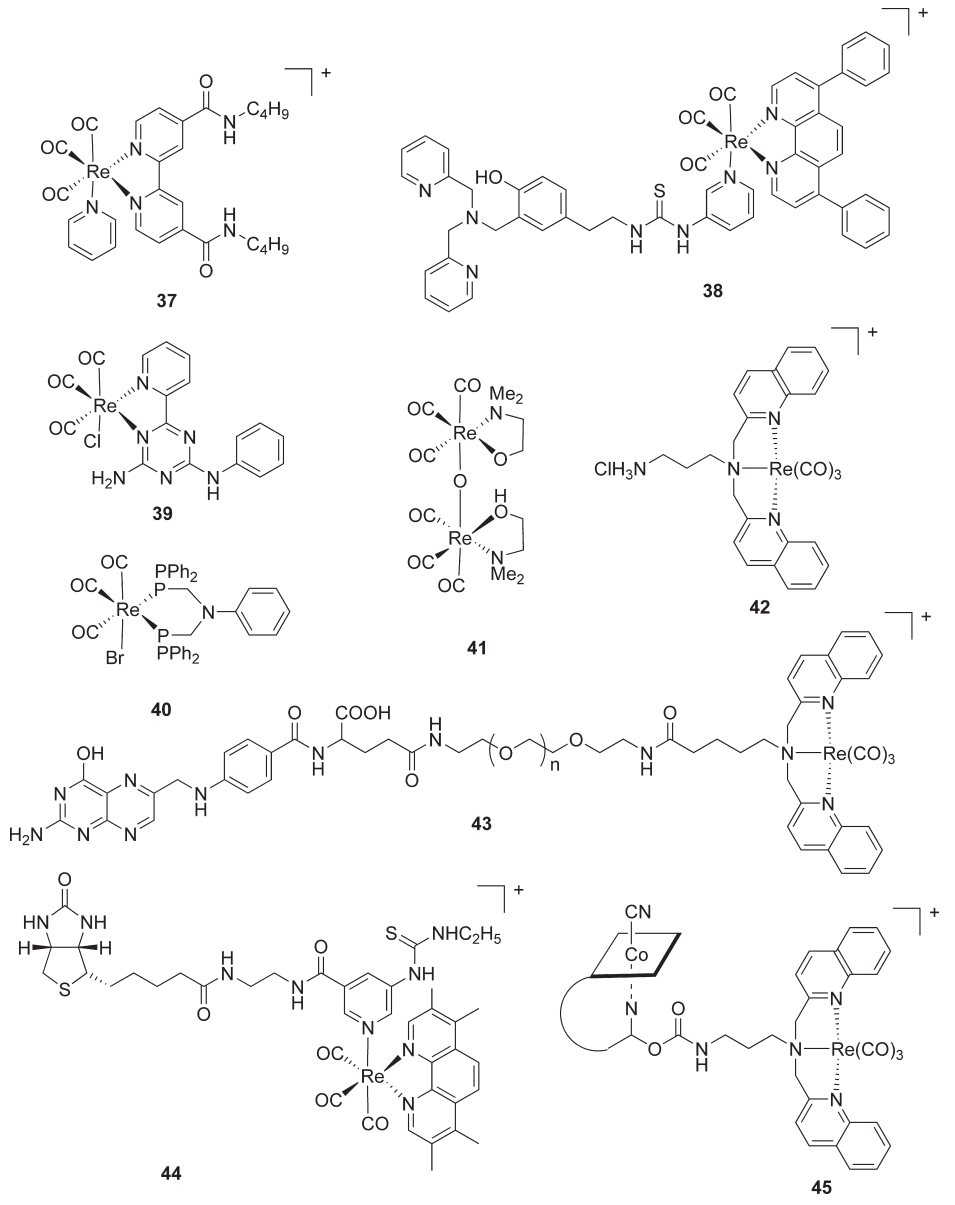


Figure 16. Cytotoxic Re organometallics.

cytotoxic than **45** (Figure 16),[171] probably because of a more rapid cellular uptake of **45** due to passive diffusion. Aggregation of **45** in the cytosol and in the nucleus was observed. The positively charged rhenium chelate component is envisaged to interact with the negatively charged DNA backbone, thus playing a role in the observed cytotoxicity.[171]

Ruthenium, Osmium, Iridium, and Platinum Organometallics as Scaffolds for Protein Kinase Inhibitors[245]

