New metal complexes as potential therapeutics

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The many activities of metal ions in biology have stimulated the development of metal-based therapeutics. Cisplatin, as one of the leading metal-based drugs, is widely used in treatment of cancer, being especially effective against genitourinary tumors such as testicular. Significant side effects and drug resistance, however, have limited its clinical applications. Biological carriers conjugated to cisplatin analogs have improved specificity for tumor tissue, thereby reducing side effects and drug resistance. Platinum complexes with distinctively different DNA binding modes from that of cisplatin also exhibit promising pharmacological properties. Ruthenium and gold complexes with antitumor activity have also evolved. Other metal-based chemotherapeutic compounds have been investigated for potential medicinal applications, including superoxide dismutase mimics and metal-based NO donors/scavengers. These compounds have the potential to modulate the biological properties of superoxide anion and nitric oxide.

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Current Opinion in Chemical Biology 2003, **7**:481–489

This review comes from a themed issue on
Next-generation therapeutics
Edited by John Kozarich and Hugh Rosen

1367-5931/$ – see front matter

DOI 10.1016/S1367-5931(03)00081-4

Abbreviations

dien	diethylenetriamine
en	ethylenediamine
ER	estrogen receptor
NAMI	Na*trans*-[Ru(Im)(Me_2SO)Cl_4]
NAMI-A	(ImH)*trans*-[Ru(Im)(Me_2SO)Cl_4]
P-gP	P-glycoprotein
SOD	superoxide dismutase
***trans*-DDP**	*trans*-diamminedichloroplatinum(II)

Introduction

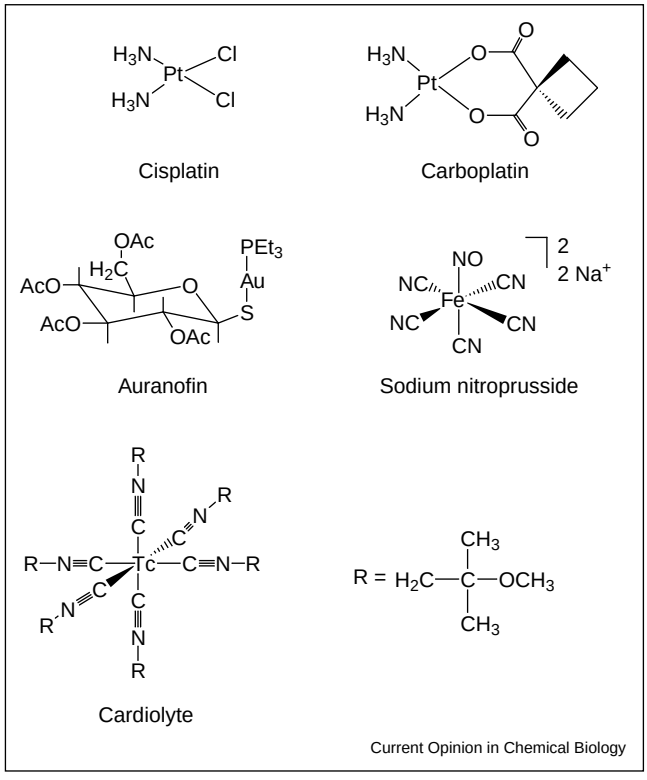
Medicinal applications of metals can be traced back almost 5000 years [1]. The development of modern medicinal inorganic chemistry, stimulated by the serendipitous discovery of cisplatin, has been facilitated by the inorganic chemist's extensive knowledge of the coordination and redox properties of metal ions. Metal centers, being positively charged, are favored to bind to negatively charged biomolecules; the constituents of proteins and nucleic acids offer excellent ligands for binding to metal ions. The pharmaceutical use of metal complexes therefore has excellent potential. A broad array of medicinal applications of metal complexes have been investigated, and several recent reviews summarize advances in these fields [2–6]. Some selected compounds that are currently used in clinical diagnosis and treatment are shown in Figure 1. Designing ligands that will interact with free or protein-bound metal ions is also a recent focus of medicinal inorganic research [7–9]. For example, chelating ligands for copper and zinc are being investigated as a potential treatment for Alzheimer's disease [10]. Developing metal complexes as drugs, however, is not an easy task. Accumulation of metal ions in the body can lead to deleterious effects. Thus biodistribution and clearance of the metal complex as well as its pharmacological specificity are to be considered. Favorable physiological responses of the candidate drugs need to be demonstrated by *in vitro* study with targeted biomolecules and tissues as well as *in vivo* investigation with xenografts and animal models before they enter clinical trials. A mechanistic understanding of how metal complexes achieve their activities is crucial to their clinical success, as well as to the rational design of new compounds with improved potency.

