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Development of anticancer agents: wizardry with osmium

Muhammad Hanif, Maria V. Babak and Christian G. Hartinger

School of Chemical Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

Platinum compounds are one of the pillars of modern cancer chemotherapy. The apparent disadvantages of existing chemotherapeutics have led to the development of novel anticancer agents with alternative modes of action. Many complexes of the heavy metal osmium (Os) are potent growth inhibitors of human cancer cells and are active *in vivo*, often superior or comparable to cisplatin, as the benchmark metal-based anticancer agent, or clinically tested ruthenium (Ru) drug candidates. Depending on the choice of ligand system, osmium compounds exhibit diverse modes of action, including redox activation, DNA targeting or inhibition of protein kinases. In this review, we highlight recent advances in the development of osmium anticancer drug candidates and discuss their cellular mechanisms of action.

Introduction

The field of inorganic medicinal chemistry has boomed in recent years following the hallmark discovery of the anticancer activity of DNA-targeting cisplatin and later its analogs carboplatin and oxaliplatin. Nowadays, these platinum-based drugs are routinely used in approximately 50% of cancer therapy regimens [1]. However, similar to many other chemotherapeutics, their therapeutic efficacy is limited by the emergence of drug resistance and severe adverse effects [2]. This has provided great impetus for investigations of metal complexes with higher selectivity and enhanced efficacy, thus creating a new paradigm in the field of anticancer drug discovery. Consequently, the antitumor properties of different metal complexes based on gold, gallium, titanium, arsenic, iron, ruthenium and osmium have been evaluated [3–5]. These metal ions display different chemical properties in terms of oxidation state, redox potential, rate of ligand exchange and binding preferences to biomolecules. Prominent examples of nonplatinum anticancer complexes are ruthenium and gallium coordination compounds and titanocenes [3,6], all of which have been evaluated in clinical trials. Moreover, arsenic trioxide (Trisenox®) was approved by the US Food and Drug Administration (FDA) in 2000 for the treatment of relapsed acute promyelocytic leukemia [7].

Corresponding authors: Hanif, M. (m.hanif@auckland.ac.nz), Hartinger, C.G. (c.hartinger@auckland.ac.nz)

The group-8 metals iron, ruthenium and osmium, have found particular interest in metal-based anticancer drug development. The most promising iron compounds are the ferrocenyl derivatives of tamoxifen, which target hormone receptors in breast cancer cells [8,9]. Ruthenium is represented by several biologically active compounds and imidazolium *trans*-[tetrachlorido(dimethylsulfoxide)(1*H*-imidazole)ruthenate(III)] (NAMI-A), indazolium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)] (KP1019) and sodium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)] (KP1339) (Fig. 1) have been tested in clinical trials [10]. In preclinical tests, NAMI-A showed strong efficacy toward solid tumor metastases, whereas the indazole complexes KP1019 and KP1339 demonstrated excellent activity in several primary tumor models. Ru(III) complexes are considered as prodrugs that can be activated by reduction to Ru(II) species *in vivo* and binding to plasma proteins is considered important in their modes of action. Ruthenium complexes in oxidation state 2+ are more labile and can bind more rapidly to biomolecules [10]. Organometallic half-sandwich Ru(II)-arene complexes have also revealed their potential as tumor-inhibiting agents. Two representative examples of these organometallic compounds are [Ru(η^6-*p*-cymene)(PTA)Cl_2] PTA = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (RAPTA-C) and [Ru(η^6-biphenyl)Ru(en)Cl]$^+$ (RM175; en = ethylenediamine) [6,11]. Whereas the ethylenediamine complex displayed *in vitro* anticancer activity similar to that of cisplatin and was more active in cisplatin-resistant *in vivo* models,

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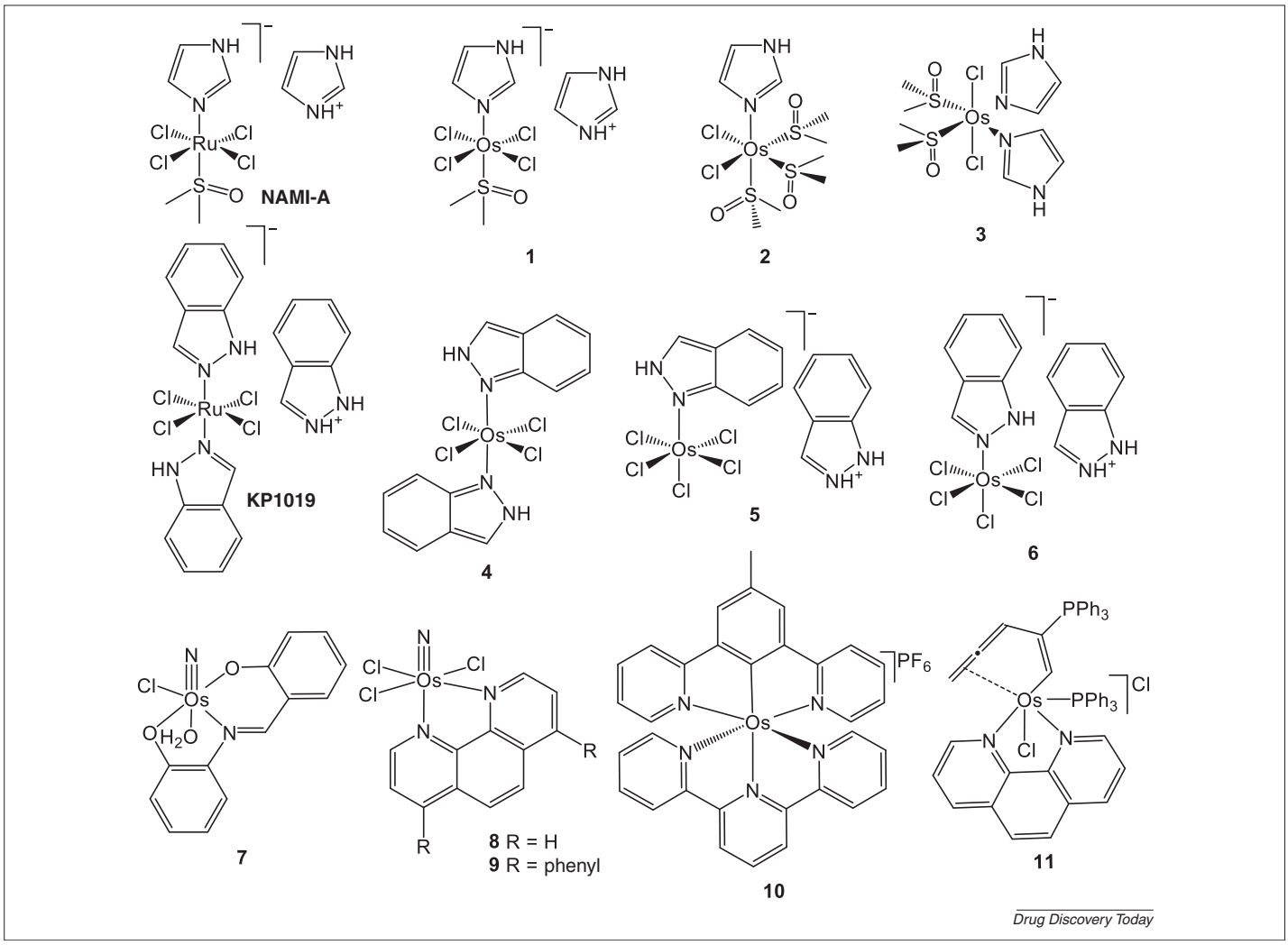

