Metal Complexes as Enzyme Inhibitors

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Contents

I. Introduction

The importance of enzyme inhibition as a mode of action for inorganic drugs has often been discovered as almost an afterthought. One approach to drug discovery is to screen potentially therapeutic complexes for biological activity and address the question of mechanism secondarily. For some drugs, the mode of action is still unclear after years of clinical use. In recent years, great advances in elucidating molecular structures have allowed precise determination of the interactions between proteins and therapeutic agents. This has clarified the mechanism for many agents and allowed informed predictions for potential drug binding sites. Identifying the exact nature of interaction for a drug is key to controlling its specificity and, thus, reducing unwanted side effects.

Detailed structural information can be employed to design molecules that bind to specific targets, and many of these efforts are aimed at enzymes. The substrate specificity of enzymes, often recognizing a single molecule only, offers the possibility of constructing a drug with a well-defined behavior. Furthermore, enzymes seem to be a natural target for inorganic drugs since metals play a key structural role for many enzymes, such as the zinc metalloenzymes. Perturbing an endogenous metal that is vital to enzymatic action can render such an enzyme inactive. These disturbances can arise from actions such as coordination of exogenous ligands to the metal, substitution of the metal, or removal of the metal. Inorganic complexes can also affect nonmetalloenzymes. Metals can coordinate to active site residues to block substrate interaction or coordinate to residues outside the active-site to affect structural integrity. While the list of enzyme inhibitors used as therapeutics agents is populated largely with organic molecules, these agents interact with the targets through the weak linkages of hydrogen bonding and van der Waals contacts. This has been known to result in undesirable side effects due to the shorter residency time of the drug at the target, allowing more nonspecific interactions with nontarget molecules. The coordination ability of metals holds the attractive promise of forming stronger attachments through covalent and ionic bonds.

We restrict our survey to work from the decade of the 1990s, including studies on metals and the metalloids, using a metalloid definition that includes boron. The term "metal complexes" is loosely interpreted to include complexes, compounds, or ions. We give limited coverage of metals such as platinum, bismuth, lithium, and gold, which are the primary topics for other reviews in this issue. The nomenclature for the metals is selected to differentiate between metal ions (e.g., Zn^{2+}) and metal atoms that are part of complexes, e.g., Zn(II)). K_i values are given as reported by the sources. Where these are not available, the reader should consult the original references for details on kinetics. The papers are classified by the medical conditions treated with the emphasis on the enzyme targets. The review is organized first by the enzyme inhibited, then grouped by metals that inhibit that particular enzyme. At times there may be multiple examples of the same metal inhibiting several enzymes but only a single reference for each enzyme. To maintain cohesiveness in this case, the primary grouping is by the metal. For ease of reference, tables are included that summarize the information by metals, complexes, and enzymes inhibited. Although it is beyond the scope of this review, we include a brief section introducing some complexes which do not themselves contain a metal but which exert their therapeutic effect by binding to a metal in an enzyme active site or whose effect is facilitated by the binding of a metal. Although these agents are not inorganic drugs, the formation of a metal complex is central to their inhibitory actions.

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Angelique Louie, a native of California, received her B.S. degree in Electrical Engineering with high honors from the University of California at Davis. This was followed by receiving her M.S. degree in Electrical Engineering (quantum electronics) from the University of California at Los Angeles under Dr. Oscar Staffsudd. With an interest in laser applications in biology and medicine, she subsequently completed her Ph.D. degree with Michael Berns at the Beckman Laser Institute on the University of California at Irvine campus. As a postdoctoral fellow at the California Institute of Technology, her research pursuits have included the inhibition of zinc finger transcription factors by cobalt complexes and the development of enzyme-activated MRI contrast agents. In 1998 she was appointed Senior Research Fellow at Caltech where she is continuing her interdisciplinary approach to imaging research and is interested in studying the aging eye.

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II. Applications to Normal and Abnormal Physiology

A. Cancer (Table 1)

One of the most notable successes for inorganic drugs has been the effectiveness of platinum complexes against testicular cancer.[1,2] These advances have spurred a surge of investigations to identify new inorganic agents for use in chemotherapy with improved specificity and decreased toxic side effects. The mechanism for the antineoplastic effect of cisplatin and its derivatives has been identified to be a direct interaction with DNA, but a number of reports suggest that platinum also has enzyme targets.[3] Since another review appearing in this issue is devoted to platinum anticancer drugs, we will only make note here that inhibition of RNA polymerase,[4] DNA polymerase (possible zinc finger disruption),[5] ileal monoamine oxidase (related to nausea and vomiting side effects of chemotherapy),[6] CTP synthase,[7] adenylate cyclase (related to ototoxicity of chemotherapy),[8,9] and other respiratory enzymes[10] have been observed. Preincubation of purified enzyme with the platinum complexes demonstrated direct interaction between them, but in many cases, Pt binding to the enzyme target also contributed to inhibition. Enzyme inhibition may contribute to some of the toxic side effects of platinum drugs, and a better understanding of the non-DNA interactions of these complexes is necessary to reduce the undesirable consequences of chemotherapy.

The platinum drugs are one example in which the therapeutic effect of the drug was noted before the mechanism of action was discovered. An alternative approach is to design a drug with a particular target in mind. Specific targets are sought which play key roles in cancer cell metabolism, which are unique to cancer cells or are differentially expressed in cancer cells. Ribonucleotide reductase is one such molecule which has emerged as a promising target for cancer therapy.