Because of space limitations, this review focuses on recent advances in developing platinum, ruthenium and gold anticancer agents with an emphasis on platinum compounds. We also cover superoxide dismutase (SOD) mimics and metal complexes as nitric oxide donors and scavengers.

Platinum-based anticancer agents

Much research attention has been paid to platinum complexes as potential anticancer drugs because of the success of cisplatin. Cisplatin, *cis*-diamminedichloro-platinum(II) (Figure 1), one of most widely used anticancer drugs, is effective in treating a variety of cancers, especially testicular cancer, for which it has a greater than 90% cure rate. Cisplatin enters cells by passive diffusion [11] and also, as recently discovered, by active transport mediated by the copper transporter Ctr1p in yeast and mammals [12••,13•]. Details about this latter mechanism remain to be elucidated. The cytotoxicity of cisplatin originates from its binding to DNA and the formation of covalent cross-links. The 1,2-intrastrand d(GpG) cross-link is the major adduct. Binding of cisplatin to DNA causes significant distortion of helical structure and results in inhibition of DNA replication and transcription [11,14•]. The distorted, platinated DNA structure also serves as a recognition binding site for cellular proteins [15,16], such as repair enzymes, transcription factors, histones and HMG-domain proteins. Binding of the HMG-domain proteins to cisplatin–DNA lesions has

Figure 1



Selected metal complexes that are currently in clinical uses.

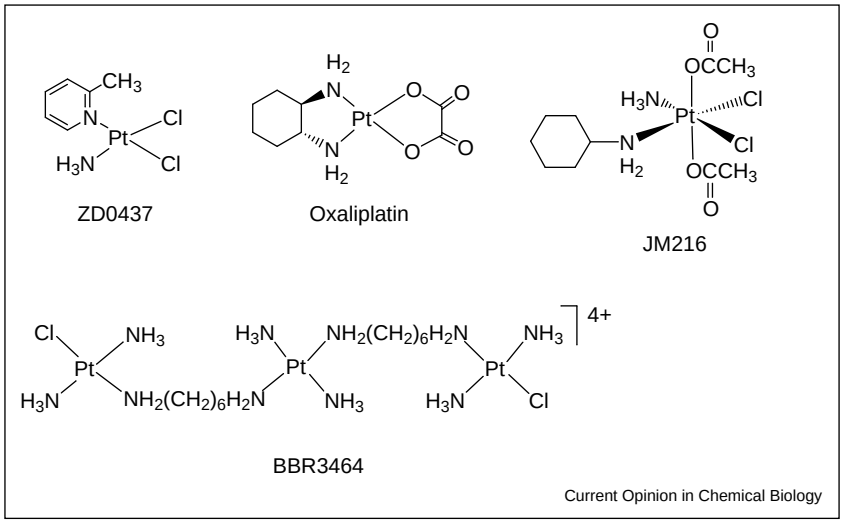
been suggested to mediate the antitumor activity of the drug [17–19]. The anticancer efficacy of cisplatin is also influenced by the efficiency of cisplatin–DNA adduct removal by the cellular repair machinery, with nucleotide excision repair being a major pathway. The repair of platinum–DNA cross-links is retarded when the DNA is bound to the histones in a nucleosome core particle [20•].