FIGURE 1

Structures of the osmium analogs of the ruthenium-based clinically tested drug candidates imidazolium *trans*-[tetrachlorido(dimethylsulfoxide)(1*H*-imidazole)ruthenate(III)] (NAMI-A; **1–3**) indazolium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)] (KP1019; **4–6**), and of osmium nitrido (**7–9**) and cycloosmate (**10**,**11**) complexes with anticancer activity.

the RAPTA complexes inhibited metastasis *in vivo*, while they were inactive *in vitro* [5,11].

Despite extensive research devoted to iron and ruthenium complexes during the past two decades, studies on biologically active osmium complexes are scarce. However, osmium offers several features distinct from ruthenium, including the preference for higher oxidation states, slower ligand exchange kinetics, stronger π-back-donation from lower oxidation states and markedly stronger spin-orbit coupling. Therefore, osmium complexes are considered interesting alternatives to ruthenium-based anticancer agents because of their relative inertness and sufficient stability under physiological conditions. Various synthetic approaches and ligand systems have been investigated, often to design structural analogs of well-established ruthenium compounds. This resulted in a structurally diverse library of osmium complexes, including mononuclear coordination complexes, multinuclear clusters and organoosmium compounds with a wide variety of chemical and biological properties [12–16]. In this review, we describe different design strategies for recent examples of anticancer osmium complexes and discuss studies on their modes of action.

Osmium azole complexes as NAMI-A and KP1019 analogs

The Ru(III) drug candidates NAMI-A, KP1019 and KP1339 are the most obvious leads to prepare structural analogs based on osmium. Indeed, a diverse series of osmium azole complexes has been reported and their anticancer activity evaluated (Fig. 1). By contrast to the Ru(III) parent compound, NAMI-A-analogous Os(II) and Os(III) complexes are highly resistant toward hydrolysis even in chloride-free solution. Notably, these chemical differences also result in different biological properties. The Os(II) complexes **2** and **3** with more than one dimethyl sulfoxide (DMSO) ligand were inactive; however, the Os(III) analog of NAMI-A **1** displayed significant antiproliferative activity compared with the parent and related compounds [17]. It is known that, in physiological buffer conditions, NAMI-A is quickly hydrolyzed into different ruthenium species that are readily taken up by cells. The pharmacological effect of NAMI-A is usually attributed to these hydrolyzed species. Therefore, the higher *in vitro* cytotoxicity of inert osmium analogs was somewhat surprising. This suggests that hydrolysis is not an essential prerequisite for biological activity of this class of

compounds [18]. Therefore, Os(III) complexes could be used as model compounds for studying the biodistribution of unhydrolyzed NAMI-A and other anticancer-active ruthenium complexes.

To establish structure–activity relationships (SAR) between KP1019 and its osmium analogs, extensive studies on different Os(III) and also Os(IV)-azole-chlorido complexes have been reported. Os(IV) analogs of KP1019 of the general formula $[Os^{IV}Cl_4(Hazole)_2]$, where Hazole is 1*H*-pyrazole, 1*H*/2*H*-indazole, 1*H*-imidazole, and 1*H*-benzimidazole were synthesized. The complexes were stable in aqueous solution and demonstrated the same order of cytotoxicity as KP1019 in human cancer cells [19]. Interestingly, indazole was coordinated to Ru(III) in KP1019 only through *N*2 (1*H*-indazole), whereas in Os(IV) complexes, it was bound either via *N*1 atom in $[Os^{IV}Cl_4(2H\text{-indazole})_2]$ **4** and $(H_2Ind)[Os^{IV}Cl_5(2H\text{-indazole})]$ **5** or *N2* in $(H_2Ind)[Os^{IV}Cl_5(1H\text{-indazole})]$ **6**. The *in vivo* anticancer activity of **5** and **6** was tested on a Hep3B SCID mouse xenotransplantation model. Overall, the complexes were well tolerated and the mice did not display any symptoms of toxicity. It was found that **6** inhibited tumor growth, whereas **5** reduced tumor necrosis and enhanced the survival time of mice [20]. To increase the water solubility of the osmium–azole complexes, the sodium salts of $[Os^{IV}Cl_5(Hazole)]^-$ complex anions were prepared. By contrast to the azolium and sodium salts of $[Ru^{III}Cl_4(Hind)_2]^-$ (KP1019 and KP1339), the sodium salts of osmium compounds were more active than the azolium salts, which might be related to differences in their cellular uptake [21].

