DOI: 10.1002/ejic.201100376

Polypyridyl Metal Complexes with Biological Activity

Luca Salassa*[a]

Keywords: Metal complexes / Polypyridyl ligands / Transition metals / Drug design / Antitumor agents

Polypyridyl metal complexes have optical properties that have been exploited in a wide range of biological and technological applications. Their structural diversity, chemical and redox properties provide a unique opportunity for designing new anticancer agents. Despite this great potential, relatively little is known about the cytotoxic effects of metal polypyridyl complexes, at least in comparison with other classes of coordination and organometallic compounds. This review uses selected examples to illustrate the most recent research carried out on this class of complexes as enzyme inhibitors and cytotoxic agents.

Introduction

The successful application of cisplatin and its analogues as anticancer drugs, as well as their therapeutic drawbacks, has fostered a growing number of studies on diverse classes of metal complexes and their biological effects. In addition to the encouraging examples of the Ru complexes NAMI-A[1] and KP1019,[2] which have reached clinical trials, a range of other metal complexes have shown interesting antiproliferative and antimetastatic properties.[3]

Organometallic compounds, in particular, have emerged as an extremely promising class of anticancer agents.[4] The bioinorganic chemistry of metallocenes and half-sandwich metal complexes (e.g. Ru, Os, Ir) is an excellent example to demonstrate the considerable achievements of metal-based chemotherapy. Only in the last two years, several specific reviews have described in detail all the most significant discoveries in the field.[5]

Metal polypyridyl complexes are well known for being coordinatively saturated, substitutionally inert and displaying unique redox properties. Furthermore, their exceptional photophysical and photochemical features have allowed them to be employed in a wide range of applications, such as catalysis,[6] molecular devices,[7] dye sensitizers for solar cells,[8] and fluorescent probes.[9]

Beginning in the 1950s, Dwyer et al. published the first articles on the biological activity of bipyridine, phenanthroline, and terpyridine complexes. They investigated a series of different metal derivatives for their in vivo toxicity and anticancer properties as well as for their antibacterial and enzyme-inhibition activity.[10] Despite these early pioneering results, metal polypyridyl complexes have received limited attention as potential anticancer agents. The reason for this can probably be found in the inertness of such classes of metal complexes, which are generally considered less suitable for coordinative, cisplatin-like, binding to DNA and/or other possible cellular targets. Surprisingly, metal polypyridyl complexes have found extensive applications as DNA intercalators and grove binders,[11] but relatively few studies have investigated their cytotoxicity in vitro.

Apart from the numerous studies on interactions between metal complexes and DNA, several promising examples of bioactive metal complexes containing bipyridines were recently reported in the literature. Many of them show how the inertness and structural versatility of polypyridyl ligands can be advantageously exploited for obtaining stable molecules that are able to form noncovalent adducts with key proteins in cells. Others highlight how a series of chemical properties (e.g. hydrophobicity, redox potential) can be properly modified by using bpy-like ligands to

[a] Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, U.K.
E-mail: L.Salassa@warwick.ac.uk

Luca Salassa completed his undergraduate studies in Chemistry at the University of Turin (Italy), where he also obtained his PhD in 2004 under the supervision of Prof. R. Gobetto. The following year, he moved to the University of Montana, where he investigated the use of metal-based fluorescent probes for biophysical applications in the groups of Prof. J. B. A. Ross and Prof. E. Rosenberg. In 2008, after a short stay in Italy, Luca Salassa was awarded a Marie Curie Intra-European Fellowship to study photoactivatable metal complexes at the University of Warwick with Prof. P. J. Sadler. Currently, he is a research fellow in the same group. His research is focused on the development of new photoactivatable metal complexes for anticancer applications and on the study of their activation mechanism by computational and spectroscopic methods.

achieve cytotoxicity of more reactive compounds in cancer cells. An increased attention to the stereochemistry of metal polypyridyl complexes in binding to potential targets is also present in several of the studies exploring the cytotoxic properties of this family of compounds.

The aim of the present contribution is to illustrate some of the most recent work done on metal polypyridyl complexes that display biomedical potential (e.g. cytotoxicity or enzyme-inhibition properties). The discussion has been narrowed to metal complexes coordinating one or more polypyridyl ligands and excludes those derivatives that contain bis(bipyridyl)-like ligands but have other distinguishing sets of ligands (e.g. metal arene complexes). The mechanism of cellular uptake and accumulation of metal complexes have been reviewed elsewhere by several authors and are not discussed here.[12]

Inert Polypyridyl Metal Complexes as Enzyme Inhibitors

Inert polypyridyl metal complexes have been used by Gray et al. to develop probes/inhibitors of the enzymes $P450_{cam}$ (compounds **1**–**3**)[13] and copper amino oxidase Arthrobacter globiformis amine oxidase (AGAO) (compounds **4**–**7**).[14] The interest in cytochrome $P450_{cam}$ is due to the relevance that P450s have in many metabolic processes and diseases, including carcinogenesis.[15] Furthermore, one of the human P450s (3A4) is responsible for the metabolism of the majority of drugs in use.[16] AGAO inhibitors can have therapeutic implications, as recent studies on the copper amino oxidase VAP-1 have found that these enzymes are implicated in tumor progression.[17]

Gray's Ru and Re complexes (Scheme 1) display one chelating ligand with an aliphatic chain of various lengths (C4 to C13) functionalized with a substrate that fits in the active site of the enzyme. Early work on these so-called molecular wires was initially oriented to probe metalloenzyme oxidation states, which would be otherwise elusive, since they are short-lived. The interactions between the metalloenzyme active sites and the metal complex can be monitored by using the intrinsic fluorescence of the metal complex;[13b] however, these systems can also be exploited to generate new transient redox states of the enzyme by light excitation and subsequently probe their characteristics by using time-resolved methods.[13c]

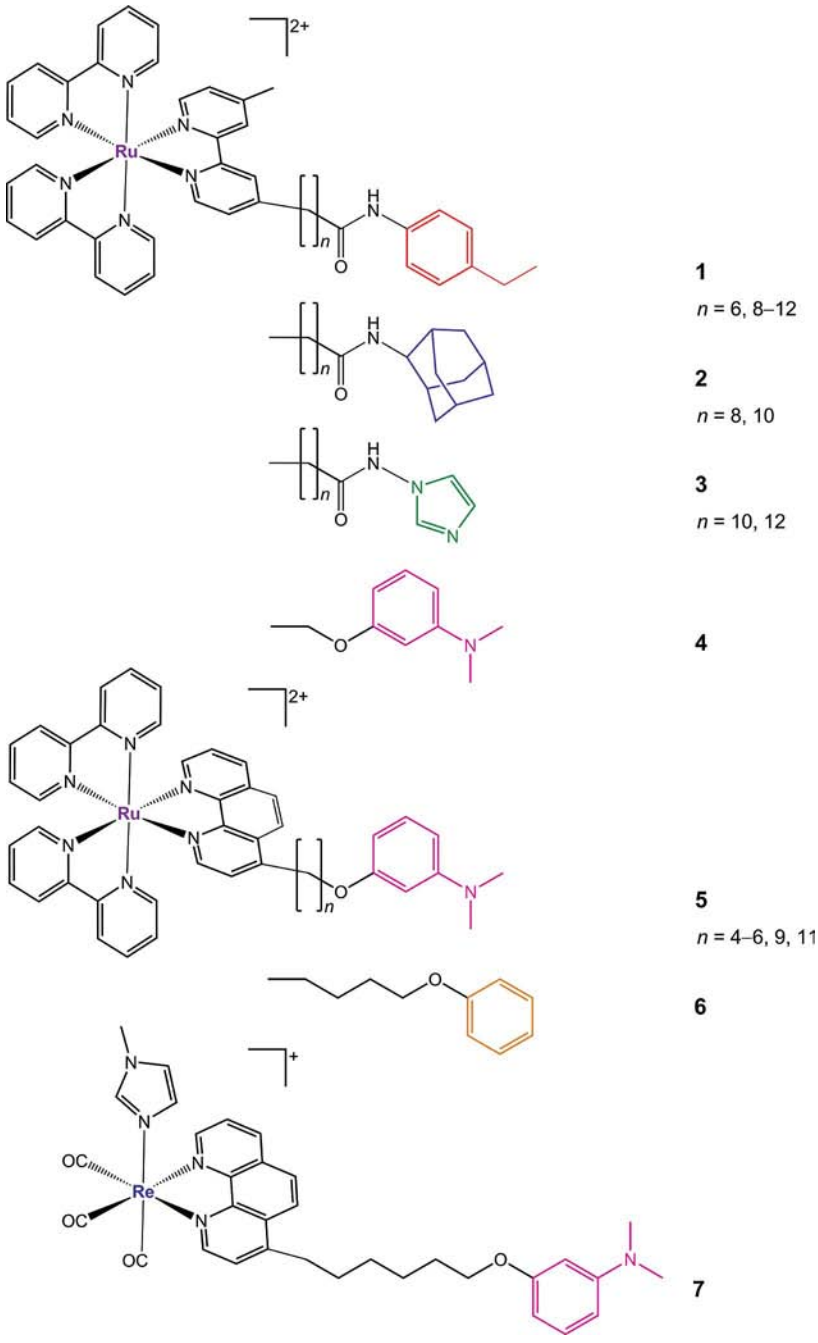
Crystal structures of metalloprotein–molecular-wire adducts[13a,13b,14a,14c] have been determined, and unique structural information on the nature of this interaction has been revealed. Such information is essential to understand at a molecular level the biological processes the enzymes are involved in, and it also provides means for designing new metal molecular wires as inhibitors of the same enzymes.

A series of $[Ru(bpy)_2(Me\text{-}bpy\text{-}linker)]^{2+}$ complexes terminating in an amido function connected to either an ethylbenzene (EB, **1**) an adamantane (Ad, **2**), or an imidazole (Im, **3**) group have been prepared for probing the active site of $P450_{cam}$. These groups were chosen since EB and Ad can strongly bind to the hydrophobic cavity of the enzyme, while Im can interact directly with the iron in the heme group. The amide function was introduced to facilitate the interaction between the wire and the Tyr96 residue present in the active site pocket. All terminal groups show the ability to bind to the heme in $P450_{cam}$ with good affinity constants. A certain length of the wire chain is necessary to allow the terminal group to extend far enough into the protein active site and reach the heme group. For example, the C11 chain is not sufficient to result in any binding by the Im wire (**3**), while the C13 chain enables a tight binding to $P450_{cam}$.[13b] All the Ru–$P450_{cam}$ adducts show that the Ru–bpy sensitizer unit is placed on the surface of the protein, at the entrance of the active site. In the case of the Ad wire, the crystal structure shows that all water molecules are displaced from the active site cavity, so that the heme Fe is pentacoordinate. On the other hand, the EB tail leaves the cavity practically unperturbed.

Besides their use as electronic structure probes, these Ru molecular wires show potential as enzyme inhibitors and therapeutic agents. Their high degree of versatility (metal, linker, tail) enables the exploration of their structure–activity relationships in a combinatorial-like fashion. Dissociation constants (K_D) of adducts of Ru molecular wires and $P450_{cam}$ are in the submicromolar range, and complexes of type **2** have a K_D of 0.7 μM.[13b] Complexes are reversibly bound to the enzyme, as demonstrated by competitive binding experiments performed with camphor, a natural substrate for $P450_{cam}$. Interestingly, these molecular wires are highly selective towards the $P450_{cam}$ enzyme. Changes in the fluorescence lifetime of the Ru chromophore demonstrated that binding to $P450_{cam}$ can occur even in a mixture containing six heme proteins.

A series of structurally similar Ru and Re derivatives (Scheme 1, **4**–**7**) were developed to asses their inhibition of AGAO and to investigate their structure–activity relationships.[14a,14c] In the case of the Ru derivative **5**-C4, the crystal structure of Ru/AGAO adducts was determined showing that the arm of the Ru molecular wire is located in the enzyme active site. The X-ray structures give insights into the topological and conformational mobility. All the prepared Ru derivatives inhibit AGAO in a totally reversible manner as shown by the complete recovery of activity after extensive dialysis. The inhibition is competitive with respect to amine substrates.