The clinical success of cisplatin is limited by significant side effects and acquired or intrinsic resistance. Therefore, much attention has focused on designing new platinum compounds with improved pharmacological properties and a broader range of antitumor activity. Several platinum complexes (Figure 2) are currently in clinical trials, but these new complexes have not yet demonstrated significant advantages over cisplatin. Oxaliplatin has been approved for clinical use in Europe, China and, for colorectal cancer, the United States. Strategies for developing new platinum anticancer agents include the incorporation of carrier groups that can target tumor cells with high specificity. Also of interest is to develop platinum complexes that bind to DNA in a fundamentally different manner than cisplatin in an attempt to overcome the resistance pathways that have evolved to eliminate the drug. These complexes may provide a broader spectrum of antitumor activity. Here we focus on recent efforts to prepare novel Pt(II) complexes using the strategies described above and review some mechanistic insights into the cytotoxic effects of these complexes. Pt(IV) compounds are not discussed here as they have been recently reviewed [21].

Cisplatin analogs with carrier groups

Drug delivery systems that can target a tumor site and/or prevent binding to non-pharmacological targets are beneficial in reducing drug toxicity and resistance. Polymer-coated micelles can protect platinum from intracellular thiols and result in prolonged circulation time in the bloodstream. Because of vascular leakage and reduced

Figure 2



Structures of a few platinum compounds that are in clinical trials or recently approved for clinical use.

lymphatic clearance, polymeric compounds tend to accumulate in tumor tissue, enhancing delivery of cisplatin to tumor sites. Recently, a poly(ethylene glycol) micelle was prepared containing a poly(aspartic acid) block to provide the chelating and leaving groups for platinum ions. It displayed a significantly longer circulation time in the bloodstream and higher accumulation in tumors, as demonstrated by an *in vivo* biodistribution assay of Lewis lung carcinoma-bearing mice [22•]. Reduced accumulation in kidney was also observed, resulting in low nephrotoxicity, one of the major side effects of cisplatin. Similarly, a series of platinum–polymer conjugates with *trans*-1,2-diaminocyclohexane as spectator ligands was investigated. *In vitro* cell survival tests of three conjugates showed their cytotoxicities to be 10-fold higher than that of cisplatin against Colo320 DM cells, a multidrug-resistant cell line [23].

To achieve tissue specificity, a homing moiety such as galactose [24,25] and bile acid [26,27] for liver and estrogen derivatives for estrogen receptor (ER) positive tissues [28] such as breast have been utilized. For example, platinum–polymer conjugates with attached galactose exhibited cell-specific cytotoxic activity against human hepatoma cells. The cytotoxicity was suggested to be mediated by galactose receptors expressed on the surface of the cells [24,25]. Similarly, a platinum–estrogen linked compound showed effective binding both as isolated receptor and in whole cell assays [28]. The antitumor activity of this complex, however, was not evaluated.

Other strategies to improve the antitumor efficacy include the use of porphyrin–platinum conjugates. The porphyrin enhances tumor specificity of the conjugates by its preferable accumulation in neoplastic tissues. In addition, porphyrins are commonly used in photodynamic therapy [4]. Thus by linking a platinum complex to a porphyrin moiety, additional toxicity against tumor cells can be achieved upon irradiation. Indeed, porphyrin–platinum complexes derivatized with either a hematoporphyrin [29] or a tetraarylporphyrin [30] exhibited enhanced cellular uptake and additional antitumor activity by the photo-induced mechanism.