With the exception of DNA-intercalating compounds, the metal center is likely to play a direct role in the anticancer activity of most compounds discussed so far by binding DNA and/or proteins. In contrast, Meggers et al. have used metal complexes as structurally inert *scaffolds* for enzyme inhibitors.[175−177] Their initial idea was that the spatial organization of the substituents around the metal center of a metal complex is much more versatile and therefore increases substantially the opportunity to build complicated three-dimensional enzyme inhibitor structures. Importantly, the metal is not playing any direct role in the inhibition; it "only" allows the spatial organization of the substituents around the metal center. Their chosen targets were protein kinases that are known to regulate many aspects of cellular physiology and pathophysiology.[178] The mutations and deregulation of protein kinases play a causal role in many human diseases, making them an important therapeutic target.[177] Eight kinase inhibitors are already clinically approved, while several more are in the pipeline. Numerous indolocarbazole alkaloid derivatives such as staurosporine were found to be potent protein kinase inhibitors through hydrogen-binding to the ATP binding site (Figure 17).[176] However, a central drawback in the design of these kinase inhibitors is the fact that kinases form one of the largest families of enzymes with highly conserved ATP binding sites, thus rendering the design of *selective* inhibitors very challenging.[177] To overcome this limitation, Meggers et al. synthesized a significant number of metal-containing enzyme inhibitors, the majority of them being Ru(II) complexes and some Pt and Os derivatives (see Figure 18 for a few examples of Ru(II) complexes). They successfully

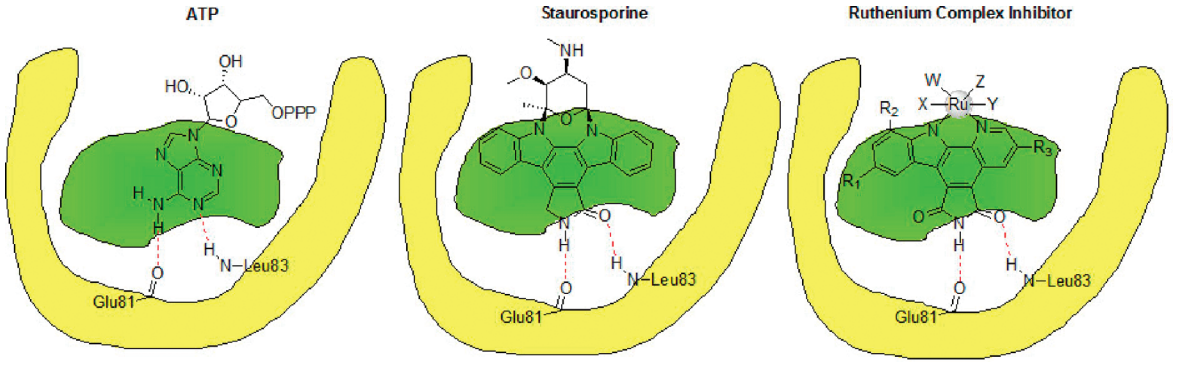


Figure 17. Binding of ATP (left), staurosporine (middle), and ruthemium complexes (right) to the ATP-binding site of cyclin dependent kinase 2 (CDK2). The green area indicates a patch of high hydrophobicity. Adapted from refs 176 and 181.

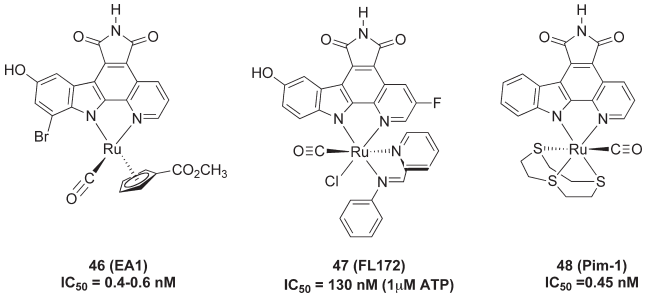


Figure 18. Examples of Ru(II) organometallics as kinase inhibitors with their IC_{50} values. IC_{50} values were measured at 100 μM ATP if not indicated otherwise.

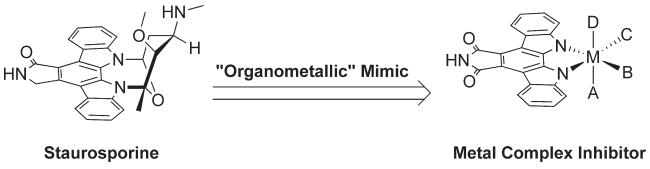


Figure 19. Schematic view of how the metal complex mimics the overall shape of staurosporine. Adapted from refs 177 and 182.

designed nanomolar and even picomolar ATP-competitive ruthenium-based inhibitors. This concept has been confirmed by, so far, six different cocrystal structures of Ru complexes with protein kinases.[179,180] As expected, the metal ion played solely a structural role (Figure 19). However, the organic ligands can be optimized to occupy the available space in the active site, as well as providing additional hydrogen bonding interactions, thus making the individual inhibitors highly specific. Moreover, physiological functions as a consequence of kinase inhibition were demonstrated within mammalian cells, *Xenopus* embryos, and zebrafish embryos.