Complex **5**-C4, having a Ru–C4–DMA unit (DMA = dimethylaniline), is the most efficient inhibitor of AGAO with a K_i (inhibition constant) of 27 nM. Interestingly, this Ru complex is able to make close contacts with nine residues of the active site pocket and in particular with the five nearest neighbors. In complexes where the Ru^{2+} head group is replaced by a Re^+ structure, an increase in K_i is observed (5-fold); if the metal head is completely omitted the increase is even more dramatic (6 μM).[14a] The charged head group has a fundamental stabilizing role of the protein–wire adduct by surface interactions, both in an electrostatic and in a hydrophobic manner. Nevertheless, when a Co^{3+} analogue is used, inhibition decreases significantly, indicating that the



Scheme 1. Schematic structural representation of the molecular wire metal complexes used as inhibitors of $P450_{cam}$ and AGAO enzymes.[13b,13c,14a,14c]

charge is not fundamental. In fact, DMA has a stabilizing effect: when it is replaced by an H atom, an 18-fold decrease in the binding constant is observed. The effect of the chain length appears to be less significant. No particular effects are observed upon moving from $(CH_2)_4$ to $(CH_2)_{11}$. Only the $(CH_2)_1$ is too short to allow interaction between the DMA and the cofactor topaquinone (TPQ), which is in close proximity of the copper center of the enzyme.[14a]

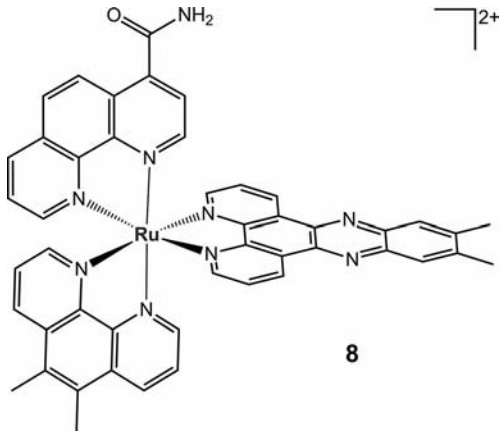
Because of its potency, Gray and others studied **5**-C4 further to determine whether the two optical isomers of this Ru molecular wire could have different inhibition potency towards AGAO.[14c] By analysis of the Δ and Λ isomers in enantiopure AGAO/**5** adducts, the authors found that the two enantiomeric structures display identical orientations of the DMA group with respect to TPQ. The chain has a slightly different orientation at the entrance of the channel and the metal complex head is at a similar position for both isomers, despite the difference in chirality. DMA interacts via H-bonding with TPQ and the Tyr284 residue of the active site pocket. Moreover, the Tyr296 residue is at an open position when the Ru molecular wire is inside the cavity, while in the native AGAO it closes the channel by interacting with the first of the three water molecules that are inside the channel.

Assays indicate that there is no significant stereocontrol in the inhibition reaction. Both Δ and Λ isomers are active (36 and 32 nM) but slightly less potent than the racemic mixture. Inhibition tests on other copper oxidases showed that the complexes are rather selective towards AGAO. Such selectivity can principally be ascribed to all the interactions occurring between the pendant arm of the wire and the active site pocket. However, in the case of **5**-C4, the charged head is on the protein surface at the end of the active site. Longer chains offer more freedom and the metal-containing head is further away from the cell surface, which causes a decrease in the inhibition properties.

Adopting concepts typical of medicinal organic chemistry, Meggers and co-workers have elegantly demonstrated how rigid and unreactive organometallic complexes can effectively be used to create stable three-dimensional structures and inhibit a series of kinases (e.g. GSK-3, Pim-1, PAK-1, PI3K, and MSK) of importance for therapeutic purposes.[18] The chemistry and activity of such derivatives has been extensively reviewed by the same group[19] and others.[5a–5d]

A similar approach was adopted for inert polypyridyl ruthenium complexes. In such studies, the synthetic work is driven by the application of combinatorial chemistry methods to medicinal inorganic compounds to obtain optically pure complexes. The biological effects (e.g. enzyme inhibition, antiproliferative properties) of different complexes and isomers obtained accordingly are investigated.[20]

For instance, complex **8** (Scheme 2) is able to inhibit acetylcholinesterase (AChE) activity at a submicromolar level with 50-fold more efficiency than the reference complex $[Ru(bpy)_3]Cl_2$. Such improvement in activity is achieved by functionalizing the periphery of the Ru polypyridyl complex. Notably, the metal complex structure was optimized by using a combinatorial-like approach based on solid-phase synthesis. The authors also investigate the effect of different solid phases and reaction conditions on the reaction yields. After identification of **8** as the best AChE inhibitor, the two diastereomers of this compound were resolved by reversed-phase HPLC. One of the two diastereomers shows higher affinity for AChE by a factor of two, displaying an IC_{50} of 200 nM.



Scheme 2. Schematic structural representation of complex **8**.

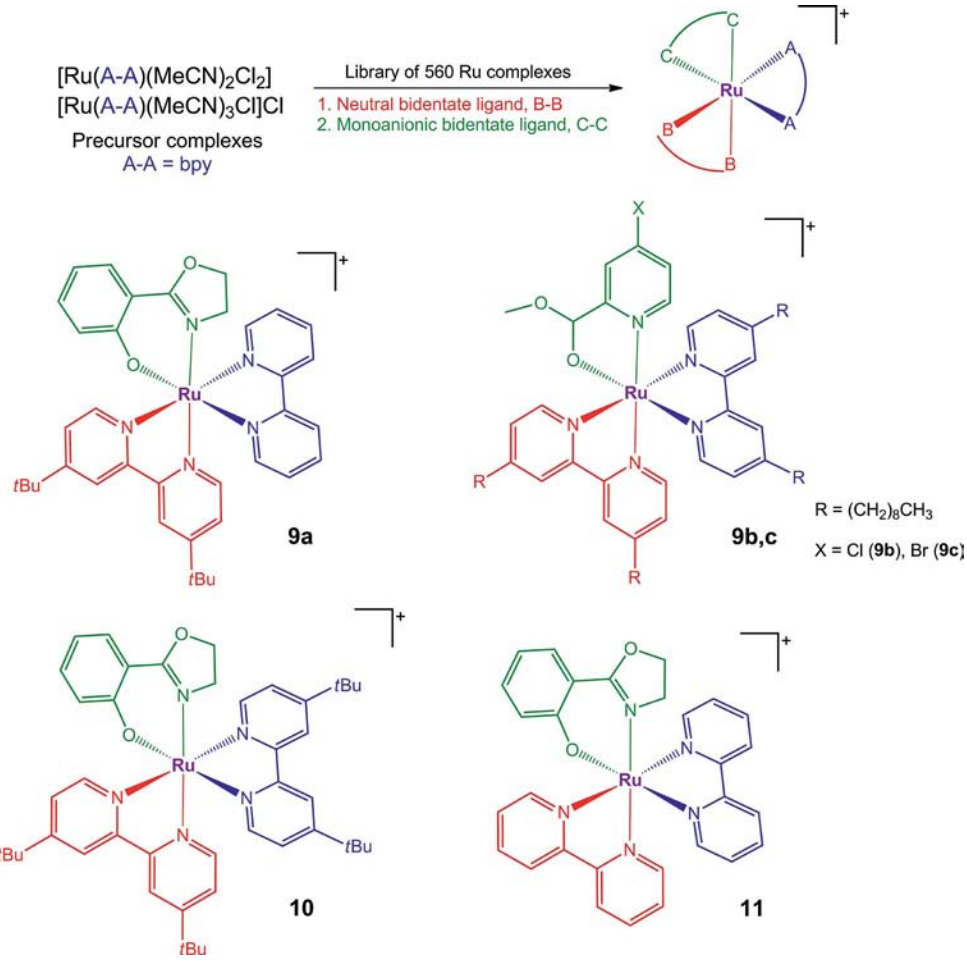
Cytotoxic Polypyridyl Metal Complexes

Expanding further the idea of combinatorial synthesis for metal polypyridyl complexes, Meggers et al. prepared a large library of derivatives, which were successively screened for anticancer activity.[21] The authors optimized a reaction scheme (Scheme 3) that allowed step-by-step coordination of three different chelating ligands to the Ru center. Reaction volumes of 100 μL and sealed propylene 96-well plates were used for the synthesis of the compounds. Reaction conditions were optimized such that side product formation was minimal, and good yields were obtained. This was confirmed by random HPLC analysis of a fraction (10%) of the reactions performed. Of the crude reaction products, the 560 most suitable were selected for cytotoxicity tests in HeLa cells (30 μM, 24 h exposure time).

Among all the screened compounds, three derivatives showed good IC_{50} values (**9a**–**c**). Complexes **9a**–**c** were individually synthesized on a larger scale and purified. Complex **9a** (Scheme 3) was found to display the lowest IC_{50} values, and it was therefore selected for further structure–activity relationship studies aimed at understanding which ligand played a key role in the cytotoxicity effect of the compound. By varying single and multiple ligands and monitoring the effect of such changes on cytotoxicity, it was observed that substitution of the bpy ligand by a tBu$_2$-bpy ligand in **9a** to prepare complex **10** resulted in an outstanding IC_{50} value (1.3 μM for 24 h drug exposure or 0.3 μM for 72 h).

Conversely, substituting the tBu$_2$-bpy ligand in **9a** with a bpy ligand led to the nontoxic compound **11** (IC_{50} > 100 μM). The difference in the cytotoxicities of compounds **9a**, **10**, and **11** shows a correlation with the hydrophobicity of the complexes, which affects their cellular uptake. Moreover, compound **10** has dose-dependent antiproliferative and apoptotic effects on Burtkitt-like lymphoma (BJAB) cells. Indeed, complex **10** is able to affect the viability of BJAB cells even at a concentration of 100 nM (24 h incubation). The mechanism of action involves the reduction of the mitochondrial membrane potential, implying the participation of the intrinsic pathway of programmed cell death. Complex **10** can also overcome induced vincristine resistance in BJAB cells, which indicates that caspase-3 is necessary for **10** in order to induce apoptosis. However, **10** cannot induce apoptosis in doxorubicin-resistant cells, which shows that the complex is not sensitive to Bcl-2.

Another interesting research line involving polypyridyl ruthenium complexes currently being developed by Meggers et al. involves the optimization of the synthesis of optically pure complexes without the need for separation steps. As demonstrated in the case of the staurosporine analogue FL172,[22] where the Λ isomer (IC_{50} = 130 nM) is a significantly better inhibitor of PAK-1 than the Δ isomer (3480 nM), asymmetric synthesis of octahedral complexes



Scheme 3. Combinatorial synthesis of a library of Ru complexes (top) and schematic structural representation of complexes **9a**–**c**, **10**, and **11**.

will prove valuable for the preparation of optically pure complexes of enhanced potency and selectivity.

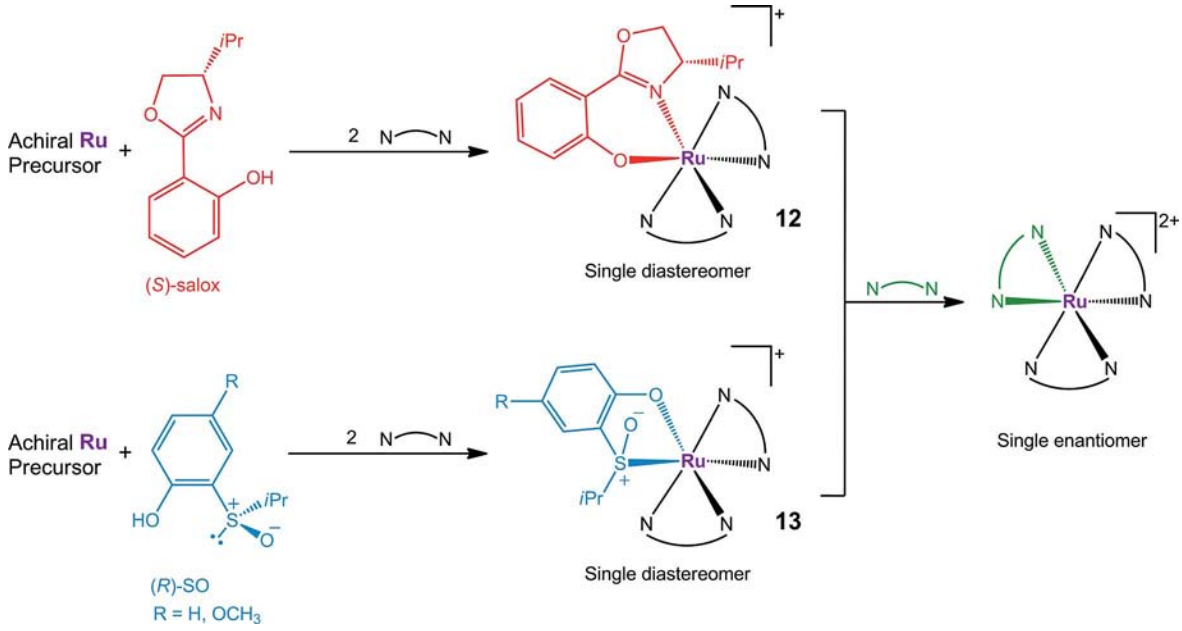
Auxiliary ligands such as salicyloxaline and isopropynylsulfynylphenol can be effectively employed for the synthesis of ruthenium complexes of high enantiopurity (Scheme 4).[23]

Chiral auxiliary ligands are used to favor the formation of intermediates, such as **12** and **13**, with controlled metal-centered configuration. Subsequently, these chiral ligands can be replaced in the presence of acid (trifluoroacetic) to afford tridentate polypyridyl derivatives with retention of configuration. The authors demonstrated the fundamental role the solvent plays in the diastereoselectivity, discussing the effect of substituents on various ligands together with other reaction parameters (concentration of reactants, temperature, and type of acid for ligand replacement reactions).