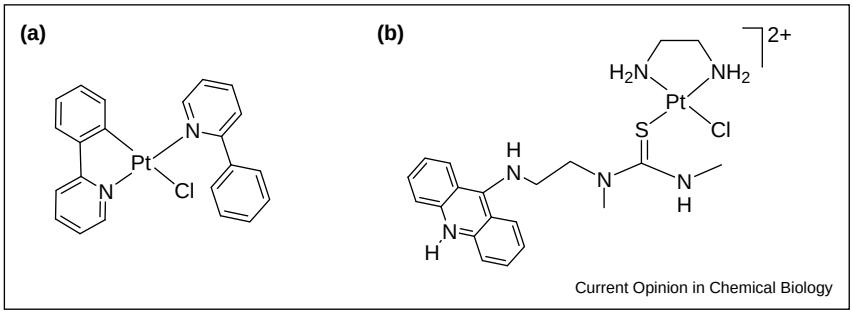
Because DNA is a key pharmacological target of platinum compounds, DNA-targeting groups such as intercalators were conjugated to the metal over a decade ago [31–33]. Such compounds exhibit enhanced antitumor activity. Renewed interest in platinum complexes with appended intercalators has produced some promising results. A series of *cis*-ethylenediamineplatinum(II) complexes with tethered 9-aminoacridine-4-carboxamides was able to overcome cross-resistance in human ovarian carcinoma cell lines *in vitro* [34]. Altered DNA sequence specificity and increased DNA binding rates compared with those of cisplatin were observed for these intercalator–platinum conjugates [35].

Trans- and multinuclear platinum complexes

Platinum complexes with distinctively different DNA binding modes from that of cisplatin may provide higher antitumor activity against cisplatin-resistant cancer cells. Among such complexes are those with amine ligands having trans stereochemistry. The trans analog of cisplatin, *trans*-diamminedichloroplatinum(II) (*trans*-DDP), is inactive, but its inertness may originate in part from kinetic instability and consequent susceptibility to deactivation. Substitution of one or both ammine ligands in *trans*-DDP with more bulky ligands has produced more toxic compounds. The bulky ligands can retard ligand substitution reactions of the two chloride ions, thereby reducing undesired reactions between platinum and cellular components and facilitating its interaction with DNA. Discovery of these properties has stimulated the development of additional complexes with trans geometry. Several classes of trans platinum complexes have been characterized, showing favorable cytotoxicity against cancer cells, especially cisplatin-resistant cells [36]. The spectator ligands in these complexes can be classified into three groups: planar aromatic amines, alkylamines and iminoethers. These compounds in general are more active than their cis analogs against cisplatin-resistant cell lines. Very recently, a series of *trans*-Pt(II)-piperazine compounds were reported that displayed significant cytotoxicity against cisplatin-resistant ovarian cancer cells [37]. These cationic complexes are more water soluble and bind more rapidly to DNA compared with cisplatin and *trans*-DDP, whereas their interactions with two cellular proteins, ubiquitin and myoglobin, are much slower than those of cisplatin and their neutral analogs (see Update).

Another class of platinum complexes that bind to DNA in a manner different from that of cisplatin are multinuclear. These compounds contain two, three or four platinum centers with both cis and/or trans configurations. Polyamines are generally utilized as linkers to connect the platinum centers. A representative trinuclear complex, BBR3464 (Figure 2), has entered a phase II clinical trial and exhibits activity against pancreatic, lung and melanoma cancers. Furthermore, this complex is effective against human tumor mouse xenografts containing mutant p53 gene [38]. The p53 gene is a tumor suppressor encoding a nuclear phosphoprotein that mediates cellular response towards genotoxic stress including cisplatin treatment [39]. Over 60% of human cancers are characterized by nonfunctional p53. Therefore, the activity of BBR3464 against cells with mutant p53 renders it a potent anticancer drug. BBR3464 is a highly charged 4+ species. It binds to DNA rapidly, forming various long-range interstrand and intrastrand cross-links. The interstrand adducts account for ~20% of the BBR3464-mediated DNA adducts. Recent mechanistic studies suggest that the interstrand cross-links, rather than intrastrand adducts, are important to the antitumor activity [40,41•]. The hypersensitivity of

Figure 3



Structures of Pt(II) complexes **(a)** with 2-phenylpyridine ligand **(b)** with acridinylthiourea ligand.