Nitric oxide (NO) is known to have key roles in several biological processes as a messenger molecule. With respect to cancer, it is involved in the mediation of tumor-induced angiogenic processes, which is a key step in the formation of metastases. One of the possible explanations of the unusual antimetastatic activity of NAMI-A is its ability to interfere with NO metabolism *in vivo*. This provided the rationale for the preparation of ruthenium complexes of NO, but their osmium analogs are relatively unexplored, although their slower ligand exchange kinetics might allow controlled NO release in the biological system.

Keppler and co-workers designed a novel series of *cis*- and *trans*-configured Ru(NO) and Os(NO) complexes of the general formula (cation)$[MCl_4(NO)(Hazole)]$, where Hazole is 1*H*-pyrazole, 1*H*-indazole, 1*H*-imidazole and 1*H*-benzimidazole, which are structural analogs of NAMI-A [22]. Contrary to the dimethylsulfoxide complexes, the ruthenium series displayed markedly higher cytotoxicity compared with their osmium congeners. This finding might be explained by different lability of the M–NO bond as compared with M–DMSO, resulting in different behavior of the complexes in biological media. For example, whereas Ru(III)–DMSO complexes were reduced by natural reducing agents, such as ascorbic acid, the NO compounds were stable for at least 24 and 72 hours for ruthenium and osmium complexes, respectively. However, Ru–NO complexes underwent hydrolysis of M–Cl, whereas their osmium counterparts remained intact in the presence of ascorbic acid.

High-valent osmium nitrido complexes: from DNA as a target to endoplasmic reticulum stress

Although the anticancer activity of osmium complexes in oxidation states 2+, 3+ and 4+ has been investigated for some time, the therapeutic potential of high-valent Os(VI) compounds remained untapped until recently it was discovered that complexes based on the $Os^{VI}{\equiv}N$ fragment displayed potent *in vitro* and *in vivo* anticancer activity [23–25]. Os^{VI}(nitrido) complexes with azole, quinolinolato and tridentate Schiff-base co-ligands were tested for their potency toward different cancer cell lines. Notably, the IC_{50} values of the most cytotoxic complexes were lower than those of cisplatin. The *in vitro* cytotoxicity of the complexes was in good correlation with their cellular accumulation. The complexes induced cell cycle arrest in S phase and premitotic G2 phase as a result of DNA damage or degradation. The nitridoosmium(VI) complex **7** (Fig. 2) significantly reduced the tumor growth in nude mice without severe adverse effects [26]. These studies demonstrated that osmium(VI) nitrido complexes are promising anticancer agents that might affect DNA as a primary target. Os^{VI}(nitrido) compounds with bidentate lipophilic *N,N*-chelating ligands, such as 1,10-phenanthroline, 3,4,7,8-tetramethyl-1,10-phenantroline or 4,7-diphenyl-1,10-phenantroline, exhibited excellent *in vitro* activity against a panel of human cancer cell lines. These complexes were selective for cancer cells (A2780) over healthy cells (MRC5) and were also active against cisplatin-resistant cell lines [25]. Complex **8** with 1,10-phenanthroline displayed the highest antiproliferative activity and induced conformational changes and the degradation of DNA, possibly involving multiple cell death pathways including p53-dependent cell death. However, **9** containing 4,7-diphenyl-1,10-phenantroline did not cause any DNA damage; instead, it induced profound endoplasmic reticulum stress. In agreement with different intracellular targets of **8** and **9**, their subcellular distribution was markedly different. Whereas **8** was predominantly localized in the cytoplasm, **9** was evenly distributed between the cytoplasm and nucleus. The latter compound is of special interest, because it is capable of inducing cell death independently of the p53 status. It seems to be the first reported example of an Os(VI) complex that does not induce apoptosis through DNA interactions [25].

Notably, complexes bearing the $Os^{VI}{\equiv}N$ fragment feature novel mechanisms of action with promising *in vitro* and *in vivo* properties; thus, they could carve out their niche in anticancer research.

Cytotoxic osmium clusters: microtubule targeting

Another class of osmium compounds with anticancer activity is that of organometallic osmium clusters [12,16]. A series of triosmium carbonyl clusters of the general formula $Os_3(CO)_{12\text{-}n}(L)_n$ and their protonated analogs (L = nitriles, acetonitrile, maltol, triphenyl phosphine, among others) were evaluated for their potency in cancer cells [13,14,27]. Studies included estrogen receptor (ER)-dependent MCF-7 and ER-independent MDA-MB-231 breast cancer cell lines and revealed that the cytotoxicity of the osmium clusters was strongly dependent on their solubility and the ligand type. Compounds having labile ligands (e.g. acetonitrile or maltol) were significantly more active compared with complexes with a nonlabile phosphine ligand. For example, the maltolato cluster exhibited strong antiproliferative activity with an IC_{50} value of 3 μM after only 24 hours incubation with MDA-MB-231 cells [27]. This can be explained by ligand exchange reactions at the metal centers that allow for subsequent binding to biological targets. Cationic clusters showed enhanced activity in comparison with neutral analogs because of the increased