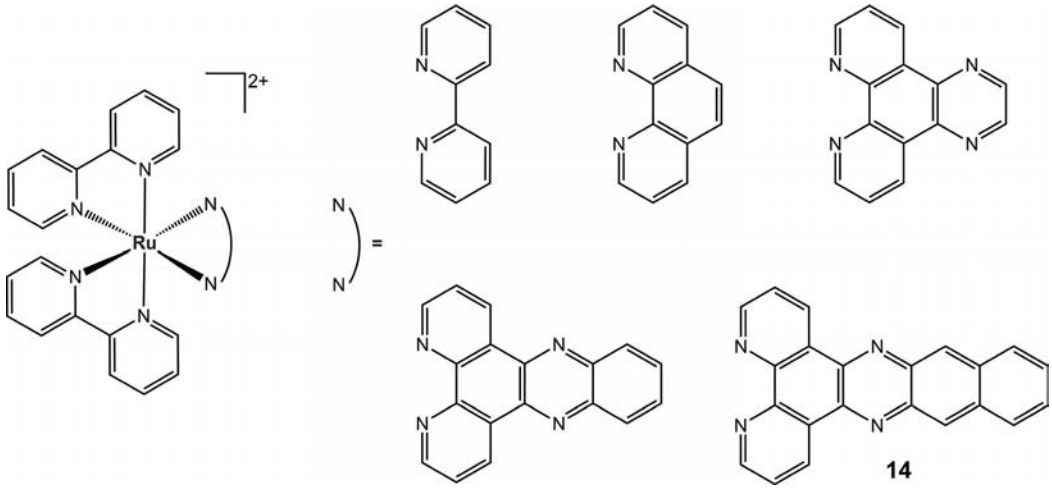
Complexes such as *cis*-[Ru(bpy)$_2$Cl$_2$], *mer*-[Ru(terpy)(bpy)Cl]Cl, and *mer*-[Ru(terpy)Cl$_3$] (where terpy = 2,2′:6′,2′′-terpyridine) were among the earliest Ru polypyridyl complexes to be tested for anticancer activity. In HeLa and L1210 cells as well as in vivo, the latter complex was found to be more cytotoxic than the other two derivatives, which is consistent with its ability to form intrastrand DNA cross-links.[24]

More recently, the cytotoxicity and metabolic effects of related [Ru(bpy)$_2$(N–N)]$^{2+}$ complexes (Scheme 5) were studied by Schatzschneider, Ott et al.[25] The dppn derivative **14** shows good antiproliferative properties against the HT-29 and MCF-7 cell lines. The complex is as cytotoxic as cisplatin for these cell lines, having IC$_{50}$ values of 6.4 μM for HT-29 and 3.3 μM for MCF-7 cells. Higher IC$_{50}$ values are found for the other derivatives investigated. The cytotoxic activity correlates well with the size of the aromatic surface in the N–N ligand. In fact, cellular uptake studies demonstrate that **14** accumulates in the cells up to 162 ng per mg of cell protein with an exposure concentration of 100 μM. Higher concentrations of the other complexes (500 μM) are required to achieve detectable Ru levels in cells.

The authors used a chip-based sensor system to investigate the effect of the complexes on the cellular metabolism and morphology (HT-29). From three different types of measurements (oxygen consumption, extracellular acidification rate, impedance of growing cell layer), it was clear that the behavior of **14** is distinctly different from that of the other complexes of the series. It is reported that this complex is able to interact directly and irreversibly with the cell membrane and/or with membrane proteins. The related morphology changes are in good agreement with the uptake



Scheme 4. Scheme for the synthesis of optically pure Ru polypyridyl complexes (N–N = diimine ligands).[23b,23c]



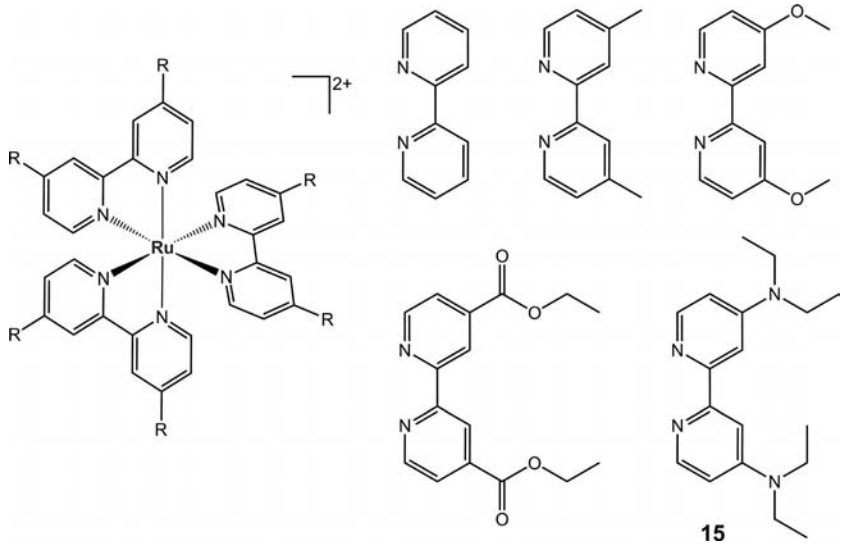
Scheme 5. Schematic representation of the $[Ru(bpy)_2(N–N)]^{2+}$ family of complexes investigated in ref.[25] Reprinted with permission from ref.[25] Copyright 2008 Wiley-VCH Verlag GmbH & Co. KGaA.

and cytotoxicity data, and the cellular membrane is likely to represent the most probable target for **14**.

Dyson and Vogel investigated the effect of five ruthenium tris(bipyridyl) dyes on the A2780 and on the cisplatin-resistant A2780cisR cell lines (Scheme 6).[26] Complex **15** is the most active with $IC_{50} < 1$ μM (72 h drug exposure time). The other derivatives are either not active or weakly cytotoxic ($IC_{50} > 85$ μM). The superior antiproliferative activity of **15** agrees well with its higher lipophilicity. Confocal microscopy fluorescence images obtained by using the emission of the complexes show that **15** is localized around the cell membrane when 10 μM solutions are employed for incubation. At lower concentration (1 μM), **15** is found in the cytoplasm as well. Uptake experiments at 4 °C indicate that the internalization is stopped at low temperature, suggesting that an active transport mechanism may be involved. However, concentration of the complex at the cell membrane is rather a passive process, since it is not blocked at 4 °C. The other tris(bipyridyl) complexes are accumulated in dot-like structures inside the cytoplasm.

Accumulation of **15** in the cell nucleus is not observed, which implies that DNA is most likely not the target for this family of complexes. Cell morphology studies on **15** revealed that the complex kills cells by necrosis, in contrast to cisplatin, which is known to induce apoptosis.

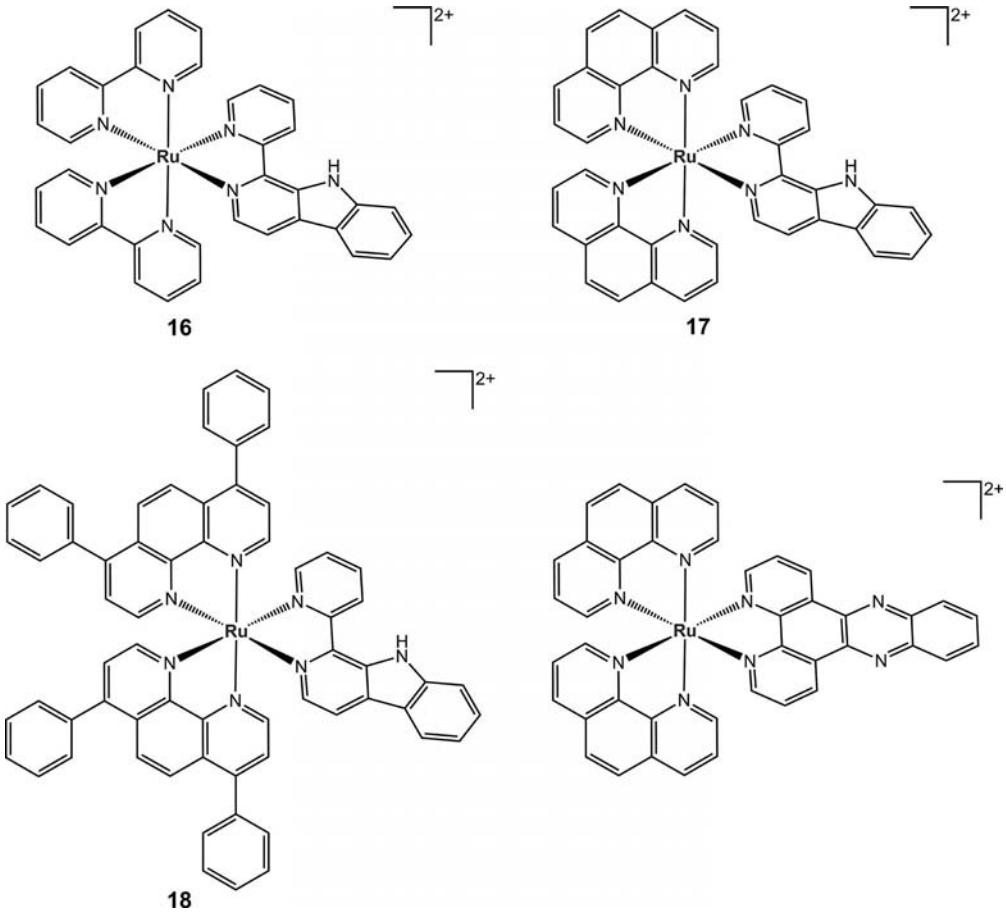
Recently, examples of inert Ru polypyridyl complexes having promising anticancer properties were published by Xu et al.[27] These derivatives contain the biologically active β-carboline ligand, an alkaloid with a broad spectrum of biological functions among which is the ability to intercalate DNA and inhibit topoisomerases (I and II) and various kinases. $[Ru(phen)_2(dppz)]^{2+}$ (where dppz = dipyrido[3,2-



Scheme 6. Schematic representation of the ruthenium tris(bipyridyl) family of complexes investigated in ref.[26] Reprinted with permission from ref.[26] Copyright 2009 Wiley-VCH Verlag GmbH & Co. KGaA.

a:2′,3′-*c*]phenazine) and the free β-carboline ligand are used as controls to assess the importance of this bioactive ligand in the cytotoxicity mechanism of compounds **16**–**18** (Scheme 7).

Notably, $[Ru(phen)_2(dppz)]^{2+}$ is not cytotoxic to the MCF-7 cell line (IC_{50} = 150–280 μM, 48 h drug exposure) or the other cell lines tested. On the contrary, the Ru-$(bpy)_2$ analogue is slightly more cytotoxic to MCF-7 cells



Scheme 7. Schematic representation of the $[Ru(bpy)_2(β\text{-carboline})]^{2+}$ family of complexes investigated in ref.[27] Reprinted with permission from ref.[27] Copyright 2010 American Chemical Society.

(IC_{50} = 90 μM with 72 h exposure), as demonstrated in the work by Schatzschneider et al.[25] mentioned above. Complexes **16**–**18** are able to reach the nucleus, especially the most lipophilic **18**. On the contrary, $[Ru(phen)_2(dppz)]^{2+}$ is confined to the cytosol. Uptake studies were performed by taking advantage of the intrinsic fluorescence of **16**–**18**. Particularly in the case of **18**, the uptake is very high, since exposure to 10 μM Ru solution gives approximately 100 ng of Ru per mg of cells. It is observed that localization in the cell nucleus causes morphological changes. Cell uptake, lipophilicity, and cytotoxicity correlate well for all complexes. The IC_{50} values were measured for HepG2, HeLa, MCF-7, and MCF-10 cells (48 h incubation), and **18** was found to have better antiproliferative activity than cisplatin (9-fold more potent against HeLa). Complexes **16** and **17** are less active than cisplatin but still better than the reference compound $[Ru(phen)_2(dppz)]^{2+}$ and the pure β-carboline ligand. In comparison to the dppz derivative, such a result highlights the importance of the β-carboline ligand coordinated to the metal center.

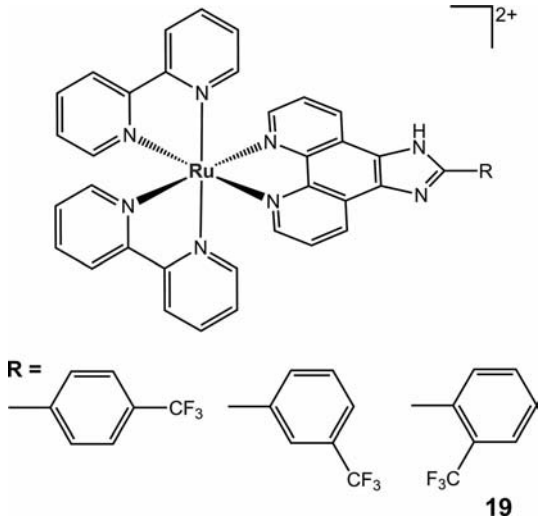
Flow cytometry studies demonstrated that compounds **16**–**18** induce apoptosis. Increase in the sub-G1 fraction is observed, which indicates apoptotic DNA fragmentation. The apoptosis induced by **18** is higher than that induced by cisplatin. Comparison between **17** and $[Ru(phen)_2(dppz)]^{2+}$ shows that the alkaloid ligand plays an important role, as more apoptotic nuclei are observed as a result of higher caspase activity.