BBR3464 to tumors with mutant p53 was investigated by a p53 binding assay [42,43], suggesting that BBR3463 bypasses p53-mediated pathways.

Monofunctional platinum compounds

In the search for *cis*-amminedichloro(2-methylpyridine)-platinum(II) (ZD0473, Figure 2) derivatives with improved antitumor activity, an unexpected monofunctional platinum(II) complex with one normal and one cyclometalated 2-phenylpyridine ligand (Figure 3a) was discovered that exhibited high antitumor efficacy against cisplatin-resistant mouse sarcoma 180 (S-180cisR) cell lines [44•]. Consistent with its higher activity in the resistant cells, more efficient cellular uptake of this new complex compared with cisplatin was demonstrated. Reduced accumulation of cisplatin mediated by P-glycoprotein (P-gP) efflux was suggested to be one of the pathways for cisplatin resistance in S-180cisR cells. As a monofunctional complex, the platinum–phenylpyrindine compound cannot form DNA cross-links, indicating a different binding mode from that of cisplatin unless a ligand is displaced intracellularly. Its high cytotoxicity in cisplatin-resistant cells may possibly be a consequence of diminished DNA repair.

Recently, a platinum(II) complex with a thiourea ligand (Figure 3b) was reported that showed excellent cytotoxicity against a leukemia cell line. The complex may bind to DNA in a dual manner involving platinum coordination and acridine intercalation. The complex exhibited activity against two ovarian cancer cell lines at micromolar concentrations, but slightly less activity than that of the free ligand [45,46].

Combinatorial approach for developing new platinum drug candidates

An approach to discovering new platinum chemotherapeutic agents is to create a library of compounds and screen them for activity. A parallel synthetic method and an efficient screening assay are required. Toward this end, we recently described a synthetic scheme and automated apparatus for preparing a large number of Pt(II) complexes. Variation of spectator ligands and leaving groups led to over 3600 Pt(II) complexes [47]. Fast screening was achieved by using a colorimetric transcription inhibition assay based on β-lactamase gene expression in BlaM HeLa cell lines. Four hit compounds were discovered. Three were previously identified as active cisplatin analogs, and the fourth, *cis*-[ammine(2-amino-3-picoline)$PtCl_2$], resembles the platinum agent ZD0473 (Figure 2), currently undergoing clinical evaluation. This combinatorial approach can be applied to prepare other classes of platinum compounds, providing an alternative strategy for platinum anticancer drug discovery.