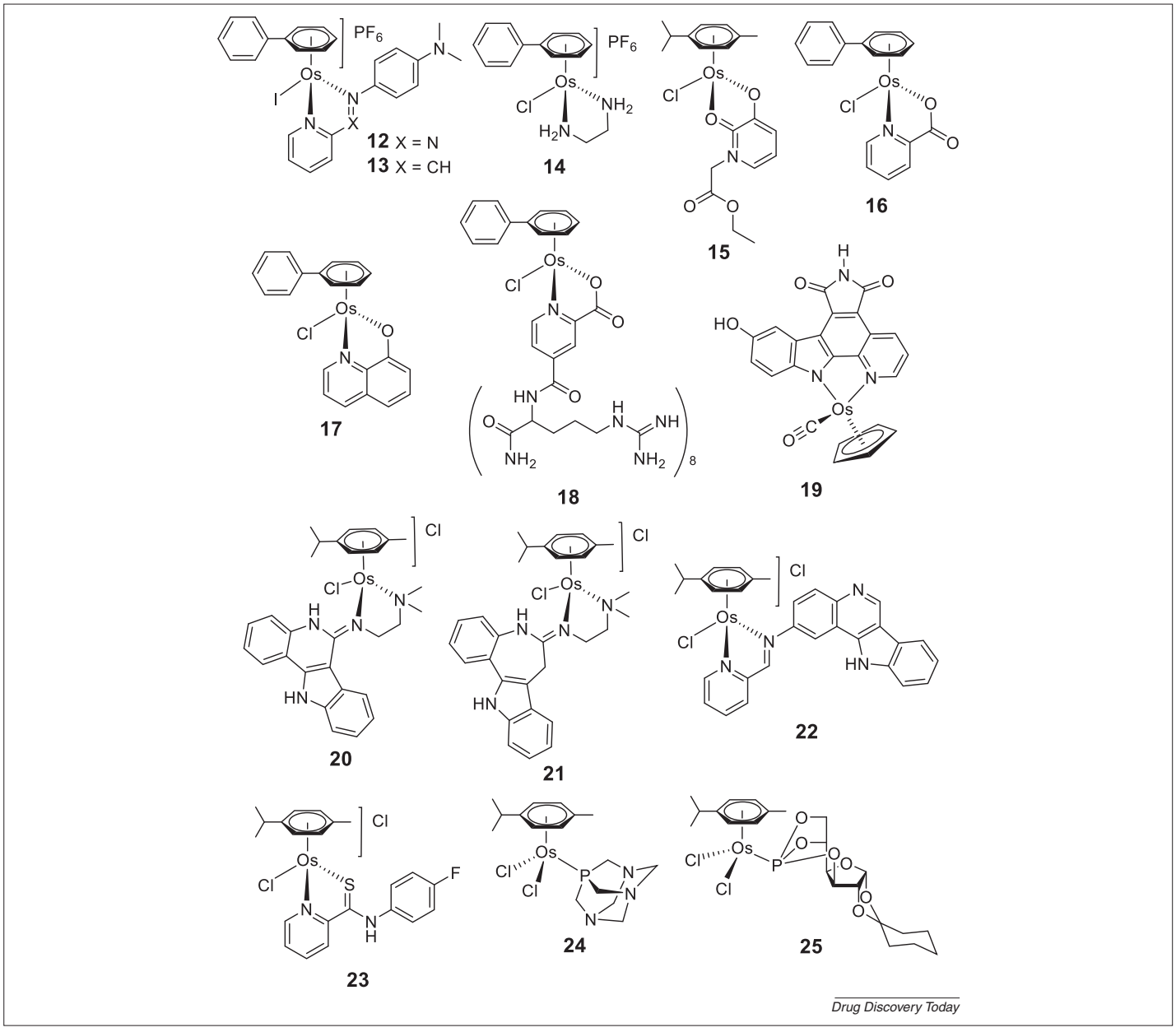

FIGURE 2

Examples of osmium–arene anticancer complexes featuring mono- and bidentate ligand systems determining their modes of action.

solubility and cell permeability. The apoptosis-inducing osmium clusters exhibited cytotoxicity in the low micromolar range and high selectivity for cancerous cells over nontumorigenic breast epithelial cells. Notably, the clusters were more potent against an ER-independent cell line compared with an ER-dependent cell line, which indicates that their modes of action involve biotargets that are different from hormone receptors [13]. In cancer cells, osmium acetonitrile clusters induced cellular events such as chromatin condensation, DNA fragmentations, caspase inhibition and an elevated level of p53. These organometallic clusters caused the disruption of the microtubule morphology revealing tubulin polymerization ability similar to taxol. It is known that proper dynamics of microtubules are required for normal cell functioning and, therefore, their hyperstabilization by osmium clusters leads to cellular dysfunction and apoptosis. At the molecular level, Os clusters interact with intra- and extracellular sulfhydryl groups, which is in agreement with their tubulin disruption ability [14].

Cycloosmate complexes: redox activation at the metal center and apoptosis

Cycloruthenated complexes induce formation of reactive oxygen species (ROS) in cancer cells [28]. Pfeffer and co-workers obtained analogous cycloosmated compounds via an intramolecular CH activation reaction of various *N*-donor ligands. This versatile synthetic route yielded a large library of ligands that acted as *C,N-*, *C,N,N-* or *C,N,C*-chelators toward the osmium center [29]. All complexes displayed good to excellent cytotoxicity toward the

human glioblastoma cell line A172 and the most active complex **10** (Fig. 1) showed IC_{50} values in the high nM range. By contrast to the Os(III) azole complexes [30], excellent correlation between the $Os^{III/II}$ redox potential and the antiproliferative activity was observed. It appeared that compounds that were more lipophilic and stable toward ligand substitution reactions before cellular uptake were the most cytotoxic. In general, compounds lacking leaving groups are believed to be less cytotoxic because of their inertness and lack of binding ability toward molecular targets. Interestingly, in these osmium compounds, the most reactive derivatives toward substitution reactions were the least cytotoxic. Similar observations were made by Meggers *et al.*, who reported structurally unusual osmacyclic complexes. The η^2-allene osmacycle **11** (Fig. 1) displayed promising antiproliferative activity at submicromolar concentration and was remarkably robust to ligand substitution reactions as well as to nucleophilic or electrophilic additions at the terminal allene. The outstanding stability of this complex was associated with the formation of a metallacycle, in which osmium is π-coordinated to an allene moiety and simultaneously σ-linked to a carbon atom of a terminal double bond. The mode of action of **11** was related to the induction of apoptosis through the intrinsic mitochondrial pathway, as evident by the reduction of the mitochondrial membrane potential, and the activation of caspases-9 and -3 in Burkitt-like lymphoma cells [31].