Interestingly, compounds **16**–**18** are also able to induce autophagy as a protective response to their cytotoxicity. Inhibition of autophagy[28] enhances the apoptotic response to **16**–**18**. All derivatives are able to cause mitochondrial dysfunction by inducing changes in the membrane potential as confirmed by confocal microscopy and flow cytometry. Reactive oxygen species (ROS) are likely to be involved in such processes, as also in autophagy. HeLa cells treated with **17** show a 7.5-fold increase in ROS production. ROS inhibition with Tiron and N-acylcysteine reduces the cytotoxicity and autophagy-inducing activity of **18**.

All the complexes showed good DNA binding properties ($K = 1 \times 10^6$–3×10^6 M^{-1}), compound **18** being the strongest binder. On the basis of the cell uptake properties of these complexes, DNA can be the preferred target, with a good correlation with nucleus penetration activity and autophagy as well.

Zheng et al. reported another interesting series of Ru compounds. These derivatives have a $[Ru(bpy)_2(N–N)]^{2+}$ structure in which the N–N is an extended phenanthroline ligand having a CF_3 substituent at different positions of the terminal phenyl group (Scheme 8).[29]

The antiproliferative activity of this family of compounds depends on the position of the CF_3 group and is cell-line-specific. Furthermore, different enantiomers display different IC_{50} values. In particular, compound Λ-**19**, in which the CF_3 group is at the *ortho* position, is more active than its Δ isomer (ca. two to five times) and all of the other isomers in the six cell lines tested. Its activity is comparable to or higher than that of cisplatin and falls in the 6–19 μM range (cisplatin 7–36 μM) for the A375, HepG2, and SW620 cell lines.



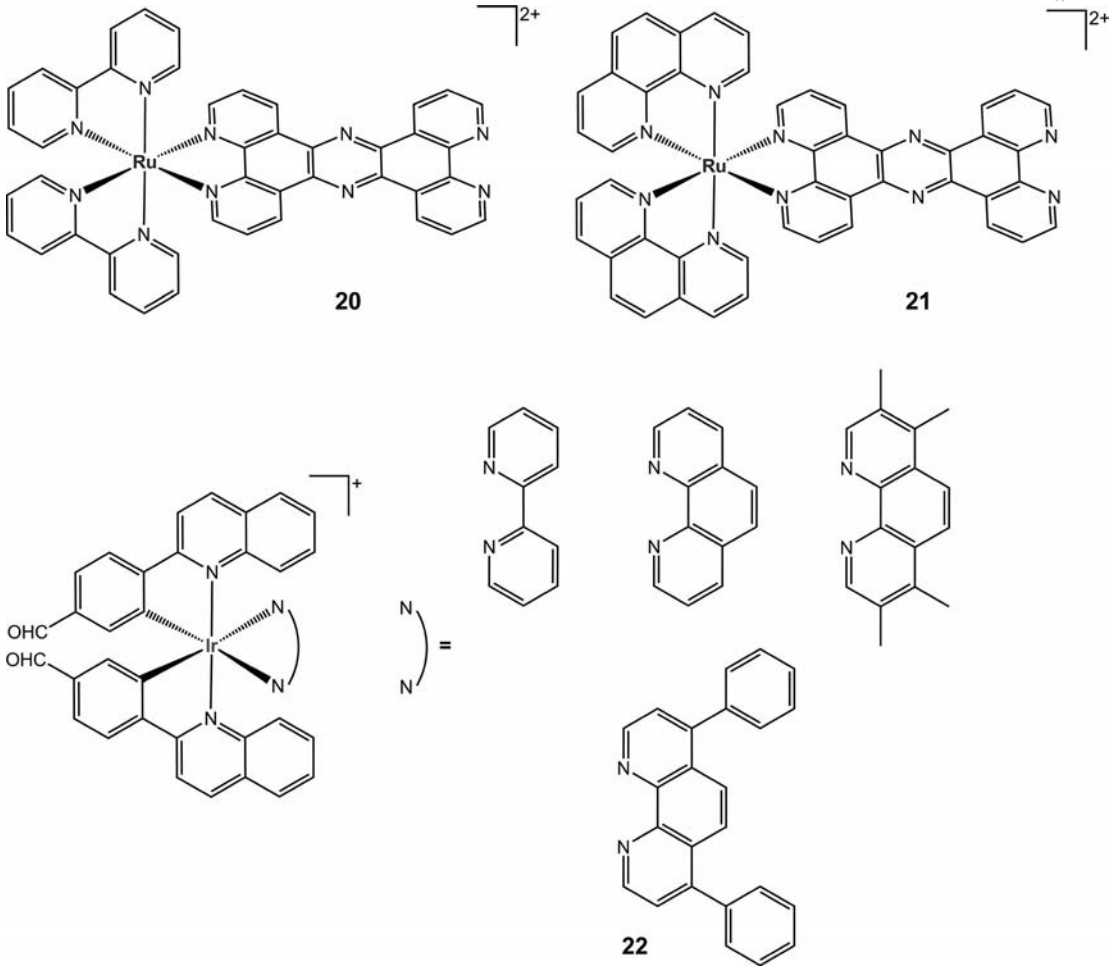
Scheme 8. Schematic representation of the $[Ru(bpy)_2(N–N)]^{2+}$ family of complexes investigated in ref.[29]

Imaging studies on A375 cells revealed that Λ-**19** is able to induce mitochondria-mediated apoptosis, most likely causing a decrease in the mitochondrial membrane potential. This effect was confirmed by the suppression of Bcl-2 and Bcl-xl proteins and the increase in pro-apoptosis Bad proteins in cells treated with Λ-**19** (Western blotting).

Thomas, Battaglia, and co-workers recently published a study of two $[Ru(N–N)_2(tpphz)]$ complexes (Scheme 9, **20** and **21**) with promising properties as cellular imaging agents and cytotoxic compounds.[30] Their anticancer activity was tested on the MCF-7 and A2780 cell lines, with the result that they were as potent as cisplatin. Complex **21** is generally better than complex **20**, in agreement with its faster uptake. Furthermore, both retain their activity in the cisplatin-resistant cells A2780-CP70, where **20** and **21** show lower IC_{50} values than cisplatin: 47 μM for **20**, 20 μM for **21**, while cisplatin has an IC_{50} of 55 μM.[30]

Complexes **20** and **21** bind to calf thymus DNA (CT DNA) by intercalation with high affinity (10^5–10^6 M^{-1}) and show light switching properties.[30,31] They can be excited at 458 nm (^{1}MLCT), and their fluorescence can be monitored at 600–640 nm (^{3}MLCT fluorescence). Complex **21** is effective in staining the nucleus of MCF-7 cells, while **20** gives fainter images, but still reaches the cell nucleus. Interestingly, Ru–dppz complexes do not seem to be able to be uptaken by the nucleus unless they are functionalized by nuclear targeting peptides. Internalization of **20** and **21** occurs by active transport (no luminescence is observed for cells at 4 °C), in agreement with their negative log *P* values (–2.08 and –1.24, respectively). Their DNA staining properties, together with their accumulation in the nucleus, indicate that DNA could be a preferential target for the inert **20** and **21**.

Ir–polypyridyl complexes containing two orthometalated ligands were prepared by Lo and co-workers for imaging of



Scheme 9. Schematic representation of fluorescent complexes **20**–**22**.[30,32] Reproduced by permission of the Royal Society of Chemistry.

cells and tested for their antiproliferative activity towards HeLa cells.[32] These complexes (Scheme 9) display intense structured luminescence in the 580–620 nm range, and their aldehyde groups can be used to bind proteins such as BSA.

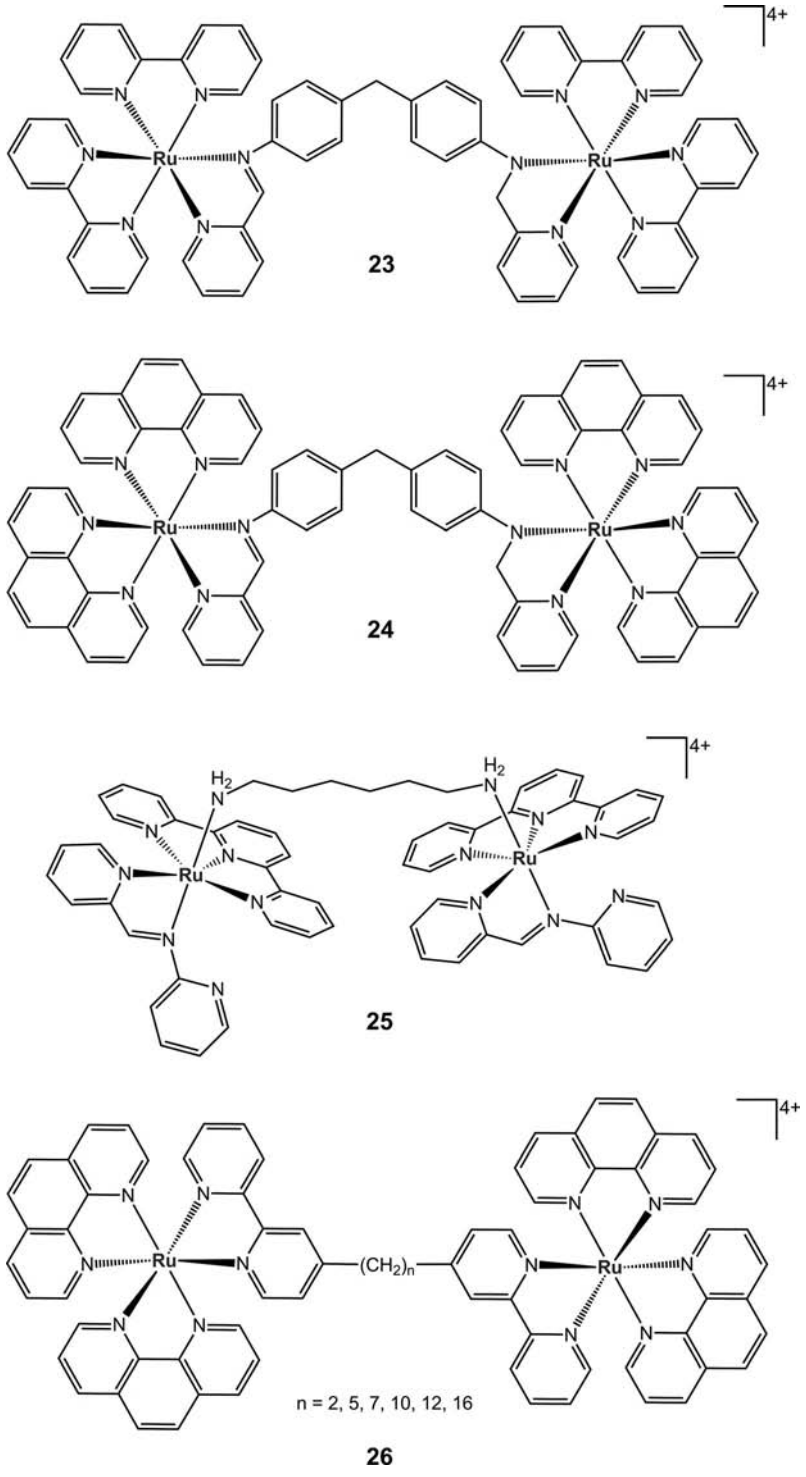
By using its emission, the authors demonstrated that complex **22** is uptaken in cells where it is likely to bind to organelles (Golgi apparatus, endoplasmic reticulum, and mitochondria). No significant uptake into the nucleus was observed. Furthermore, variable-temperature experiments and inhibition of metabolic and ATP pathways showed that uptake takes place through energy-requiring pathways such as endocytosis. All these complexes display higher cytotoxicity than cisplatin in HeLa cells (4–9 μM vs. 23 μM for cisplatin) under the same testing conditions.

Dinuclear Polypyridyl Metal Complexes

Amongst the impressive number of polypyridyl derivatives that have been developed for their DNA intercalation and binding properties,[11] there are examples of active dinuclear polypyridyl derivatives specifically designed to kill cancer cells through interaction with DNA.