Ru complexes seem like ideal candidates for this purpose, as they are chemically stable in air, water, and buffer containing millimolar concentrations of thiols, as well as being configurationally stable against ligand exchange or scrambling around the metal center.[177] Other advantages are the well established synthetic chemistry of Ru complexes, a moderate price of the starting material ($RuCl_3$), and low toxicity of such compounds.[177] Recently this concept of drug design was extended to inert iridium(III) species featuring a highly potent and selective inhibitor of the kinase Flt4, which also demonstrated strong antiangiogenic effects in zebrafish embryos.[183]

Metal NHC Complexes

Transition metal carbene complexes are organometallic compounds featuring a divalent organic ligand, which is coordinated to the metal center (see Figure 1). N-Heterocyclic carbenes (NHCs) are generally derived from the so-called persistent carbenes, which are stable compounds of divalent carbon. As they are strongly stabilized by π-donating substituents, NHCs are good σ-donors. However, π-bonding with the metal is weak. Metal NHC complexes are well-known for their catalytic properties. Additionally, their high stability and ease of derivatization make them suitable candidates for drug development.[184,185]

Initial reports on the biological application of NHC complexes dealt with the discovery of new antimicrobal compounds and have also stimulated the evaluation of these compounds as antiproliferative agents.[186−191] For example, the cationic gold imidazolidine derivative **49** displayed excellent activity against the growth of several species including *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Figure 20).[187] Most efforts in the development of carbenes as antibiotics have been focusing on NHC silver complexes. Silver complexes have a long tradition as anti-infectives; however, their mode of action is not yet completely understood. Interactions with the bacterial cell walls and the related biochemistry seem to be of relevance.[189] The pyridine-linked silver carbene complexes **50** and **51** exhibited higher bacteriostatic effects than silver nitrate.[188] Promising antibacterial activities were also obtained with complex **52**, which showed substantially higher stability in aqueous media than structurally related silver NHC complexes without Cl substituents.[192] Interestingly, **52** and structurally related species were also active against the growth of certain cultured tumor cells and preliminary studies on **52** using an ovarian cancer xenograft model also indicated in vivo antitumor activity.[193] Indeed, as outlined in more detail below, metal NHC complexes are promising candidates for the development of bioorganometallic anticancer therapeutics. In most cases, structures described as antiproliferative are closely related to the abovementioned antibacterial agents, thereby highlighting the broad applicability of the class of transition metal species.

In 2004, Barnard et al. reported the induction of mitochondrial permeability transition in isolated rat liver mitochondria by dinuclear Au(I) carbene complexes.[194] This result is of special interest, as gold complexes have been discussed as agents with an antimitochondrial mode of action since early studies on Au(I) phosphine drugs. Moreover, evidence that the impairment of mitochondrial functions is a major route of gold

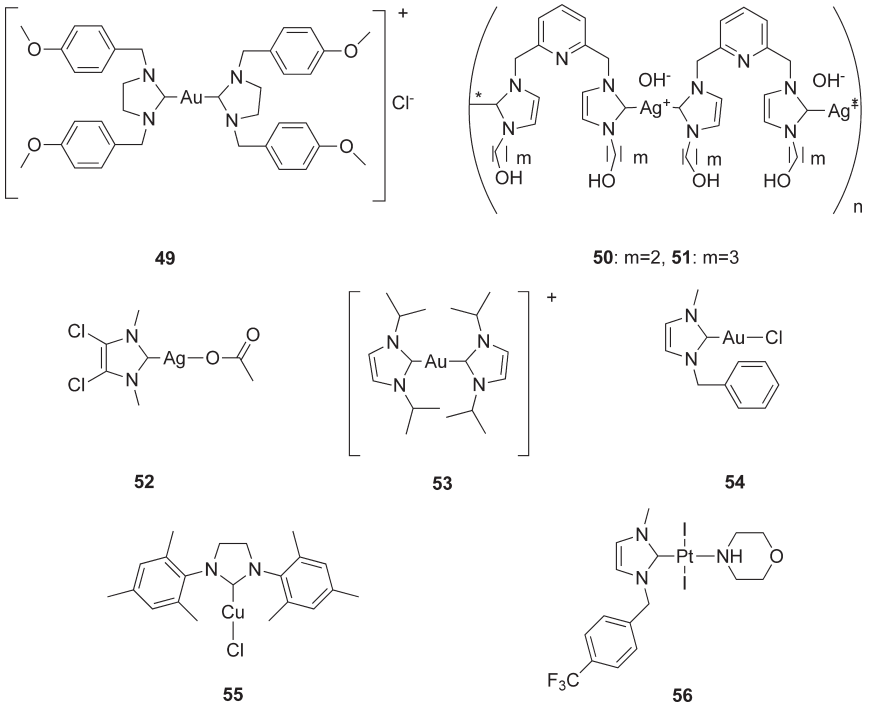


Figure 20. Metal NHC complexes.