Osmium–arene complexes and redox activity in a biological environment

Redox activity of compounds is often associated with the formation of ROS in cells and, in the case of ruthenium complexes, can lead to activation in the hypoxic environment of solid tumors [32]. Sadler and co-workers reported a library of Os(II) complexes of the general formula $[Os(\eta^6\text{-arene})(XY)Z]^+$ (XY = azopyridine derivatives, Z = Cl or I, arene = *p*-cymene or biphenyl). The bidentate azopyridine (azpy) ligand acts as a σ-donor and a strong π-acceptor; thus, there is a strong back-donation of electrons from the metal center to azpy and this has a pronounced effect on the overall reactivity of the complexes. Azopyridine ligands *per se* are not cytotoxic toward A2780 human ovarian cancer cells up to 100 μM. However, when coordinated to Os^{II}(arene) fragments, they exhibited anticancer activity in the nanomolar range against human ovarian, breast, prostate, lung, colon and bladder cancer cell lines [33]. Notably, $[Os(\eta^6\text{-}p\text{-cymene})(Azpy\text{-}NMe_2)I]PF_6$ (FY026) **12** (Fig. 2) demonstrated at least tenfold higher anticancer potency compared with cisplatin against all tested cancer cell lines. *In vivo* tests showed that **12** delayed the growth of HCT-116 human colon cancer xenografts in mice and no signs of off-target toxicity were observed. Furthermore, the potency of **12** toward A2780 ovarian and A549 lung cancer cells was significantly enhanced in combination with L-buthionine-sulfoximine, which is known to reduce the level of the cellular reductant glutathione [34,35].

SAR analysis revealed that changing the arene from *p*-cymene to biphenyl and the monodentate ligand from chlorido to iodido resulted in a significant increase of anticancer activity. Azopyridine complexes with electron-donating substituents on the phenyl ring (e.g. OH or NMe_2) or electron-withdrawing groups on the pyridine ring (e.g. F, Cl, Br or I) were an order of magnitude more active than their unsubstituted analogs. This might be because of the involvement of redox processes associated with the azo functionality [33] (e.g. by interacting with glutathione [36]). These complexes were markedly stable and appeared to be hydrolytically inert, because they did not undergo the substitution of the halide with a neutral water molecule in aqueous solution. Therefore, in contrast to established platinum anticancer agents, the activation by hydrolysis and subsequent binding to DNA does not seem to be the major mode of action of these compounds. Switching from azopyridine to iminopyridine *N,N′*-chelators resulted in equally potent Os(II) complexes, although they undergo hydrolysis of the halido ligands and subsequently bind to the nucleobase guanine. Active iminopyridine complexes, such as **13** (Fig. 2), induced a remarkable increase in the ROS level in A549 lung cancer cells. Studies on their redox activity revealed that they oxidize NADH to NAD^+. The oxidation of NADH might occur through the formation of an Os–H intermediate in aqueous solution, which causes interference in the redox signaling pathways in cancer cells [37]. Notably, iodido complexes were more active *in vitro* and no cross-resistance with cisplatin or oxaliplatin was detected. They were selective for cancer cells over healthy cells and demonstrated higher accumulation in cell membranes. Their mode of action appeared to be related to cell growth arrest in G1 phase and caspase 3 activation, and their activity was independent of p53 status [38].

DNA-targeting osmium–arene complexes

The organometallic $Ru(\eta^6\text{-biphenyl})(ethylenediamine)Cl$ complex RM175, designed by Sadler *et al.*, preferentially targets DNA [34]. To understand the effect of the metal on the activity and the mode of action of this complex, its osmium analog $[(\eta^6\text{-biphenyl})Os(en)Cl]PF_6$ **14** was prepared. It showed promising antiproliferative activity with IC_{50} values in the low micromolar range against human lung A549 and ovarian A2780 cancer cells [39,40]. Notably, the replacement of the neutral *N,N*-chelator ethylenediamine with anionic *O,O*-chelators yielded osmium–arene complexes with remarkably low cytotoxicity and fast hydrolysis rates. The low activity of osmium complexes with *O,O*-ligands was attributed to their often low stability in aqueous solution and subsequent formation of inactive hydroxy-bridged dimers $[(\eta^6\text{-arene})Os(\mu\text{-}OH)_3Os(\eta^6\text{-arene})]^+$ (Fig. 3) [41]. The stability of the osmium complexes can be influenced by the choice of *O,O*-chelating ligand. For example, pyridones formed more stable complexes (see **15** in Fig. 2) but were still significantly deactivated at biologically relevant micromolar concentrations by the formation of dimeric species [42]. Despite the higher stability of dinuclear osmium complexes in aqueous solutions, they were about three times less active *in vitro* than their ruthenium congeners [43,44]. They showed interesting biological properties in terms of cross-linking capability of DNA duplexes and DNA with proteins [45]. Consequently, Os(II)–arene complexes with anionic *N,O*-chelating ligands were synthesized and were significantly more stable in aqueous media. Os^{II}(picolinato) complex **16** exhibited cytotoxicity similar to carboplatin against human ovarian cancer cells [46–48]. The pharmacophore was also conjugated with cell-penetrating peptides (CPPs) as cell delivery vectors to enhance the cell uptake and efficacy of osmium complexes. Incorporation of polyarginine (Arg) with adjustable chain lengths into Os^{II}(picolinato) complexes resulted in a significantly enhanced cellular and nuclear uptake as well as DNA binding and cytotoxicity. Osmium