Recently, Hannon and co-workers have investigated the cytotoxicity of $[Ru(bpy)_2L]_2^{4+}$ (**23**) and $[Ru(phen)_2L]_2^{4+}$ (**24**) (where L denotes the bis(pyridyl)imine ligand with a diphenylmethane spacer).[33] The flexible **23** and **24** (Scheme 10) were designed to induce DNA binding modes analogous to other metallosupramolecular cylinders developed by the same group.[34] Although it was not possible to obtain the absolute IC_{50} value for these complexes for solubility reasons, it was observed that **23** and **24** would have an IC_{50} of approximately 80–100 μM for HBL100 cells. No significant difference in cytotoxicity is present between the mixture and the enantiopure complexes. Complex **24** was also tested in SKOV-3 cells, where a modest antiproliferative activity was determined; however, in this case, a slight difference of activity between the *meso* and *rac* forms and the pure enantiomers was found.

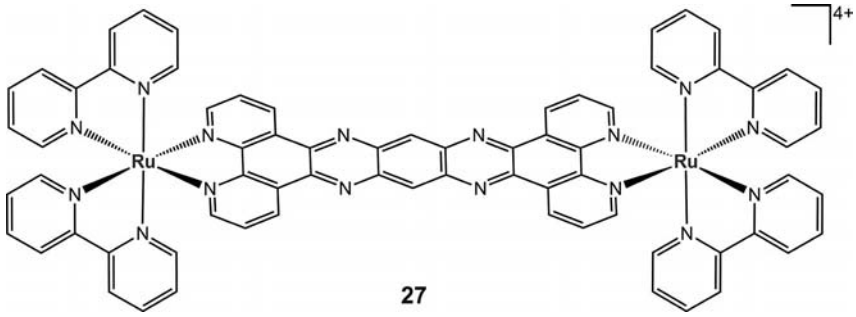
The substitutionally inert dinuclear complex of formula $[\{Ru(apy)(terpy)\}_2\{\mu\text{-}H_2N(CH_2)_6NH_2\}]^{4+}$ (Scheme 10, **25**) (where apy denotes 2,2′-azobispyridine) displays antiproliferative properties against a series of eleven cell lines, including A2780 (33 μM) and the cisplatin-resistant A2780R (28 μM) cells.[35] Mononuclear analogues bearing a labile monodentate ligand (e.g. Cl, H_2O, CH_3CN) instead of the bridging chain were also prepared. They are able to bind nucleobases, and have IC_{50} values as low as those found for **25**. Although **25** and its mononuclear analogues likely have



Scheme 10. Schematic representation of complexes **23**–**26**.

different modes of action, neither are able to induce structural distortions in DNA that are typical of cisplatin or dimetallohelicates.

Another series of dinuclear ruthenium polypyridyl complexes has been developed by Keen and Collins (Scheme 10, **26**). The complexes have general formula $[\{Ru(phen)_2\}_2\{\mu\text{-}bb_n\}]^{4+}$ where bb_n denotes bis[4(4′-methyl-2,2′-bipyridyl)]-1,*n*-alkane and *n* = 2, 5, 7, 10, 12, 16. Cytotoxicity tests in L1210 cells showed that complexes with longer chains have higher anticancer activity, $\mathbf{26}_{n=16}$ having an IC_{50} of 5 μM (4 h incubation), comparable to carboplatin. There is evidence that the cytotoxicity of this class of compounds increases with the chain length and therefore with the lipophilicity. Also, it is found that the ΔΔ enantiomer in the



Scheme 11. Schematic structural representation of complex **27**.

case of $\mathbf{26}_{n=16}$ is more active than the ΛΛ; however, such a trend is less clear in the case of other chain lengths, where the differences are less pronounced.[36]

Uptake into L1210 and isolated B cells was assayed for $\mathbf{26}_{n=16}$ by flow cytometry at different concentrations according to its IC_{50} values. Compound ΔΔ-$\mathbf{26}_{n=16}$ showed a high level of uptake in L1210 as compared to other complexes. Generally, higher uptake corresponds to longer chains. In healthy B cells, the level of accumulation is lower at all tested concentrations. Complex $\mathbf{26}_{n=16}$ is 16-fold more abundant in L1210 cells at 50 μM.

Complexes $\mathbf{26}_{n=10}$ and $\mathbf{26}_{n=16}$ are selectively accumulated in mitochondria with no staining of the cytoplasm or other organelles. Furthermore, no difference in uptake was observed in L1210 cells between the ΔΔ and ΛΛ enantiomers of $\mathbf{26}_{n=7}$.

Studies on the cellular uptake mechanism of $\mathbf{26}_{n=16}$ indicate that the complex is mostly uptaken by an energy-independent process, most probably passive diffusion. This is consistent with its activity in cancer cells, which can be related to their increased leakiness and metabolic activity. Apoptosis is the mechanism of cell death induced by this class of complexes as revealed by cellular morphology studies.

These dinuclear Ru complexes also have potential as antibacterial agents. Minimum inhibitory concentrations in the order of 1–2 $\mu g\,mL^{-1}$ were found for these derivatives against gram positive and gram negative bacteria. Tests for $\mathbf{26}_{n=7,10,12,16}$ in the presence of two human cell lines, fresh blood cells and THP-1 cells, showed that these complexes are significantly less toxic to human cells than bacteria. The most promising complex as antibacterial agent is $\mathbf{26}_{n=12}$.[37]

Complexes **26** can all bind to the minor groove of oligonucleotides.[38] CT DNA binding studies (ΔΔ enantiomers) demonstrated that derivatives with $n = 5, 7, 11$ have the best binding affinity, while lower values were observed for $\mathbf{26}_{n=2}$ and $\mathbf{26}_{n=16}$. DNA binding does not correlate with the biological activity; nevertheless, it is possible that interaction with mitochondrial DNA can inhibit mitochondrial activity.[36]

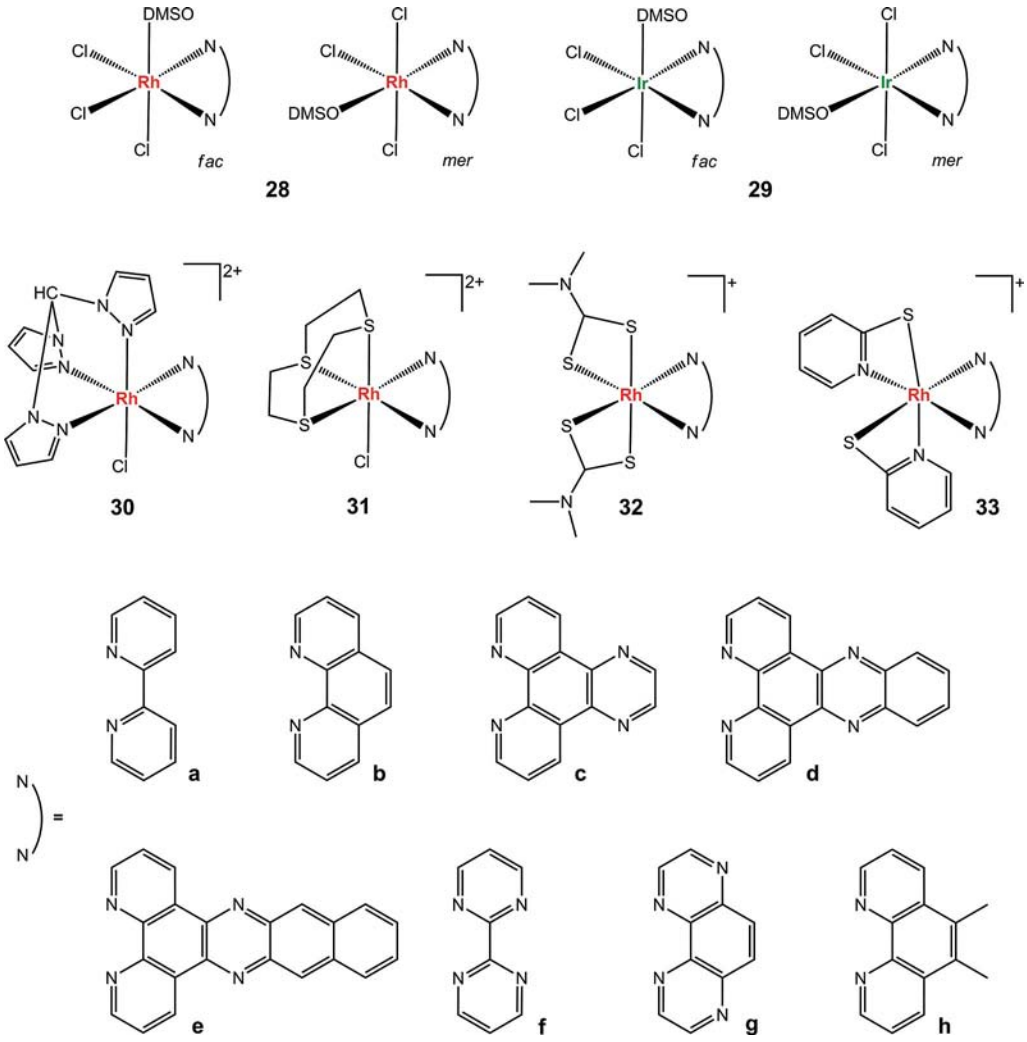
Derivatives in which the alkane chain is substituted by a polyether or polyamine residue present lower cytotoxicity.[38a] Also tri- and tetranuclear analogues have interesting IC_{50} values for L1210 cells (ca. 10 μM) despite their high charge, which is probably the reason for their negative (–1.2 and –1.9) log *P* values.[38a]

Similarly to tirapazamine and other metal complexes (e.g. Cu and Co) currently developed as hypoxia-activated agents,[39] the binuclear Ru polypyridyl complex [$(phen)_2$-Ru(tatpp)Ru$(phen)_2$]$^{4+}$ {**27**; tatpp = 9,11,20,22-tetraazatetrapyrido[3,2-*a*:2′,3′-c:3′′,2′′-1:2′′′,3′′′-*n*]pentacene} in Scheme 11 displays promising hypoxia-selective properties. Complex **27** has marked DNA-cleaving properties.[40] In the presence of glutathione, **27** is reduced and cleaves DNA, while under an O_2 atmosphere the cleaving reaction is significantly less efficient, despite the fact that the derivative is still able to bind tightly to DNA ($K = 1.1\ 10^7\ M^{-1}$ in 25 mM NaCl). As confirmed by UV/Vis and electrochemistry,[41] the reduction reaction with glutathione (pH = 7.0) is a two-electron process that provides the species H_2Ru^{4+}. This species is a strong single-strand nicking agent, and its mechanism of action is likely to involve the formation of C-based radicals. This was confirmed by trapping experiments with the TEMPO trapping agent. O-based radicals seem not to be part of the mechanism, as adding DMSO (up to 5%) to the reaction mixture does not reduce DNA cleavage. The activity of **27** is reduced in the presence of O_2 probably because C radicals are quenched by O_2. Unfortunately, to the best of our knowledge, no cytotoxicity data on **27** have been reported so far in the literature.

Rhodium and Iridium Polypyridyl Complexes

Rhodium carboxylate complexes have raised interest for their cytotoxic properties;[42] however, also polypyridyl derivatives of this metal have recently been shown to be highly active towards a series of cancer cell lines. In a number of studies,[43] Sheldrick and co-workers investigated solution behavior and in vitro effects of the Rh and Ir complexes shown in Scheme 12.