metallodrug activity keeps steadily increasing.[195,196] This antimitochondrial mode of action of many gold species is commonly related to the inhibition of the mitochondrial form of thioredoxin reductase (TrxR), a protein closely related to glutathione reductase. TrxR is involved in various physiological processes (including proliferation) and is overexpressed in several cancerous tissues. The active site of mammalian TrxR contains a selenocysteine residue, which is considered to be the target of gold metallodrugs.[196,197]

Antimitochondrial effects (induction of Ca^{2+}-sensitive mitochondrial swelling) were also noted for a series of mononuclear, linear, cationic $[Au(NHC)_2]^+$ complexes.[198] The onset of mitochondrial swelling was most rapidly induced by the complexes with the highest lipophilicity, which was in line with previous studies on gold(I) species demonstrating that the bioactivity can be influenced by fine-tuning of the lipophilicity. A complex (**53**) with intermediate lipophilicity was selected for further studies confirming the significant antimitochondrial properties. It was found that **53** induced apoptosis via caspase 9 and caspase 3 activation. Furthermore, **53** inhibited TrxR activity in MDA-MB-231 cells and accumulated in mitochondria.[199,200] Structurally related Au(I) NHC complexes containing one NHC ligand and one chloro ligand (see **54** in Figure 20 for a relevant example) exhibited potent inhibitory activities against protein tyrosine phosphatases, a family of enzymes involved in various physiological processes.[201] In analogy to the interaction of various gold complexes with cysteine and selenocysteine residues of TrxR or glutathione reductase (GR),[196,197] a cysteine residue within the catalytic site of protein tyrosine phosphatases is most probably the main molecular target for this kind of NHC complexes.

Recently, Lemke et al. reported a series of gold NHC complexes with promising antiproliferative poteny including Au(III) species as well as derivatives containing cysteine thiolate ligands.[202] Both the Au(III) and cysteine-modified NHC derivatives showed similar biological activities compared to related Au(I) NHC complexes without cysteine-derived ligands. This therefore strongly suggests that the development of structurally diverse bioactive gold NHC species is possible and that activity as well as pharmacokinetic properties can be optimized by appropriate choice of the oxidation state of the metal and more sophisticated ligands. In this context, NHC complexes can be functionalized with peptide ligands, which opens the possibility of developing metal NHC derivatives for targeted drug delivery.[203]

Besides the mentioned gold and silver derivatives, NHC complexes with palladium,[204] nickel,[205] copper,[206] or platinum[207] have also been recently reported to exhibit antiproliferative properties. Thus, the copper NHC complex **55** was more cytotoxic than cisplatin. Complex **55** induced apoptosis and, unlike cisplatin, arrested the cell cycle progression in the G1 phase. Concerning a plausible mode of action for this compound, its nuclease-like activity and O_2-activating properties, which led to DNA strand breaks, appear to be of high relevance.[185,206] *Trans*-configured square planar platinum(II) species (see **56** in Figure 20 for a relevant example) also demonstrated promising activity in a cisplatin-resistant cell line.[207]

Overall, metal NHC complexes display promising pharmacological properties as novel antibacterial and antitumor drugs. Regarding their mode of action, the choice of the coordinated metal most probably determines the respective main biological target, e.g., thioredoxin reductase or other enzymes containing (seleno)cysteine residues in their active site for gold or DNA for copper NHC complexes.

Metal Carbonyl Complexes

Metal CO complexes (or metal carbonyls) are organometallic complexes containing one or more carbon monoxide ligands. So far, a large variety of different metal carbonyl complexes with promising antiproliferative properties have been reported including the above-mentioned rhenium and osmium derivatives but also various cobalt,[208] iron,[40] chromium

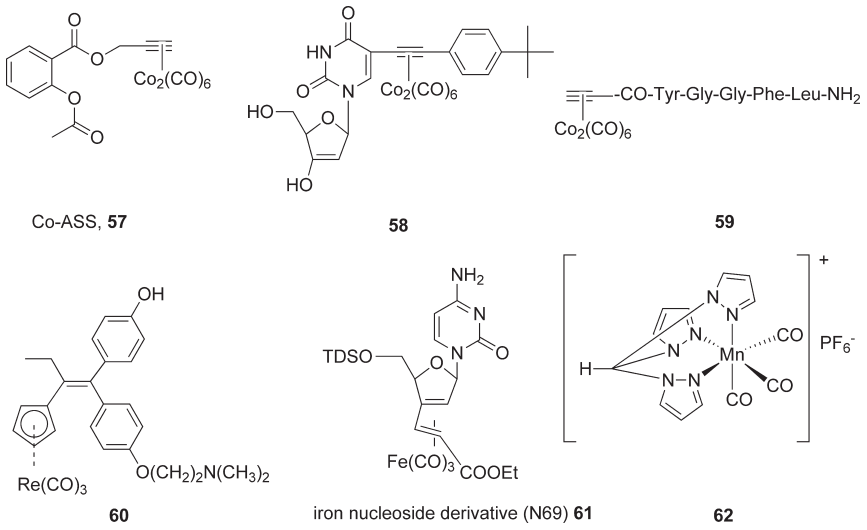


Figure 21. Metal CO complexes.