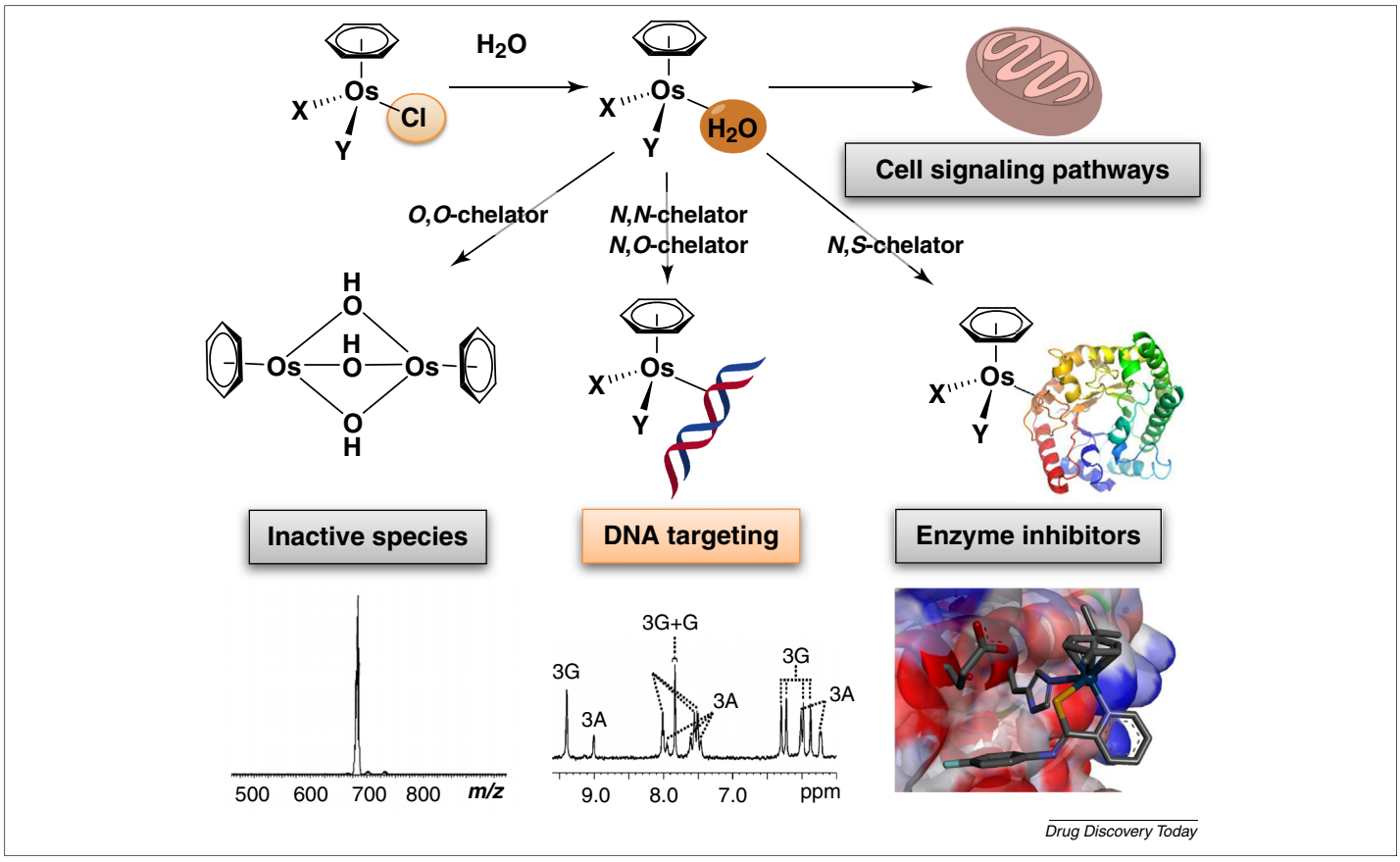

FIGURE 3

Schematic representation of the mechanisms of action of osmium–arene complexes bearing different bidentate chelators and monohalido ligands. Parts of the figure were adapted, with permission, from [39,58,66].

conjugate **18** with octaarginine (Arg8) displayed a tenfold increase in cell uptake and 15-fold enhanced DNA binding, compared with the monoarginine (Arg1) analog, although at the expense of decreased cytotoxicity. This might be attributed to the cationic nature of the peptide that can bind to other cellular targets [49]. In a different approach, the same pharmacophore was functionalized with a dicarba analog of octreotide, a potent somatostatin agonist. Receptors of somatostatin (particularly subtype 2) are overexpressed on the membrane of various tumor cells. Chemically, the novel osmium conjugate behaved similarly to the parent compound **16**, but again its biological activity markedly decreased. In general, the conjugation of osmium fragments with peptides resulted in enhanced biological properties of the peptides, but did not reach the efficacy of the parent osmium compounds [50].

Protein- or enzyme-targeting osmium–arene complexes

Coordination of biologically active molecules to metal centers is an attractive approach to achieve synergistic effects of both components and to endow multitargeting properties to the drug molecules. Coordination of bioactive ligands, such as paullones or indolocarbazole derivatives, to metal ions resulted in the modulation of their physical and biological properties, such as solubility, bioavailability and biological activity [11]. Meggers *et al.* used this approach by designing a metal-based structural analog of the known kinase inhibitor staurosporine. The ruthenium derivative DW12 displayed excellent kinase inhibitory activity in the nanomolar range [51]. Swapping ruthenium with osmium yielded the isostructural complex **19** (DW12-Os) with almost indistinguishable biological properties and almost identical binding behavior to kinases (Fig. 4a). However, compound **19** was a slightly more potent inhibitor of glycogen synthase kinase3 (GSK-3β) with an IC_{50} value in the sub-nM range. The metal center in these complexes has merely a structural function in maintaining rigid 3D structures that can perfectly fit into the active site pocket of the protein [52].