The [Rh(N–N)$(Cl)_3$(DMSO)] complexes (**28a**–**e**) (where N–N = bpy, phen, dpq, dppz, dppn) are prepared by reacting the precursor *mer,cis*-[Rh$(Cl)_3$(DMSO-κ*S*)$_2$(DMSO-κ*O*)] with the chosen diimine ligand. Products are formed as *mer* isomers and are stable in dichloromethane solution, although in the case of the bpy and phen derivatives fast *mer*/*fac* isomerization occurs. ^{1}H NMR spectroscopic stud-



Scheme 12. Schematic structural representation of Rh and Ir polypyridyl complexes.

ies highlight that substitution of DMSO in CH_3OH and D_2O occurs readily and isomerization is also rapid in the case of the bpy and phen derivatives.[43b]

For the HT-29 and MCF-7 cell lines, *mer*-[Rh(N–N)$(Cl)_3$(DMSO)] complexes display high cytotoxicity, which reaches values smaller than 0.1 μM in the case of the most extended chelating ligand. Interestingly, it is found that the ligands alone are also significantly cytotoxic to HT-29 and MCF-7 cells. For example, dppz (**28d**) and dppn (**28e**) complexes have IC_{50} values that are smaller than that of cisplatin. Nevertheless, the ligands are always approximately one order of magnitude less cytotoxic than the corresponding complex. By exposing a DMSO solution of *mer*-[Rh(N–N)$(Cl)_3$(DMSO)] to light, partial *mer*-to-*fac* conversion is observed. When tested, a solution containing a mixture of *mer* and *fac* isomers generally gives higher IC_{50} values. The IC_{50} of [Rh(dpq)$(Cl)_3$(DMSO)] (**28c**) changes from 0.069 μM for the pure *mer* isomer to 0.312 μM for the *mer*/*fac* mixture (40:60%). Also, *mer*-**28d** slowly isomerizes (5 days) to *fac*-**28d**, but no influence is observed on its IC_{50}value for the HT-29 or MCF-7 cells.[43a]

The analogues [Rh(N–N)$(Cl)_3(CH_3OH)$] can be prepared as a mixture of *mer* and *fac* isomers by starting from $RhCl_3{\cdot}3H_2O$. They display either low or no cytotoxicity as compared to their DMSO counterparts.[43a]

UV/Vis studies on the DMSO derivatives show that DNA is not likely to be the major cellular target of these compounds. In fact, only [Rh(dpq)$(Cl)_3$(DMSO)] is able to interact weakly with CT DNA, while the others do not show any interaction. This result is somewhat surprising, particularly in the case of [Rh(dpq)$(Cl)_3$(DMSO)], in view of both its hydrolysis and intercalative properties. No binding with model nucleobases was observed for the rhodium complexes. The neutral charge of the complexes can play an important factor in their lack of interaction with DNA.[43a]

Analysis of the cellular accumulation by atomic absorption spectroscopy (AAS) shows that the amount of Rh found in cells is in good agreement with the cytotoxicity of complexes **28a**–**h**. Increasing the size of the chelating ligand should facilitate the passage of the complexes through the cellular membrane (e.g. dpq < dppz < dppn); however, this trend is only partially observed, and Rh levels in cells follow

the order **28a** < **28b** < **28c** ≈ **28d** ≈ **28e**.[43b] Uptake values (in units of ng Rh/mg cell protein) range between 42 and 92 in MCF-7 cells and between 25 and 70 in HT-29 cells. In HT-29 cells, **28d** displays an extremely high molar cellular concentration of 133 μM (exposure concentration of 1 μM).[43b]

Investigating the cellular metabolism and morphological changes for this class of complexes revealed that cellular oxygen consumption, extracellular pH changes, and cellular impedance were all affected. Interestingly, **28c** has marked influence on the oxygen consumption, indicating that mitochondria might be targeted by the complex.[43b]

Complexes **28c** and **28d** have evidenced an ability to induce apoptosis in BJAB cells superior to cytostatic drugs such as doxorubicin and vincristine. Fluorescence microscopy in the case of dppz shows that the cells undergo apoptosis by shrinking and fragmentation. Rh complexes appear to trigger the mitochondrial pathway.[43a]

In contrast to Rh complexes, the *mer* isomers are less active than the *fac* isomers in Ir derivatives such as **29**. [Ir(N–N)$(X)_3$(L)] complexes display IC_{50} values that depend on both the X (Cl or Br) and the L ligand (DMSO, H_2O). It is reported that IC_{50} decreases upon going from Cl to Br and from H_2O to DMSO. The IC_{50} values for MCF-7 and HT-29 cells are in the μM range. The Ir derivatives do not hydrolyze but slowly isomerize from *fac* to *mer* in DMSO. Furthermore, these complexes do not interact with DNA, but they react with S-containing biomolecules such as N-acetylmethionine. *fac*-[Ir(phen)$(Cl)_3(H_2O)$] was also tested for apoptosis activity and was shown to be able to induce it by the intrinsic mitochondrial pathway. [Ir(tpy)$(X)_3$] complexes are the most active towards MCF-7 and HT-29 cells (IC_{50} < 0.33 μM), and the X = Cl derivative is able to induce apoptosis, although it also causes some necrotic activity,[43a] whereas [Ir(N–N)$(X)_3$(L)] does not. The case of *fac*/*mer*-**28h** and *fac*-**29h** is particularly interesting, as both of these derivatives are highly cytotoxic towards MCF-7 and HT-29 cells (submicromolar) and display selectivity towards these cell lines in comparison to normal HFF-1 and immortalized HEK-293 cells. The cytotoxicity of these two compounds is related to their methylated ligand, which also shows activity on its own. Furthermore, **28h** and **29h** can induce apoptosis by oxidative stress, as observed by the high level of reactive oxygen species in Jurkat leukemia cells.[44] The Ir derivative **29h** is less potent than the Rh analogue **28h**, but it is also selective towards malignant leukemia (Jurkat) and lymphoma (BJAB) cells as compared to ex vivo healthy leukocytes. This improved selectivity and lower cytotoxicity in healthy cells could be an advantage for this potential class of anticancer agents.

Complexes **30a**–**g** have been synthesized from *mer*,*cis*-[Rh$(Cl)_3$(DMSO-κ*S*)$_2$(DMSO-κ*O*)] in a two-step reaction with the N–N ligand and the tripodal ligand tris(pyrazolyl)-methane (tpm). Complexes **30c** and **30d** are potently cytotoxic towards MCF-7 and HT-29 cells with IC_{50} values of 4.0 and 6.7 μM, respectively, in the case of the dpq derivative and 0.43 and 0.37 μM, respectively, in the case of the dppz complex.[43c] The phen derivative **30b** is mildly active, while the others are all inactive. Complex **30d** interacts strongly with CT DNA and is able to induce photocleavage when irradiated with 311 nm light in the presence of plasmid pBR322. The bpy analogue has less interaction with CT DNA, but it can totally convert the plasmid into its nicked form in the presence of light irradiation. No effects of the two corresponding Rh complexes were observed on plasmid pBR322 in the dark.[43c]

The IC_{50} values of compounds **30a**–**g** correlate well with their hydrophobicity. Compound **30c** shows high levels of cell accumulation. Values of 24.0 and 11.8 ng Rh per mg cell protein are obtained in MCF-7 and HT-29 cells, respectively, after 6 h of incubation. This represents a 10-fold increase in Rh accumulation compared to that of the arene analogue [(η^5-C_5Me_5)Ir(Cl)(dpq)]$^+$.

The dependence of the IC_{50} value on the size of the N–N ligand is less marked when the tpm ligand is substituted by the cyclic thioether ligand, [9]aneS_3, as in complexes **31a**–**g**. These have good IC_{50} values, and **31b** shows the highest values, 36 μM for MCF-7 cells and 72 μM for HT-29 cells. The other derivatives display values in the range 4–20 μM. For this family of compounds, the anticancer activity within the series phen → dppz is consistent with the size of the N–N ligand, but also bpy, bpm, and tap have good cytotoxicity. Complex **31a** has nuclease activity under light irradiation, while **31c** already converts supercoiled DNA to nicked DNA in the dark (although moderately).[43d]

The cellular uptake in the case of **31** (**a**, **g**, **f**) is lower than that for **28** and **30**, and no correlation with cytotoxicity could be made. The bpm and dppz complexes were employed for cell mechanism studies. Both induce apoptosis in BJAB cells by the mitochondrial pathway, causing only negligible necrosis.

Comparing complexes bearing η^5-C_5Me_5, tpm, or [9]-aneS_3 ligands, the authors could establish a clear structure–activity relationship in the case of the subseries phen-dpq-dppz, finding that the activity follows the order η^5-C_5Me_5 < [9]aneS_3 < tpm. On the contrary, in the case of the bpy complex, tpm induces much less activity than [9]aneS_3. Also the trichlorido complexes [Rh(tpm)Cl_3] and [Rh([9]aneNS_2)-Cl_3] are known for their cytotoxicity towards HCV29T cells, while the pure tpm and [9]aneS_3 ligands are not active.

Recently, Sheldrick and co-workers[45] have extended the work to new Rh polypyridyl complexes by investigating the cytotoxicity of [Rh(N–N)$(Me_2NCS)_2$]$^+$ (**32**, where N–N = bpy, phen, 5,6-Me_2phen, dpq, dppz) and [Rh(N–N)(2-S-py)$_2$]$^+$ (**33**, where 2-S-py = pyridine-2-thiolate and N–N = dpq, dppz) to MCF-7 and HT-29 cells. In HT-29, **32d** and **33d** have similar IC_{50} regardless of the S ligand employed (ca. 0.055 μM), while the dpq analogue of **33** is much more active (0.078 μM) than that of **32** (0.285 μM). Cellular uptake of these two complexes is very high compared to that of other established metallodrugs, and it has been demonstrated that **33d** is able to induce apoptosis by involving the mitochondrial pathway, as demonstrated by cellular oxygen consumption tests and detection of a high level of reactive oxygen species.

Gold Polypyridyl Complexes

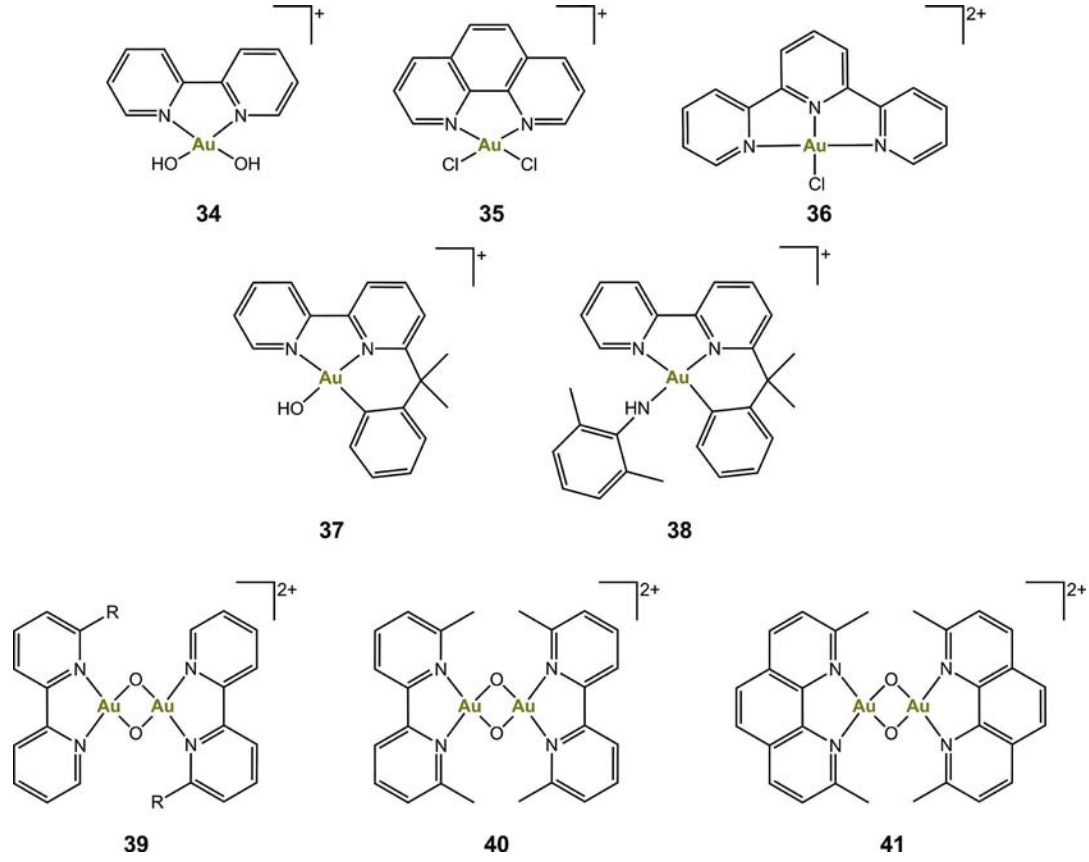
Initially, gold complexes attracted great interest for their cytotoxicity in vitro; however, animal studies demonstrated that these early derivatives were less effective in vivo than expected. The low stability of these first gold complexes in solution can account for their failure in vivo, which is likely to be due to their binding to serum proteins and subsequent inactivation. In addition, high system toxicity was another reason for abandoning gold compounds.[46]

More recently, new classes of Au compounds[47] with improved stability have been synthesized and tested for their biological properties. Such derivatives were found to display promising anticancer properties, both in terms of potency and selectivity. Among others, the Au^{III} polypyridyl complexes (Scheme 13) studied in detail by Casini, Messori et al. were found to be extremely active towards a number of cell lines.[46,48]

Complexes **34**–**41** have IC_{50} values in the low μM range, some of them are cytotoxic even at nM concentrations. It has been shown that these derivatives are also active towards the cisplatin-resistant A2780 cell line. This result, together with DNA-binding studies performed by different techniques, suggests that Au^{III} polypyridyl complexes have a different mechanism of action from that of cisplatin and that DNA is not their major target. As further confirmation, cell cycle studies showed that Au drugs affect the cell cycle very little, in contrast to cisplatin, demonstrating that these agents cannot disrupt DNA functions efficiently.

Observations on the cytotoxicity mechanism suggest that proteins are the preferred targets of Au systems. The preference of Au complexes for protein binding can be easily explained by the fact that both Au^{I} and Au^{III} can bind to thiols and imidazole groups in amino acids and proteins. In particular, proteins involved in mitochondrial functions are likely to be targeted by this class of compounds. Selectivity for mitochondria is responsible for the observed apoptosis of cells treated with Au complexes.