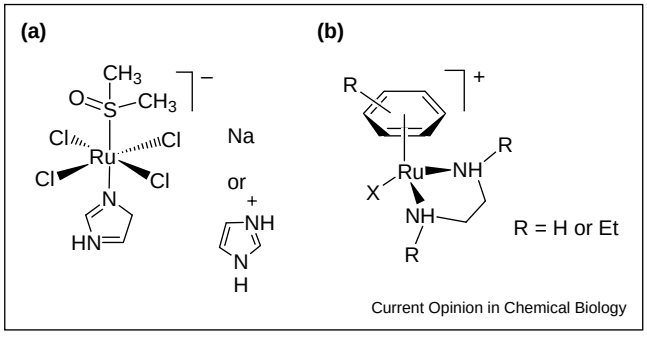
Non-platinum anticancer agents

Ruthenium complexes

Many ruthenium complexes with oxidation state 2+ or 3+ display antitumor activity, especially against metastatic cancers [48]. The Ru(III) complex Na*trans*-[Ru(Im)(Me_2SO)Cl_4] (NAMI) is currently in a clinical trial. For Ru(III) compounds, *in vivo* reduction to Ru(II) may be required for activity. Cellular uptake of many ruthenium complexes appears to be mediated by the iron transport protein transferrin [49]. In general, the cytotoxicity of ruthenium complexes correlates with their ability to bind DNA. As an exception, the antimetastatic activities of NAMI and its analog (ImH)*trans*-[Ru(Im)(Me_2SO)Cl_4] (NAMI-A) (Figure 4a) do not appear to involve DNA binding. Instead, they interfere with type IV collagenolytic activity and reduce the metastatic potential of the tumors [48]. Angiogenesis is crucial for metastatic tumor growth. The effect of NAMI-A on a transformed human endothelial cell line ECV304 was recently investigated [50]. The results show that the ruthenium complex triggers apoptosis in the ECV304 cells.

Organometallic Ru(II) complexes with arene ligands represent a relatively new group of ruthenium compounds with antitumor activity. Since the initial discovery that [Ru(η^6-C_6H_6)(dmso)Cl_2] can inhibit topoisomerase

Figure 4



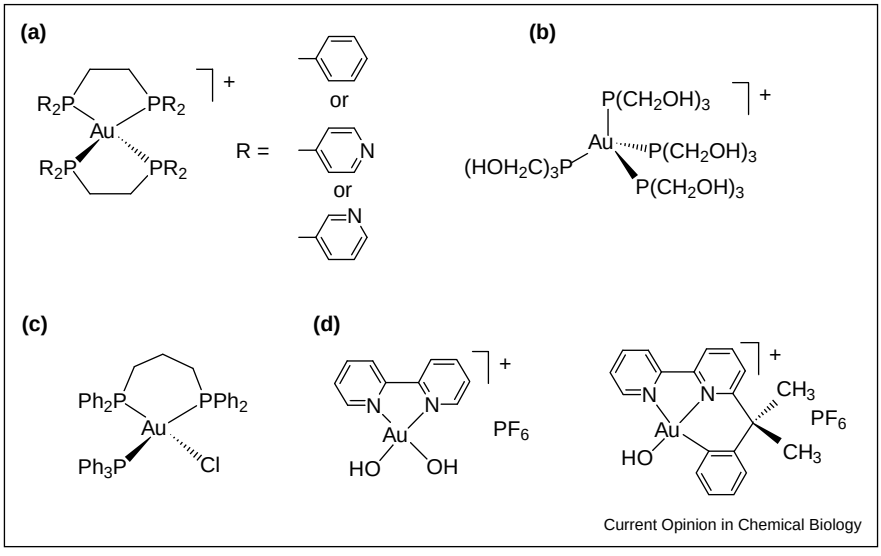
Structures of **(a)** NAMI and NAMI-A, and **(b)** $[Ru^{II}(\eta^6\text{-arene})(en)X]^+$ (X = Cl or I, arene = *p*-cumene or biphenyl, en = ethylenediamine or *N*-ethylethylenediamine).

II activity, three derivatives have been prepared by replacing the dmso ligand with 3-aminopyridine, *p*-aminobenzoic acid or aminoguanidine. These analogs show enhanced efficacy of topoisomerase II inhibition and higher cytotoxicity against breast and colon carcinoma cells compared to the parent compound [51]. Recently, several Ru(II) arene compounds with the formula $[Ru^{II}(\eta^6\text{-arene})(en)X]^+$ (X = Cl or I, arene = *p*-cumene or biphenyl, en = ethylenediamine or *N*-ethylethylenediamine, Figure 4b) were demonstrated to inhibit the proliferation of human ovarian cancer cells. Some of the IC_{50} values were comparable with that of carboplatin [52]. These complexes do not inhibit topoisomerase II activity, however. One representative compound binds strongly to DNA, forming monofunctional adducts selectively with guanine bases. Further studies led to the synthesis of 13 Ru(II) analogs, six of which are quite active against human ovarian cancer cells. No cross-resistance was observed in cisplatin-resistant cells. Cross-resistance did occur, however, with the multi-drug-resistant cell line 2780^{AD}, possibly mediated through the P-gP efflux mechanism [53•].