(half-sandwich),[209,210] ruthenium,[211] or manganese[212,213] bioorganometallic species.

For example, an increasing number of reports deal with the biological properties of alkyne hexacarbonyldicobalt ($Co_2(CO)_6$) species,[208] a class of bioorganometallic complexes whose cytotoxic properties had been mentioned first in 1987[214] and then studied in more detail again since 1997.[215] During subsequent structure−activity studies, a complex containing an acetylsalicylic acid (aspirin) derived ligand emerged as a lead compound for this class of drugs (**57** in Figure 21).[216,217] The importance of the aspirin partial structure for the biochemical properties of this compound is also reflected in its name Co-ASS, which includes the German abbreviation for aspirin, ASS. Clinical studies on aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) have indicated a correlation between the long-term intake of NSAIDs and positive effects for cancer patients (mainly concerning a substantial decrease in recidive risks), thereby making NSAIDs interesting candidates for chemoprevention and combination chemotherapy.[218] On the basis of these observations, the NSAID-like character of Co-ASS and related complexes was studied in more detail and appeared to contribute significantly to the activity of this lead compound.[217] Co-ASS strongly inhibits the NSAID main target enzymes COX-1 and COX-2,[217] and on the basis of its good stability,[219] it could be concluded that the active species was indeed the intact organometallic complex. Recently, it was confirmed that Co-ASS exhibited several biochemical properties related to the reported antitumoral effects of NSAIDs including induction of apoptosis, inhibition of PGE_2 formation, and triggering of antiangiogenic effects.[220] Interestingly, it could be shown that Co-ASS acetylated several lysine residues of its putative main target COX-2, in contrast to aspirin, which acetylates a serine residue in the active site of the enzyme.[220]

In this context, it should be noted that a COX-2 related mechanism, as seen with Co-ASS, most probably does not exist for other hexacarbonyldicobalt species not containing NSAID derived ligands. A variety of hexacarbonyldicobalt species with interesting biological properties have been reported including nucleosides (e.g., **58**),[221] carbohydrates,[222] peptide derivatives (e.g., **59**),[223] and complexes with hormonally active ligands (Figure 21).[224−226] On the basis of the chemical structures of these compounds, it is very likely that different biological structures are targeted, and one might speculate that the $Co_2(CO)_6$ moiety modifies the interaction with those biomolecules. Thus, for complexes with hormone derived ligands, it was demonstrated that the hormonal activity and the receptor binding were retained. For nucleoside ligand-containing derivatives, preliminary studies indicated that the uptake of the compounds into the tumor cells might correlate with their cytotoxic activity. Because a similar dependence was also observed for the uptake into the nuclei, it can be concluded that for the nucleoside derivatives, a possible mode of action might involve an interaction with the DNA or the DNA related enzyme machinery.[221]

Hormonal activity has also been described for metal CO complexes other than hexacarbonyldicobalt alkynes, mainly by the group of Jaouen.[227,228] Thus, several metal carbonyl derivatives of estradiol or hydroxytamoxifen exhibited good estradiol receptor binding affinity, and for some derivatives antiproliferative activity was also observed. For example, hydroxytamoxifene derivatives containing a cyclopentadienylmetal tricarbonyl moiety (see **60**) were well recognized by both ERα and ERβ and triggered antiproliferative effects (see also the section Metallocenes and Rhenium Organometallics above).[227]

In the case of iron-containing metal carbonyl complexes, nucleoside containing derivatives have been the subject of major attention. Thus, the iron CO complex N69 (**61** in Figure 21) significantly induced apoptosis in tumor cells but did not trigger unspecific necrotic effects (see also compound **2** in Figure 3).[40] These properties are highly relevant for further development of these compounds into suitable drug candidates. Additionally, further unspecific effects of the iron diene unit of **61** could be ruled out, as close analogues of the lead compound and the (non-iron-containing) free ligand were not active.[40] Interestingly, the apoptosis induction in melanoma cells by **61** was independent of caspase activation but could be related to ROS formation. It was suggested that the N69-mediated ROS production could be due to its capacity as an iron donor with the nucleoside ligand functioning as a carrier.[229]