Paullones are another class of natural products well known for their ability to inhibit cyclin-dependent kinases (CDKs). Os^{II}(arene) complexes of different indolo[3,2-*d*]benzazepines (paullones) and indolo[3,2-*c*]quinolines were designed (see **20** and **21** in Fig. 2) [53–56]. The coordination sphere of the metal center was completed with the *N,N′*-chelating ethylenediamine moiety incorporated at the lactam site of the bioactive moiety. In the case of uncoordinated paullones, the lactam moiety appears to be essential for CDK inhibition activity; however, metal complexes of paullones modified at the lactam site were the most cytotoxic. Uncoordinated indolo[3,2-*c*]quinolones were potent cytotoxins with IC_{50} values against A549, SW480 and CH1 human cancer cells in the upper nanomolar range, whereas corresponding indolo[3,2-*d*]benzazepines were in the low micromolar range. This

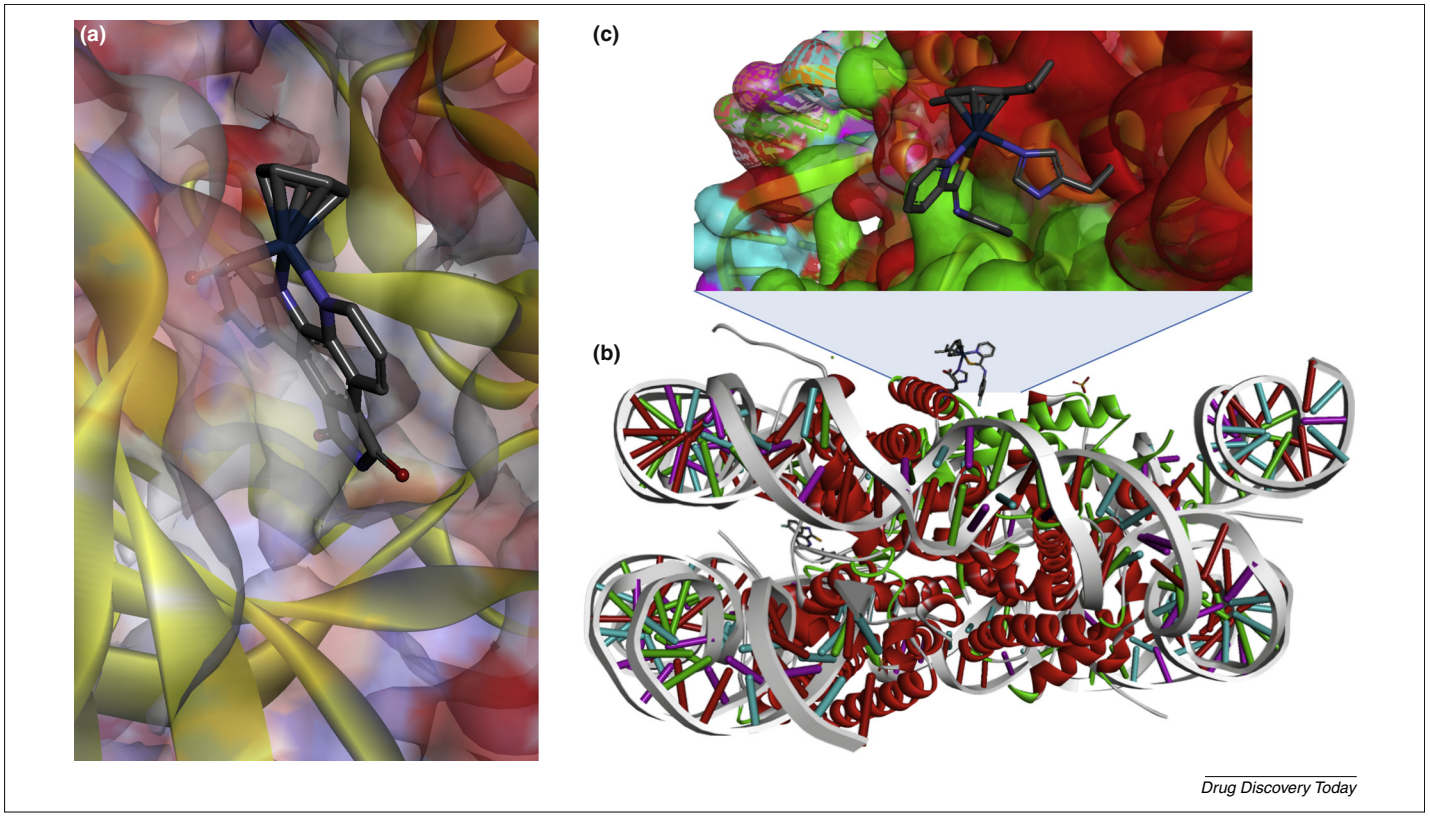

FIGURE 4

Protein targeting osmium organometallic compounds. **(a)** Binding of an osmium-based kinase inhibitor **19** into the ATP-binding pocket of Pim-1 (PDB code: 3BWF); **(b)** Adduct formation of **23** at a histone protein of the nucleosome core particle through binding to a His-imidazole residue **(c)**. Adapted from [52,58].

is possibly because of the higher DNA intercalation ability of the planar six-membered quinolone ring compared with the related azepine derivatives featuring a seven-membered, nonplanar ring.

Coordination of the indoloquinoline ligands to Os(II) resulted in slightly increased antiproliferative activity in comparison with uncoordinated ligands. By contrast, coordination of indolobenzazepine ligands to Os(II) fragments significantly enhanced their cytotoxicity up to tenfold depending on the cell line. In cell-free conditions, indoloazepines and their osmium complexes exhibited substantially higher kinase inhibition (CDK2/cyclin E) compared with the indoloquinoline counterparts. This might indicate involvement of intracellular targets other than CDKs [53]. To enhance the stability of such compounds, the diamine motif was replaced with iminopyridine *N,N*-chelators for coordination at the metal center (see **22** in Fig. 2). In some cases, this modification also resulted in improved cytotoxicity [54,55]. Derivatization of the indoloquinoline scaffold in position 2, which maintains the lactam unit in the compound, gave complexes with IC_{50} values in the micromolar range against A549, SW480 and CH1 cancer cells. In an *in vivo* study using the murine colon cancer model CT-26, the osmium compounds were well tolerated and significant tumor growth inhibition was observed after both intraperitoneal and oral administration [56].

Another class of kinase inhibitors is quinoxalinone and its derivatives. Their Os^{II}(arene) complexes were significantly more cytotoxic compared with the ligands, but did not display any significant effect on the cell cycle distribution of cancer cells [57].