COMPARE analysis[49] was performed on Au polypyridyl complexes **34**–**41** (among others) for thirty-six cell lines. This analysis confirmed that the mechanism is DNA-independent and differs from that of cisplatin. Also, the test indicated that Au derivatives target several biochemical systems, for example, histone deacetylase, protein kinase C/staurosporine and cyclin dependent kinases. Different compounds possibly give diverse mechanisms. However, **40** and **41** showed superior and excellent antiproliferative properties.[48,50] For example, complex **41** is selective towards lung cancer, prostate, and ovarian cell lines, but less so towards renal cells. This derivative exhibits a mean IC_{50} value of 0.032 μM and a mean IC_{70} of 0.22 μM. Solution studies indicate that the dimeric structure might be lost after 6 h in buffer. On the basis of the COMPARE analysis, **41** might be targeting the histone deacetylase since it acts like known



Scheme 13. Schematic structural representation of complexes **34**–**41**. Reprinted with permission from ref.[48] Copyright 2009 Springer.

inhibitors. Also superoxide dismutase (SOD) appears to be a possible target. Metalation of this enzyme is preferred in the case of competitive studies with ubiquitin and cytochrome c.

Except **38**, all the derivatives reported in Scheme 12 are stable in solution, and only minor changes in their absorption profile is observed over 24 h at 37 °C in phosphate buffer (pH = 7.4). Such changes correspond to hydrolysis of the monodentate ligands and formation of aqua/hydroxido species. No redox reaction occurs at the Au^{III} center. In contrast, complex **38** readily loses a xylidine ligand, although also in this case no redox reactions are observed.[51] Dissociation of monodentate ligands can be responsible for activation of **34**–**36**, while in the case of the oxo-bridged **39**–**41**, reduction to Au^{I} seems to be more likely. Cooperation of the two modes of activation is possible. Interestingly, a correlation is found between the redox potentials and cytotoxicity. It is reported that all dinuclear complexes **39**–**41** are reduced in solution by glutathione and ascorbic acid.

On the basis of the knowledge acquired on the antiarthritic gold drug auranofin, a proposed mechanism of action for Au^{III} prodrugs in mitochondria involves the selenoenzyme thioredoxin reductase (TrxR).[52] The selenocysteine in the active site of TrxR[53] has a high affinity for the soft Au^{I}. Many of the Au^{III} derivatives in Scheme 12 are efficient inhibitors of the cytosolic form of TrxR (TrxR1) with IC_{50} values in the nM to μM range. The most efficient inhibitors appear to be the ones that bear a Cl or a monodentate ligand, probably because they can easily release a ligand or undergo reduction. It was observed that Au^{I} derivatives tend to be more active than those of Au^{III} and that the cytosolic isoforms of the enzyme are more responsive than the mitochondria TrxRs. The softness of the metal center is a key parameter; in fact, Au^{I} has higher affinity for Se than Au^{III}. Moreover, the release of a ligand from the metal is important, since Au^{I} derivatives can form Au–Se bonds easily and have increased cytotoxicity. Attack on the selenol group of TrxR is confirmed by the drop in cytotoxicity when the mutant having a cysteine instead of the selenocysteine is used.

Binding to TrxR has been extensively studied at a molecular level by using MALDI-TOF spectrometry and biochemical assays.[52] Messori and co-workers found that Au complexes can metalate TrxR at a number of sites. In particular, strong binding of Au^{I} complexes seems to occur favorably, while in the case of Au^{III} oxidation of the selenol/cysteine group prevails. Au^{III} has a lower selectivity, and it is plausible that other mechanisms, such as oxidative stress, are also operative. The dimeric complex **41** reacts with the synthetic peptide Ac-Gly-[Cys-Sec]-Gly-NH_2, which models the C-terminal motifs of TrxR.[54] ESI-MS data indicate that Au^{I}_2–tetrapeptide species are formed, which confirms that Au^{III} prodrugs are activated through reduction.

Finally, the same group showed that **34** and **35** are extremely good inhibitors of poly(ADP-ribose) polymerases (PARPs),[55] key enzymes in DNA repair and cancer resistance to chemotherapeutic agents. PARPs are characterized by two Zn fingers, which are important in the DNA recognition process. Au derivatives can compete with Zn and can be coordinated by three cysteine and one histidine residue. Compared to cisplatin and other Ru anticancer agents, the Au derivatives are definitely more active (ca. 10 nM vs. ca 10 μM).

Concluding Remarks and Outlook

Metal polypyridyl complexes are characterized by high structural versatility due to the different coordination modes of transition metals and to the wide range of diimine ligands commercially and synthetically accessible. In principle, such flexibility can be exploited to obtain a variety of shapes and physicochemical properties (e.g. hydrophobicity) and reactivities, which can be tuned to improve the effectiveness of this class of compounds in chemotherapy. In this sense, the synthetic strategies developed by Meggers and co-workers can considerably advance the role of polypyridyl complexes in chemical biology and medicinal inorganic chemistry. Moreover, biofunctionalization of the diimine ligand provides further possibilities for improvement by employing drug delivery techniques.[56]

Pharmacologically, the chemical stability (but not necessarily inertness) of metal polypyridyl complexes in aqueous solution is an advantage. It facilitates the investigation of the cytotoxicity mechanism under physiological conditions, and the fate of polypyridyl complexes in biological systems can be more easily determined than those of less stable systems. In the case of the described Au^{III} complexes, the stability acquired by coordination to diimine ligands can be associated to the promising properties of these derivatives. They can reach mitochondria where they can be activated by hydrolysis/reduction and carry out their cytotoxic action. Conversely, first-generation Au complexes were too reactive, hence undergoing biochemical reactions responsible for complex speciation and severe toxic effects. Notably, combination of these features with the unique photophysical and photochemical properties of polypyridyl complexes is envisaged to promote new advances in the field of medicinal inorganic chemistry.

Recent impressive progress in the field of cellular optical imaging has been achieved by using several transition metal diimine complexes.[57] In addition, encouraged by the success of photodynamic therapy in the treatment of superficial cancers and diseases, photoactivation of metal complexes has increasingly been investigated as an activation mode[58] to promote the interaction of inert complexes with target biomolecules (e.g. DNA bases)[59] or for releasing bioactive molecules (e.g. CO).[60] The aim of such an approach is to provide spatial and temporal control of the biological effects of the therapeutic agent. For example, promising photocytotoxic Pt[61] and Rh polypyridyl[62] systems have been reported in the last few years, and the field has been recently reviewed by us[63] and by Schatzschneider[64] in this journal.

Acknowledgments

The author is grateful to European Research Council (ERC) BIOINCMED (Prof. Peter J. Sadler) and Marie Curie Intraeuropean Fellowship (220281 PHOTORUACD) for financial support and to Prof. Peter J. Sadler for fruitful discussions. Many thanks also to Dr. A. Habtemariam, Dr. A. M. Pizarro, Dr. C. Sanchez-Cano, Dr. C. Garino, T. Ruiu, and Isolda Romero for their helpful comments.

[1] E. Alessio, G. Mestroni, A. Bergamo, G. Sava, *Curr. Top. Med. Chem.* **2004**, *4*, 1525–1535.
[2] C. G. Hartinger, S. Zorbas-Seifried, M. A. Jakupec, B. Kynast, H. Zorbas, B. K. Keppler, *J. Inorg. Biochem.* **2006**, *100*, 891–904.
[3] a) W. H. Ang, P. J. Dyson, *Eur. J. Inorg. Chem.* **2006**, 4003–4018; b) P. C. A. Bruijnincx, P. J. Sadler, *Curr. Opin. Chem. Biol.* **2008**, *12*, 197–206.
[4] G. Jaouen in *Bioorganometallics*, Wiley-VCH, Weinheim, **2006**.
[5] a) G. Gasser, I. Ott, N. Metzler-Nolte, *J. Med. Chem.* **2011**, *54*, 3–25; b) T. Gianferrara, I. Bratsos, E. Alessio, *Dalton Trans.* **2009**, 7588–7598; c) C. G. Hartinger, P. J. Dyson, *Chem. Soc. Rev.* **2009**, *38*, 391–401; d) E. A. Hillard, G. Jaouen, *Organometallics* **2011**, *30*, 20–27; e) A. M. Pizarro, A. Habtemariam, P. J. Sadler, *Med. Organomet. Chem.* **2010**, *32*, 21–56.
[6] a) J. J. Concepcion, M. K. Tsai, J. T. Muckerman, T. J. Meyer, *J. Am. Chem. Soc.* **2010**, *132*, 1545–1557; b) T. P. Yoon, M. A. Ischay, J. N. Du, *Nat. Chem.* **2010**, *2*, 527–532.
[7] K. Kalyanasundaram, M. Grätzel, *Coord. Chem. Rev.* **1998**, *177*, 347–414.
[8] a) M. K. Nazeeruddin, M. Grätzel, *Photofunctional Trans. Met. Compl.* **2007**, *123*, 113–175; b) A. S. Polo, M. K. Itokazu, N. Y. M. Iha, *Coord. Chem. Rev.* **2004**, *248*, 1343–1361.
[9] a) C. Garino, S. Ghiani, R. Gobetto, C. Nervi, L. Salassa, V. Ancarani, P. Neyroz, L. Franklin, J. B. A. Ross, E. Seibert, *Inorg. Chem.* **2005**, *44*, 3875–3879; b) J. R. Lakowicz in *Principles of Fluorescence Spectroscopy*, Kluwer Accademic/Plenum Publishers, New York, **1999**.
[10] a) F. P. Dwyer, E. C. Gyarfas, W. P. Rogers, J. H. Koch, *Nature* **1952**, *170*, 190–191; b) F. P. Dwyer, E. Mayhew, E. M. F. Roe, A. Shulman, *Br. J. Cancer* **1965**, *19*, 195; c) F. P. Dwyer, I. K. Reid, A. Shulman, G. M. Laycock, S. Dixson, *Aust. J. Experim. Biol. Med. Sci.* **1969**, *47*, 203.
[11] a) J. K. Barton, E. D. Olmon, P. A. Sontz, *Coord. Chem. Rev.* **2011**, *255*, 619–634; b) M. J. Hannon, *Chem. Soc. Rev.* **2007**, *36*, 280–295.
[12] a) M. D. Hall, M. Okabe, D. W. Shen, X. J. Liang, M. M. Gottesman, *Ann. Rev. Pharmacol. Toxicol.* **2008**, *48*, 495–535; b) A. V. Klein, T. W. Hambley, *Chem. Rev.* **2009**, *109*, 4911–4920; c) C. A. Puckett, R. J. Ernst, J. K. Barton, *Dalton Trans.* **2010**, *39*, 1159–1170.
[13] a) A. R. Dunn, I. J. Dmochowski, A. M. Bilwes, H. B. Gray, B. R. Crane, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 12420–12425; b) I. J. Dmochowski, B. R. Crane, J. J. Wilker, J. R. Winkler, H. B. Gray, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12987–12990; c) J. J. Wilker, I. J. Dmochowski, J. H. Dawson, J. R. Winkler, H. B. Gray, *Angew. Chem.* **1999**, *111*, 93; *Angew. Chem. Int. Ed.* **1999**, *38*, 89–92.
[14] a) S. M. Contakes, G. A. Juda, D. B. Langley, N. W. Halpern-Manners, A. P. Duff, A. R. Dunn, H. B. Gray, D. M. Dooley, J. M. Guss, H. C. Freeman, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 13451–13456; b) E. C. Glazer, Y. H. L. Nguyen, H. B. Gray, D. B. Goodin, *Angew. Chem.* **2008**, *120*, 912; *Angew. Chem. Int. Ed.* **2008**, *47*, 898–901; c) D. B. Langley, D. E. Brown, L. E. Cheruzel, S. M. Contakes, A. P. Duff, K. M. Hilmer, D. M. Dooley, H. B. Gray, J. M. Guss, H. C. Freeman, *J. Am. Chem. Soc.* **2008**, *130*, 8069–8078.
[15] F. P. Guengerich, *Cytochrome P450: Structure, Mechanism and Biochemistry* (Ed.: P. R. Ortiz de Montellano), Plenum, New York, **1995**, pp. 473–536.
[16] a) F. P. Guengerich, *Mutat. Res.-Rev. Mutat. Res.* **2001**, *488*, 195–209; b) F. P. Guengerich, *Chem. Res. Toxicol.* **2008**, *21*, 70–83; c) D. C. Lamb, M. R. Waterman, S. L. Kelly, F. P. Guengerich, *Curr. Opin. Biotechnol.* **2007**, *18*, 504–512.
[17] a) S. Jalkanen, M. Salmi, *Embo J.* **2001**, *20*, 3893–3901; b) F. Marttila-Ichihara, K. Castermans, K. Auvinen, M. Egbrink, S. Jalkanen, A. W. Griffioen, M. Salmi, *J. Immunol.* **2010**, *184*, 3164–3173; c) M. Salmi, S. Jalkanen, *Trends Immunol.* **2001**, *22*, 211–216.
[18] J. Baselga, *Science* **2006**, *312*, 1175–1178.
[19] a) E. Meggers, *Chem. Commun.* **2009**, 1001–1010; b) S. P. Mulcahy, E. Meggers, *Med. Organomet. Chem.* **2010**, *32*, 141–153.
[20] S. P. Mulcahy, S. Li, R. Korn, X. L. Xie, E. Meggers, *Inorg. Chem.* **2008**, *47*, 5030–5032.
[21] S. P. Mulcahy, K. Grundler, C. Frias, L. Wagner, A. Prokop, E. Meggers, *Dalton Trans.* **2010**, *39*, 8177–8182.
[22] J. Maksimoska, L. Feng, K. Harms, C. L. Yi, J. Kissil, R. Marmorstein, E. Meggers, *J. Am. Chem. Soc.* **2008**, *130*, 15764–15765.
[23] a) L. Gong, S. P. Mulcahy, D. Devarajan, K. Harms, G. Frenking, E. Meggers, *Inorg. Chem.* **2010**, *49*, 7692–7699; b) Z. J. Lin, L. Gong, M. A. Celik, K. Harms, G. Frenking, E. Meggers, *Chem. Asian J.* **2011**, *6*, 474–481; c) E. Meggers, *Chem. Eur. J.* **2010**, *16*, 752–758.
[24] O. Novakova, J. Kasparkova, O. Vrana, P. M. van Vliet, J. Reedijk, V. Brabec, *Biochemistry* **1995**, *34*, 12369–12378.
[25] U. Schatzschneider, J. Niesel, I. Ott, R. Gust, H. Alborzinia, S. Wolfl, *ChemMedChem* **2008**, *3*, 1104–1109.
[26] O. Zava, S. M. Zakeeruddin, C. Danelon, H. Vogel, M. Grätzel, P. J. Dyson, *ChemBioChem* **2009**, *10*, 1796–1800.
[27] C. Tan, S. Lai, S. Wu, S. Hu, L. Zhou, Y. Chen, M. Wang, Y. Zhu, W. Lian, W. Peng, L. Ji, A. Xu, *J. Med. Chem.* **2010**, *53*, 7613–7624.
[28] K. H. Maclean, F. C. Dorsey, J. L. Cleveland, M. B. Kastan, *J. Clin. Invest.* **2008**, *118*, 79–88.
[29] T. Chen, W.-J. Mei, Y.-S. Wong, J. Liu, Y. Liu, H.-S. Xie, W.-J. Zheng, *MedChemComm.* **2010**, *1*, 73–75.
[30] M. R. Gill, H. Derrat, C. G. W. Smythe, G. Battaglia, J. A. Thomas, *ChemBioChem* **2011**, *12*, 877–880.
[31] D. A. Lutterman, A. Chouai, Y. Liu, Y. Sun, C. D. Stewart, K. R. Dunbar, C. Turro, *J. Am. Chem. Soc.* **2008**, *130*, 1163–1170.
[32] P.-K. Lee, H.-W. Liu, S.-M. Yiu, M.-W. Louie, K. K.-W. Lo, *Dalton Trans.* **2011**, *40*, 2180–2189.
[33] U. McDonnell, J. Kerchoffs, R. P. M. Castineiras, M. R. Hicks, A. C. G. Hotze, M. J. Hannon, A. Rodger, *Dalton Trans.* **2008**, 667–675.
[34] a) A. C. G. Hotze, B. M. Kariuki, M. J. Hannon, *Angew. Chem.* **2006**, *118*, 4957; *Angew. Chem. Int. Ed.* **2006**, *45*, 4839–4842; b) G. I. Pascu, A. C. G. Hotze, C. Sanchez-Cano, B. M. Kariuki, M. J. Hannon, *Angew. Chem.* **2007**, *119*, 4452; *Angew. Chem. Int. Ed.* **2007**, *46*, 4374–4378.
[35] E. Corral, A. C. G. Hotze, H. den Dulk, A. Leczkowska, A. Rodger, M. J. Hannon, J. Reedijk, *J. Biol. Inorg. Chem.* **2009**, *14*, 439–448.
[36] M. J. Pisani, P. D. Fromm, Y. Mulyana, R. J. Clarke, H. Koerner, K. Heimann, J. G. Collins, F. R. Keene, *ChemMedChem* **2011**, *6*, 848–858.
[37] F. Li, Y. Mulyana, M. Feterl, J. M. Warner, J. G. Collins, F. R. Keene, *Dalton Trans.* **2011**, *40*, 5032–5038.
[38] a) Y. Mulyana, D. K. Weber, D. P. Buck, C. A. Motti, J. G. Collins, F. R. Keene, *Dalton Trans.* **2011**, *40*, 1510–1523; b) C. B. Spillane, J. A. Smith, D. P. Buck, J. G. Collins, F. R. Keene, *Dalton Trans.* **2007**, 5290–5296.
[39] T. W. Hambley, *Aust. J. Chem.* **2008**, *61*, 647–653.
[40] T. K. Janaratne, A. Yadav, F. Ongeri, F. M. MacDonnell, *Inorg. Chem.* **2007**, *46*, 3420–3422.
[41] N. R. de Tacconi, R. O. Lezna, R. Konduri, F. Ongeri, K. Rajeshwar, F. M. MacDonnell, *Chem. Eur. J.* **2005**, *11*, 4327–4339.