In the above-mentioned examples, the structural influence of the CO ligands on the bioactivity and molecular receptor interaction is not yet clear but increasing evidence exists that the presence of these ligands is crucial for the efficacy of the compounds. In this context, it is important to note that the position of a CO ligand in the active site of the target

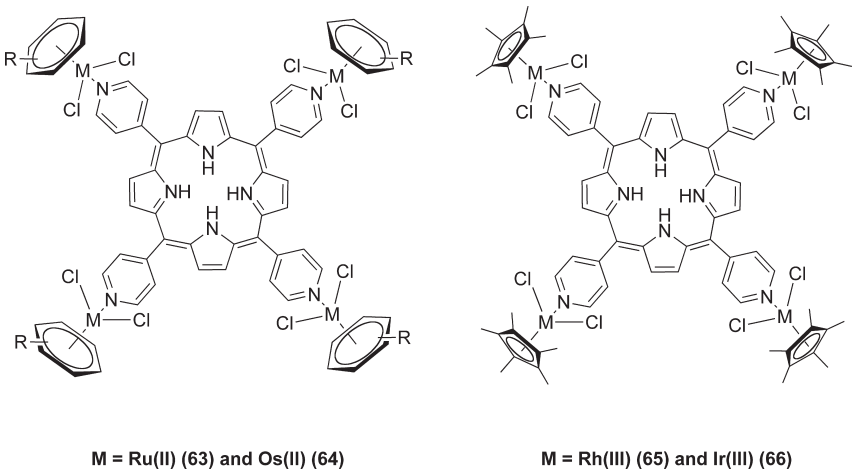


Figure 22. Examples of Ru(II), Os(II), Rh(III), and Ir(III) organometallic porphyrin compounds.

enzyme Pim-1 could be confirmed by X-ray crystallography for a staurosporine-derived ruthenium kinase inhibitor (see Figures 17 and 18 for more details on this class of complexes). From this crystal structure it is obvious that the CO ligand (together with a cyclopentadienyl ring) occupies a binding pocket in the active site of the enzyme, which is usually filled by the carbohydrate moiety of staurosporin.[175] On the basis of these data, it can be speculated that similar biomolecular target interactions might also be relevant for other metal CO complexes.

Besides their potential role in the molecular interaction with biological targets, another relevant feature of CO complexes is their high lipophilicity, which led to increased cellular uptake levels in a number of studies.[217,221,222] It can thus be additionally speculated that stable CO ligands trigger an enhanced bioactivity because the cellular uptake of the (bioactive) ligand of the complex is increased.

Another interesting concept for the biomedical use of metal carbonyl species is the fact that the CO ligands can be released under appropriate conditions, enabling the released carbon monoxide to trigger pharmacological effects.[230] Thus, CO releasing properties have also been recently reported for hexacarbonyldicobalt complexes[231] and for manganese carbonyl derivatives, where photoinduced cytotoxic effects were observed (see **62**).[212] These properties are elegantly used in a broader context by the design of carbon monoxide releasing molecules (CORMs), which might find future application in medicine because of their powerful vasodilatory, anti-inflammatory, and antiapoptotic properties. For a more detailed description of the group of CORMs the reader is referred to recent reviews on this topic.[232,233]

Metal carbonyl species have also shown potential for application as diagnostics. While this topic is also beyond the scope of this Perspective, the concept shall be mentioned briefly here: The fact that intensive IR vibrations of the CO ligands are suitable for detection purposes found use in the so-called carbonylmetallo immunoassay (CMIA).[234] For example, it was possible to develop assays for the sensitive detection of hormones or antiepileptics and to obtain metal-carbonyl-dendrimer-antibody bioconjugates with a broad range of applications.[235]

Miscellaneous

Other strategies and ideas for the treatment of cancer involving the use of organometallics were recently employed

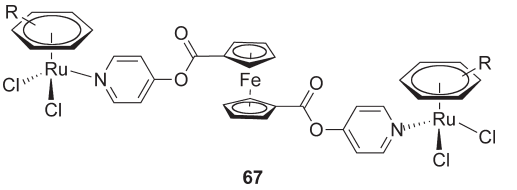


Figure 23. Diarene ruthenium compound bridged by a ferrocene.[238]

by Therrien et al. They investigated the possibility of combining the chemotherapeutic activity of organometallics with photosensitizing agents for photodynamic therapy (PDT). With this in mind, they prepared a series of Ru(II) (**63**), Os(II) (**64**), Rh(III) (**65**), and Ir(III) (**66**) complexes containing porphyrin derivatives, which are known to be efficient photosensitizing agents (Figure 22).[236] The photoactivation produces singlet oxygen and radical species, which results in tumor cell death. All complexes had similar moderate cytotoxicity toward cancer cells with the exception of the Rh complex, which was found to be nontoxic. Importantly, the Ru(II) complexes exhibit excellent phototoxicity toward melanoma cells when exposed to laser light at 652 nm.[236] The exact mechanism of action of these Ru complexes is not yet determined, but it has been shown by fluorescence microscopy that they were not accumulating in the nucleus, suggesting a non-DNA mode of action. In a similar perspective, very recently, the same group described the use of sawhorse-type diruthenium tetracarbonyl complexes containing porphyrin-derived ligands as highly selective photosensitizers for female reproductive cancer cells.[237]