Gold complexes

Gold complexes are well known pharmaceuticals, the main clinical application being to treat rheumatoid arthritis. They are also active as antitumor agents [54]. Tetrahedral Au(I) complexes with 1,2-bis(diphenylphosphino)ethane and 1,2-bis(dipyridylphosphino)ethane ligands (Figure 5a) display a wide spectrum of antitumor activity *in vivo*, especially in some cisplatin-resistant cell lines. Mechanistic studies suggest that, in contrast to cisplatin, DNA is not the primary target of these complexes. Rather, their cytotoxicity is mediated by their ability to alter mitochondrial function and inhibit protein synthesis. Very recently, a hydrophilic tetrakis((tris(hydroxymethyl))phophine)gold(I) complex (Figure 5b) was reported to be cytotoxic to several tumor cell lines. With HCT-15 cells, derived from human colon carcinoma, cell cycle studies revealed that inhibition of cell growth may result from elongation of the G1 phase of the cell cycle [55]. A Au(I) complex having both monophosphine and diphosphine ligands (Figure 5c) has recently been prepared that is highly cytotoxic against several tumor cell lines. Its IC_{50} values are in the micromolar range [56]. Au(III) complexes, with their metal centers being isoelectronic and isostructural to Pt(II), are thus promising

Figure 5



Structures of several Au(I) and Au(III) complexes. **(a)** Au(I) complexes with 1,2-bis(diphenylphosphino)ethane and 1,2-bis(dipyridylphosphino)ethane ligands. **(b)** Tetrakis((trishydroxymethyl)-phosphine)gold(I) complex. **(c)** Chlorotriphenylphophine-1,3-bis(diphenylphosphino)propanegold(I) complex. **(d)** $[Au(bipy)(OH)_2]PF_6$ and $[Au(bipy^c\text{-}H)(OH)]PF_6$.

candidates as anticancer agents. Indeed, several Au(III) compounds with multidentate ligands such as en, dien and damp (*N*-benzyl-*N*,*N*-dimethylamine) are active against human cancer cell lines [57,58]. A recent *in vitro* cytotoxicity study demonstrated promising activity of two Au(III) complexes with bispyridyl ligands, [Au(bipy)-$(OH)_2$]PF_6 and [Au(bipy^c-H)(OH)]PF_6 (Figure 5d) [59]. Low cisplatin cross-resistance was observed. Both complexes are quite stable under physiological conditions, with [Au(bipy^c-H)(OH)][PF_6] being resistant to sodium ascorbate reduction. Mechanistic studies indicated that DNA is not the primary cellular target mediating antitumor activity of these complexes.

SOD mimics for cardiovascular, inflammatory, and neurological disorders

As a product of oxygen metabolism, superoxide anion can trigger oxidative injury to tissues. This activity has been suggested to be associated with reperfusion and inflammatory diseases as well as neurological disorders such as Parkinson's and Alzheimer's disease. In living systems, a natural defense mechanism against superoxide-mediated oxidative damages involves SODs, enzymes that catalytically deplete O_2^- to form O_2 and H_2O_2. The Cu/Zn-SOD predominates in extracellular spaces, whereas Mn-SOD functions in mitochondria. Therapeutic application of natural SODs is limited by their short plasma half-life, inability to cross cell membranes, and immunogenic responses [60]. Therefore, low-molecular-weight SOD mimics have been vigorously pursued as potential pharmaceutical agents for treating such diseases.