[42] A. M. Angeles-Boza, H. T. Chifotides, J. D. Aguirre, A. Chouai, P. K. L. Fu, K. R. Dunbar, C. Turro, *J. Med. Chem.* **2006**, *49*, 6841–6847.
[43] a) M. Dobroschke, Y. Geldmacher, I. Ott, M. Harlos, L. Kater, L. Wagner, R. Gust, W. S. Sheldrick, A. Prokop, *ChemMedChem* **2009**, *4*, 177–187; b) M. Harlos, I. Ott, R. Gust, H. Alborzinia, S. Woelfl, A. Kromm, W. S. Sheldrick, *J. Med. Chem.* **2008**, *51*, 3924–3933; c) R. Bieda, I. Ott, R. Gust, W. S. Sheldrick, *Eur. J. Inorg. Chem.* **2009**, 3821–3831; d) R. Bieda, I. Ott, M. Dobroschke, A. Prokop, R. Gust, W. S. Sheldrick, *J. Inorg. Biochem.* **2009**, *103*, 698–708.
[44] Y. Geldmacher, I. Kitanovic, H. Alborzinia, K. Bergerhoff, R. Rubbiani, P. Wefelmeier, A. Prokop, R. Gust, I. Ott, S. Woelfl, W. S. Sheldrick, *ChemMedChem* **2011**, *6*, 429–439.
[45] F. Hackenberg, L. Oehninger, H. Alborzinia, S. Can, I. Kitanovic, Y. Geldmacher, M. Kokoschka, S. Woelfl, I. Ott, W. S. Sheldrick, *J. Inorg. Biochem.* **2011**, *105*, 991–999.
[46] a) A. Casini, C. Hartinger, C. Gabbiani, E. Mini, P. J. Dyson, B. K. Keppler, L. Messori, *J. Inorg. Biochem.* **2008**, *102*, 564–575; b) S. Nobili, E. Mini, I. Landini, C. Gabbiani, A. Casini, L. Messori, *Med. Res. Rev.* **2010**, *30*, 550–580.
[47] a) D. Fregona, L. Ronconi, D. Aldinucci, *Drug Discovery Today* **2009**, *14*, 1075–1076; b) L. Ronconi, D. Fregona, *Dalton Trans.* **2009**, 10670–10680.
[48] A. Casini, G. Kelter, C. Gabbiani, M. A. Cinellu, G. Minghetti, D. Fregona, H. H. Fiebig, L. Messori, *J. Biol. Inorg. Chem.* **2009**, *14*, 1139–1149.
[49] D. W. Zaharevitz, S. L. Holbeck, C. Bowerman, P. A. Svetlik, *J. Mol. Graphics* **2002**, *20*, 297–303.
[50] M. A. Cinellu, L. Maiore, M. Manassero, A. Casini, M. Arca, H.-H. Fiebig, G. Kelter, E. Michelucci, G. Pieraccini, C. Gabbiani, L. Messori, *ACS Med. Chem. Lett.* **2010**, *1*, 336–339.
[51] L. Messori, G. Marcon, M. A. Cinellu, M. Coronnello, E. Mini, C. Gabbiani, P. Orioli, *Bioorg. Med. Chem.* **2004**, *12*, 6039–6043.
[52] A. Bindoli, M. P. Rigobello, G. Scutari, C. Gabbiani, A. Casini, L. Messori, *Coord. Chem. Rev.* **2009**, *253*, 1692–1707.
[53] K. Fritz-Wolf, S. Urig, K. Becker, *J. Mol. Biol.* **2007**, *370*, 116–127.
[54] A. Pratesi, C. Gabbiani, M. Ginanneschi, L. Messori, *Chem. Commun.* **2010**, *46*, 7001–7003.
[55] F. Mendes, M. Groessl, A. A. Nazarov, Y. O. Tsybin, G. Sava, I. Santos, P. J. Dyson, A. Casini, *J. Med. Chem.* **2011**, *54*, 2196–2206.
[56] a) M. Gottschaldt, U. S. Schubert , S. Rau, S. Yano, J. G. Vos, T. Kroll, J. Clement, I. Hilger, *ChemBioChem* **2010**, *11*, 649–652; b) K. K.-W. Lo, K. H.-K. Tsang, K.-S. Sze, C.-K. Chung, T. K.-M. Lee, K. Y. Zhang, W.-K. Hui, C.-K. Li, J. S.-Y. Lau, D. C.-M. Ng, N. Zhu, *Coord. Chem. Rev.* **2007**, *251*, 2292–2310; c) C. Sanchez-Cano, M. J. Hannon, *Dalton Trans.* **2009**, 10702–10711.
[57] a) V. Fernandez-Moreira, F. L. Thorp-Greenwood, M. P. Coogan, *Chem. Commun.* **2010**, *46*, 186–202; b) M. R. Gill, J. Garcia-Lara, S. J. Foster, C. Smythe, G. Battaglia, J. A. Thomas, *Nat. Chem.* **2009**, *1*, 662–667; c) X. H. Tian, M. R. Gill, I. Canton, J. A. Thomas, G. Battaglia, *ChemBioChem* **2011**, *12*, 548–551.
[58] a) L. Cubo, A. M. Pizarro, A. G. Quiroga, L. Salassa, C. Navarro-Ranninger, P. J. Sadler, *J. Inorg. Biochem.* **2010**, *104*, 909–918; b) F. S. Mackay, N. J. Farrer, L. Salassa, H. C. Tai, R. J. Deeth, S. A. Moggach, P. A. Wood, S. Parsons, P. J. Sadler, *Dalton Trans.* **2009**, 2315–2325; c) L. Salassa, C. Garino, G. Salassa, C. Nervi, R. Gobetto, C. Lamberti, D. Gianolio, R. Bizzarri, P. J. Sadler, *Inorg. Chem.* **2009**, *48*, 1469–1481.
[59] a) S. Betanzos-Lara, L. Salassa, A. Habtemariam, P. J. Sadler, *Chem. Commun.* **2009**, 6622–6624; b) L. Salassa, T. Ruiu, C. Garino, A. M. Pizarro, F. Bardelli, D. Gianolio, A. Westendorf, P. J. Bednarski, C. Lamberti, R. Gobetto, P. J. Sadler, *Organometallics* **2010**, *29*, 6703–6710.
[60] a) J. Niesel, A. Pinto, H. W. P. N′Dongo, K. Merz, I. Ott, R. Gust, U. Schatzschneider, *Chem. Commun.* **2008**, 1798–1800; b) U. Schatzschneider, *Inorg. Chim. Acta* **2011**, *374*, 19–23.
[61] a) N. J. Farrer, J. A. Woods, L. Salassa, Y. Zhao, K. S. Robinson, G. Clarkson, F. S. Mackay, P. J. Sadler, *Angew. Chem. Int. Ed.* **2010**, *49*, 8905–8908; b) F. S. Mackay, J. A. Woods, P. Heringova, J. Kasparkova, A. M. Pizarro, S. A. Moggach, S. Parsons, V. Brabec, P. J. Sadler, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 20743–20748; c) A. F. Westendorf, L. Zerzankova, L. Salassa, P. J. Sadler, V. Brabec, P. J. Bednarski, *J. Inorg. Biochem.* **2011**, *105*, 652–662.
[62] L. E. Joyce, J. D. Aguirre, A. M. Angeles-Boza, A. Chouai, P. K. L. Fu, K. R. Dunbar, C. Turro, *Inorg. Chem.* **2010**, *49*, 5371–5376.
[63] N. J. Farrer, L. Salassa, P. J. Sadler, *Dalton Trans.* **2009**, 10690–10701.
[64] U. Schatzschneider, *Eur. J. Inorg. Chem.* **2010**, 1451–1467.

Received: April 7, 2011
Published Online: October 6, 2011