It was also shown by the same group that the bridging of two Ru organometallics through a ferrocene moiety (**65**, Figure 23) increased considerably the cytotoxicity of the compounds compared to the monoruthenium analogue.[238] A difference in redox potential of the ferrocene units has been proposed as a possible cause of the increased cytotoxicity. Remarkably, these compounds were equally potent against cisplatin-resistant and -nonresistant cell lines, which is indicative of a mode of action different from that of cisplatin.[238] The concept of multinuclearity for the improvement of anticancer activity has also been demonstrated by Hartinger et al.[239−241]

Another original example of the use of organometallics for cancer therapy was to employ an "organometallic cage" to transport metal complexes, namely, $[Pd(acac)_2]$ and $[Pt(acac)_2]$

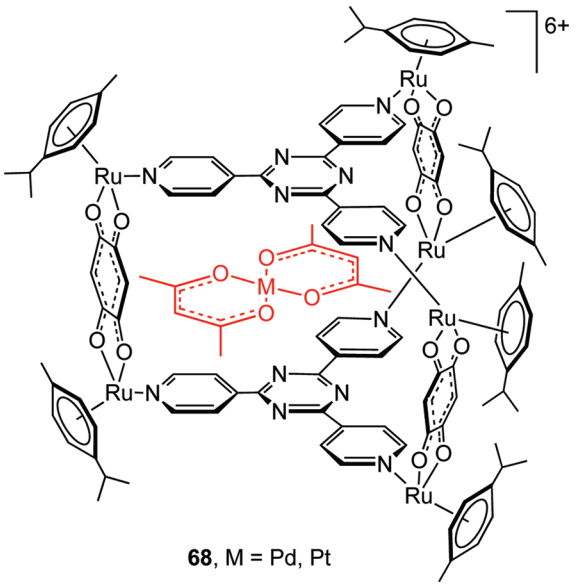


Figure 24. Structure of the "complex-in-a-complex" cations $[(acac)_2MRu_6(p\text{-}^iPrC_6H_4Me)_6(tpt)_2(dhbq)_3]^{6+}$ (**66**). Reproduced, with modification, with permission from *Angewandte Chemie, International Edition*.[242]. Copyright 2008 Wiley-VCH Verlag GmbH & Co. KGaA.

(acac = acetylacetonato) into cancer cells "by encapsulation". The trigonal-prismatic "cage" molecule consists of six half-sandwich (η^6-arene)Ru (**68**, Figure 24) or (η^5-pentamethylcyclopentadienyl)Rh units held together by two trigonally substituted triazine and additional chloro or oxaloto bridges. Interestingly, once inside the cells, $[Pd(acac)_2]$ or $[Pt(acac)_2]$ is released and exerts a cytotoxic effect.[242] It was shown that these cages were extremely stable, even at high temperatures. It was further demonstrated that the empty cage and the Pd and Pt complexes by themselves were less toxic than their "complex in a complex".[242] The exact mechanism of action for these compounds is still unknown, but it is postulated that the organometallic cage facilitates the cellular uptake. The use of such metallaboxes is currently being investigated by this group.[243]

Sadler, Brabec, and co-workers also investigated the photoactivation of dinuclear ruthenium(II) arene complexes to trigger DNA binding and fluorescence.[244] They showed that upon irradiation with UV-A light, some of their complexes underwent arene loss. Interestingly, the fluorescence of the unbound arene is roughly 40 times greater than when it is complexed to the Ru center, therefore enabling visualization of the intracellular localization of the arene moiety. Furthermore, irradiation also had a significant effect on DNA binding in that the formed ruthenium adducts strongly block RNA polymerase. These complexes therefore have the potential to combine photoinduced cell death and fluorescence imaging of the location and efficiency of the photoactivation process.

Conclusion and Perspective

In this Perspective, we summarized recent developments toward the use of organometallic compounds as anticancer drug candidates. The general notion that organometallic compounds would be sensitive to air and water and therefore unstable under physiological conditions and unsuitable for medicinal purposes has been disproved. Rather, our above analysis demonstrates a broad range of classes of compounds that are stable and well characterized for biological applications. Organometallic compounds are frequently kinetically inert and amenable to (multiple) derivatization reactions. They are thus suitable for conventional structure-based drug design, including computer docking experiments similar to those for the more traditional organic drug candidates. The successful development of ruthenium kinase inhibitors by Meggers and co-workers impressively demonstrates this capacity. A recent multistep synthesis of chromium-based antibiotics modeled after the natural lead structure platensimycin further demonstrates that even complicated lead structures can be realized with organometallic cores.[210] With the overcoming of the long neglect of these so-called bioorganometallics by both industrial and academic drug research, an increasing number of emerging drug classes impressively demonstrates that the field offers a broad variety of unexplored options for synthetic medicinal chemistry.