Among metal complexes (Cu, Fe, Mn) capable of catalyzing dismutation of the superoxide anion, those of manganese are a current focus for developing SOD mimics as drugs because of the low *in vivo* toxicity of this metal ion. Mn(III)-porphyrinato and Mn(II)-pentaazacyclopentadecane complexes exhibited particularly promising biological activities, with high stability and catalytic efficacy. Results from systematic modification of the porphyrin ligand demonstrate that placement of four positively charged *ortho*-(*N*-alkyl)pyridyl groups (alkyl = methyl and ethyl) in the *meso* positions of porphyrin can strongly facilitate the disproportion of O_2^-, owing to favorable electrostatic contributions [61]. These manganese complexes reduced oxidative stress injury *in vivo* [62•,63]. In the search for a lipophilic manganese SOD mimic, a dinuclear manganese(III) complex of biliverdin IX dimethyl ester was discovered to have such activity. This example is the first whereby O_2^- dismutation is effected by a Mn(III)/Mn(IV) redox couple [64]. In addition, the manganese complex does not bind to NO and reacts very slowly with H_2O_2, demonstrating specificity towards O_2^-. Interactions of SOD mimics with NO and H_2O_2 can contribute to their toxicity by reducing free NO and H_2O_2 levels, both of which can cause high blood pressure and weaken the immune system.

Metal-based nitric oxide donors and scavengers

The discovery of diverse biological roles for NO has stimulated and facilitated the development of NO-targeted pharmaceuticals. Physiological processes mediated by NO include neurotransmission, blood pressure regulation and immunological responses. NO is an excellent ligand for metal ions. As a consequence, metal nitrosyl complexes have therapeutic values. Sodium nitroprusside, $Na_2[Fe(CN)_5NO]\cdot 2H_2O$, is used clinically to treat cardiovascular disorders and lower blood pressure through release of NO. Toxicity involving cyanide accumulation has limited its application, however. A search for new metal nitrosyl complexes led to the discovery of several classes of ruthenium complexes with promising biological activity [48]. Among these, *trans*-[$Ru(NH_3)_4P(OEt)_3$-(NO)]$(PF_6)_3$ exhibited reduced toxicity and similar hypertensive activity compared with sodium nitroprusside in animal studies [65]. Prolonged blood pressure reduction was observed for *trans*-[Ru^{II}(cyclam)(NO)Cl]-$(PF_6)_2$ in both normotensive and acute hypertensive Wistar rats [66•], demonstrating its beneficial ability in controlled release of NO. Overproduction of NO contributes significantly to various diseases such as sespis, arthritis, diabetes and epilepsy. Ruthenium–polyaminocarboxylate complexes are efficient NO scavengers [48,67,68], demonstrating their therapeutic potential.

Conclusion

Recent advances in medicinal inorganic chemistry demonstrate significant prospects for the utilization of metal complexes as drugs, presenting a flourishing arena for inorganic chemistry. Significant progress in platinum-based anticancer agents has been achieved, based in part on a mechanistic understanding of the DNA-binding and pharmacological effects of cisplatin. Several new compounds with reduced toxicity and high specificity have been developed. Ruthenium complexes with antitumor activity are also emerging rapidly. Besides their established use to treat arthritis, gold complexes exhibiting anticancer potency have evolved. Because of the relevance of superoxide anion and nitric oxide to human disease, synthetic SOD mimics as well as metal-based NO donors and scavengers offer several possibilities for therapeutic applications. The future development of medicinal inorganic chemistry requires an understanding of the physiological processing of metal complexes, to provide a rational basis for the design of new metal-based drugs. Application of new methodologies such as combinatorial chemistry, extensively used in organic drug discovery, will be beneficial for the development of inorganic compounds as therapeutics.

Update

A recent study on globally platinated DNAs with *trans*-DDP analogs showed that substitution of one ammine ligand in the platinum complex with piperidine, piperazine

or 4-picoline resulted in more stable 1,3-intrastrand DNA cross-links. Increased efficiency for the formation of interstrand cross-links was also observed [69].

Acknowledgements

Work from our laboratory reviewed in this article was supported by a grant from National Cancer Institute.

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