A novel series of Os(II)-arene complexes of the *N*-substituted neutral *S,N*-chelators 2-pyridinecarbothioamides (PCAs), for example **23**, were recently reported (Fig. 2). PCAs are non-toxic gastric mucosal protectants. Coordination of PCAs to organometallic osmium (and Ru) fragments resulted in promising antiproliferative agents with exceptional stability in 60 mM hydrochloric acid. This makes these derivatives suitable for oral administration. In mode of action studies, the X-ray diffraction structures of the nucleosome core particle with [chlorido(η^6-*p*-cymene)(*N*-phenyl-2-pyridinecarbothioamide)osmium(II)] and [chlorido(η^6-*p*-cymene)(*N*-fluorophenyl-2-pyridinecarbothioamide)osmium(II)] were solved. These complexes bind to histone proteins by ligand exchange of the chlorido ligand. The Os(η^6-*p*-cymene)(PCA) fragment was located at histidine side chains on the surface and the inner cleft of the nucleosome core particle (Fig. 4b,c). These observations might indicate interference with chromatin activity as a possible mode of action of such Os(II) complexes [58].

RAPTA-inspired osmium complexes

RAPTA-C, [Ru(η^6-*p*-cymene)Cl_2(pta)], is an antimetastatic agent that interacts preferentially with proteins over DNA. The osmium analog [Os(η^6-*p*-cymene)Cl_2(pta)] **24** (Fig. 2) and its cationic derivative [Os(η^6-*p*-cymene)Cl_2(pta-Me^+)] were prepared and tested for anticancer activity. The Os(pta) complexes were shown to be selective toward tumorigenic cells, whereas the cationic analog indiscriminately damaged both healthy and diseased cells [59–61]. In an attempt to enhance the selectivity of such

compounds, the pta ligand was replaced with sugar-derived phosphite ligands. In this series of osmium compounds, the most lipophilic complex [dichlorido(η^6-*p*-cymene)(3,5,6-bicyclophosphite-1,2-*O*-cyclohexylidene-α-D-glucofuranoside)osmium(II)] **25** was the most cytotoxic, with IC_{50} values in the low micromolar concentration range [62]. In biologically relevant media, the complexes exhibited hydrolysis significantly slower than that of their ruthenium analogs. However, ruthenium complexes were more active than their osmium counterparts [63,64]. Dyson and co-workers prepared osmium–arene complexes containing functionalized 1,2,3-triazolylidene *N*-heterocyclic carbenes (tzNHCs), of the general formula [Os(η^6-*p*-cymene)(tzNHC)Cl_2]. The complexes underwent hydrolysis and the carbene ligands remained coordinated to the metal center under physiologically relevant conditions. These compounds exhibited an excellent activity profile against a range of human cancer cell lines. The activity was dependent on the substituents on the tzNHC ligand, which is a versatile scaffold to introduce a variety of substituents [65].

Concluding remarks

Metal complexes are a versatile scaffold for the design and discovery of new therapeutic agents that are not accessible in purely organic molecules. During the past decade, considerable attention has been paid to the development of anticancer drugs based on ruthenium, arsenic, gold, titanium, iron and other metals; however, the antitumor potential of osmium complexes has received undeservedly little attention. Significant advances in the field of ruthenium-based anticancer agents initiated the interest in osmium complexes because of the close proximity of osmium and ruthenium in the periodic table. As a result, osmium analogs of the widely investigated ruthenium-based anticancer agents RAPTA-C, RM175, NAMI-A and KP1019 were synthesized and their biological properties were investigated. In general, osmium complexes displayed considerable stability and inertness toward ligand substitution. Therefore, they might serve as inert models to understand the elusive modes of action of ruthenium drugs. However, the interest in osmium complexes goes much further because the unique properties of osmium can be used for the design of novel complexes with interesting biological properties. In some compounds, osmium has only a structural role by maintaining the 3D rigid structure of molecules, subsequently enhancing their interactions with biotargets. By contrast, the osmium fragment is not always an innocent spectator and it is capable of binding to different targets, such as DNA, proteins or enzymes, with a wide variety of options for ligand selection and compound design, which allow introducing the desired properties into the molecules.

The field has also broadened with the expansion from coordination compounds to organometallic pharmacophores, for which extensive studies on their SARs were performed. It was shown that the fate of half-sandwich osmium complexes in the body is markedly dependent on the ligands coordinated to the metal center. Whereas complexes with *O,O*-chelating ligands were often relatively unstable and formed inactive dimeric species, stable *N,O*-complexes were shown to target preferentially DNA. For kinetically inert Os^{II}(arene) complexes of *N,N*-ligands, DNA binding was ruled out as the mode of action. However, they might be capable of inducing apoptosis through intracellular redox chemistry. The incorporation of other *N,N*-ligands, such as ethylenediamine, into the osmium scaffold resulted in the formation of DNA targeting osmium complexes. The coordination of *S,N*-chelating ligands to the Os^{II}(arene) scaffold endowed complexes with unique properties, such as exceptional stability in hydrochloric acid, thus making them suitable for oral applications. These complexes formed stable adducts with histone proteins of nucleosome core particles in preference over DNA.

It should be noted that the analysis of different classes of ruthenium and/or osmium complexes does not result in a uniform picture. In some cases, the variation of the metal from ruthenium to osmium resulted in significantly enhanced cytotoxicity, as in case of NAMI-A analogs, whereas in other cases, the ruthenium complexes were markedly more active or the biological properties were independent of the nature of the metal center.

To conclude, the past few years witnessed the bloom in the field of osmium anticancer agents. It was demonstrated that osmium complexes were capable of inducing apoptosis by interfering with diverse cellular pathways; however, currently, no universal picture about the mechanism of action of osmium complexes can be drawn. Further studies are required to gain comprehensive knowledge of biological processes interfered by osmium complexes in the body. The interest in biologically active osmium complexes has been continuously growing and we believe that several novel osmium complexes with exciting biological properties will be developed in the future.

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