By combination of modern organometallic synthesis with state-of-the-art biochemical studies, the field has advanced markedly from the rather crude "synthesis-and-cytotoxicity-screening" approach that was common just a few years ago. Organometallic chemists frequently collaborate with medicinal chemists, biochemists, and molecular or cell biologists. Arriving at the forefront of medicinal chemistry research, they employ the whole toolbox of modern biomedical research, including structural biology, computer-aided design, and biochemical and cell-based assays to gain a deep insight into possible cellular targets and the molecular details of target interactions. For a number of compounds, even in vivo testing is in progress. We have pointed to prior work and mentioned in vivo results in the respective sections. More studies are currently underway but not yet publicly available. However, we would certainly expect that this is the next frontier for medicinal organometallic chemistry, on the way to bringing at least some of the most promising organometallic drug candidates described herein as drugs to the market.

In this Perspective, we have tried to emphasize such biochemical studies (where available) to elucidate molecular targets and modes of action. While it is clear that for many organometallic complexes interesting bioactivities were observed, the molecular modes of target interaction or the targets themselves are not perfectly clear for each class of compounds at this stage. It is clear, however, that DNA is *not* the target for most bioorganometallics and protein interactions (e.g. with cyclooxygenases, kinases, thioredoxin reductase or hormonal receptors) are major modes of action. Moreover, some metal complexes may even exhibit completely novel, *metal-specific* modes of action, such as the ferrocifen derivatives in which the metallocene acts as a redox antenna for intramolecular redox activtion. Clearly, exploitation of the distinct properties of metal complexes for biologically active compounds deserves more attention. It is hoped that the advent of organometallic complexes in clinical trials will improve acceptance of such compounds in the pharmaceutical industry and support further research into the fascinating field of organometallic drugs and their biological targets.

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generous access to all the facilities of the Institute of Inorganic Chemistry of the University of Zurich.

Biographies

Gilles Gasser received his B.Sc. Hons. in Chemistry in 2000 (University of Neuchâtel, Switzerland) and then worked for 1 year in the research and development division of the agropharmaceutical company Lonza Ltd. (Visp, Switzerland). Gilles returned to Neuchâtel to carry out a Ph.D. in Supramolecular Chemistry with Prof. Helen Stoeckli-Evans (2001−2004) before heading south to undergo a postdoc in Bioinorganic Chemistry with Prof. Leone Spiccia (Monash University, Australia, 2004−2007). In 2007, Gilles was awarded an Alexander von Humboldt Fellowship that he undertook at the Ruhr-University Bochum (Germany) in Prof. Metzler-Nolte's group. Since 2010, Gilles has started his independent research group at the University of Zurich (Switzerland). His current research interests involve the use of metal complexes to understand, identify, and/or influence biological processes in living cells.

Ingo Ott graduated with a degree in Pharmacy from the University of Innsbruck (Austria) in 1999, aquired a pharmacist's license ("Approbation") in 2000, and obtained his Ph.D. in the group of Prof. R. Gust at Freie Universität Berlin (Germany) in 2004. Afterwards he focused on several projects in bioinorganic and bioorganometallic medicinal chemistry at the same institution and performed postdoctoral studies in the group of Prof. X. Qian at East China University of Science and Technology in Shanghai (China). In 2009 Ingo was appointed Professor for Pharmaceutical/Medicinal Chemistry at Technische Universität Braunschweig (Germany). His current research interests involve the development of novel anticancer therapeutics with a focus on bioinorganic and bioorganometallic compounds as well as the study of the biological functions of transition metal complexes in general.

Nils Metzler-Nolte obtained his Ph.D. from LMU Munich (Germany) in 1994, did a postdoc with Prof. M. L. H. Green at Oxford (U.K.), and started his independent research on bioorganometallic chemistry at the Max-Planck-Institut für Strahlenchemie (nowadays MPI for Bioinorganic Chemistry) in Mülheim, Germany. He was appointed Associate Professor at the University of Heidelberg (Germany) in 2000 and Full Professor at Ruhr-University Bochum (Germany) in 2006. He is Speaker of the DFG-funded Research Unit "Biological Function of Organometallic Compounds", member of the COST Action D39 "Metallodrug Design and Action". He is a member of the international advisory boards of several journals. With research interests in medicinal organometallic chemistry and functional metal bioconjugates, the group is running a full program from inorganic synthesis to cell biology.

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