Ribonucleotide reductase performs a key, rate-limiting step in DNA synthesis by controlling the production of the four deoxyribonucleotide building blocks. The enzyme reduces the 2′ carbon on ribose at the diphosphate level.[11] The enzyme is composed of two dimeric subunits (termed M1 and M2 in older reports or R1 and R2 in more recent reports) which are encoded by two different genes under separate regulatory control. The enzyme itself is under complex allosteric regulation by the various nucleotides. R1 contains binding sites for substrates and regulatory effectors, while R2 contains a binuclear, nonheme iron center and a tyrosyl free radical that are necessary for enzyme function. Ribonucleotide reductase controls the balance of the deoxyribonucleotide pools, and changes in its activity can alter the spontaneous mutation rate of cells.[11] Increased ribonucleotide reductase activity has been associated with disease states including cancer,[11,12] and inhibition of this enzyme is an attractive target for cancer therapy.

Some success has been reported for the use of gallium nitrate in inhibition of ribonucleotide reductase.[13−15] Gallium has a similar ionic radius to iron and is believed to interfere with the availability of iron to the R2 subunit. The inhibitory effect of gallium is enhanced when it is delivered in a complex with the essential iron transport molecule, transferrin. Cellular studies demonstrate that treatment with transferrin−gallium (Tf−Ga) blocked cellular uptake

Table 1. Cancer Applications[a]

complex	metal	enzyme inhibited	ref
transferrin-Ga, ions	Ga	ribonucleotide reductase	14, 15
carbothioamide, Figure 1	Fe, Cu	ribonucleotide reductase	16, 17
thiosemicarbazones	Cu	ribonucleotide reductase, RNA-dependent DNA polymerases	19
ions	Zn	ribonucleotide reductase	12
ions	Ga	ribonucleotide reductase	13
anthracycline, ions, Figure 2	Cu	protein kinase C	21
selenocystine/glutathione	Se	protein kinase C	22, 25
metallocenes	Mo, V	protein kinase C, topoisomerase II	27
bipyridine phenanthroline	Pt, Pd	RNA polymerase	4
streptonigrin, Figure 14	Fe, Cu, Pd, Cd, Zn	topoisomeraseIII, reverse transcriptase (by free radical generation)	148
$PtCl_2$(en), *cis*−DDP	Pt	DNA polymerase	5
cisplatin, copper sulfate	Pt, Cu	ileal monoamine oxidase	6
cis-DDP, K_2PtCl_4	Pt	CTP synthase	7
ions	Zn	Caspase-3	35, 36
various	Ga	ornithine decarboxylase	33
cis-DDP, tetraplatin, carboplatin	Pt	respiratory enzymes	10
cisplatin	Pt	adenylate cyclase	8
tetraplatin	Pt	adenylate cyclase	9
vanadate, peroxovanadium	V	protein tyrosine phosphatases	30, 31
ion	Zn	l Ser/Thr phosphoprotein phosphatase	32
metal chelator	Cd	matrilysin	34

[a] Note: In this and all subsequent tables, the oxidation states for the metal ions are not shown, for purposes of clarity. Refer to the text or reference for more information.

of ^{59}Fe and inhibited proliferation.[14] Decreased ribonucleotide reductase activity is reflected in the inhibition of the ESR signal from the tyrosyl free radical on the ribonucleotide reductase R2 subunit. These effects can be reversed by exposure to ferrous ammonium sulfate, indicating that inhibition affects an iron-dependent process. These results do not exclude effects on other iron-dependent pathways that may also influence cell proliferation, however. Studies in a cell free system assaying directly for enzyme activity demonstrate that gallium inhibits enzyme activity, and the authors postulate that gallium forms gallium−CDP or gallium−ADP complexes to competitively inhibit the interaction of substrate with the enzyme.[15] Neither study addresses whether gallium may substitute for iron in the R2 subunit and whether such a substituted enzyme is still active. Gallium has also been shown to act synergistically with a number of other ribonucleotide reductase inhibitors to suppress cell proliferation, while it acts antagonistically with other ribonucleotide reductase inhibitors.[13] These observations have important ramifications for combination therapy, but the exact mechanism for synergism is not known.

Other ions have also been investigated for their effects on ribonucleotide reductase. Iron, copper, and zinc are known to influence cell growth and regulate components of the iron transport cycle. Studies on the effect of these ions on ribonucleotide reductase in freshly isolated normal and leukemic human lymphocytes found that zinc acts to inhibit the enzyme in both cell types while Fe and Cu had stimulatory effects.[11,12] These results may have implications for the modulation of the effects of ribonucleotide reductase inhibitors by trace metals.

Metal complexes of carbothioamides[16−18] and thiosemicarbazones[19] have been found to inhibit ribonucleotide reductase and possess anticancer activity. Metal complexes of α-(*N*)-heterocyclic carboxyaldehyde thiosemicarbazones (α-HCATs) were found to inhibit cell growth to a greater extent than the free ligand.[17] Metal complexes of 2,2′-bipyridyl-6-carbothioamide (BPYTA, Figure 1A), structurally and functionally similar to α-HCAT (Figure 1B), do not show such a clear effect. Studies on iron and copper complexes of BPYTA found that BPYTA has a higher affinity for copper and the copper complex demonstrates higher antitumor activity than the free ligand or the iron complex. Studies with the free ligand are unclear, however, since the ligand is likely to complex with available metal ions in solution. The dosimetry of the BPYTA complexes seemed to affect performance. For example, the copper complex has the strongest antiproliferative effect on cultured murine leukemia cells after pulse contact but in the case of continuous contact it is less active than the free ligand. In addition, the different metal complexes seem to act by distinct mechanisms. While the antiproliferative effect of the iron complex can be explained by inhibition of ribonucleotide reductase and is believed to actually destroy the R2 subunit, the copper complex seems to also act on other targets besides ribonucleotide reductase.[18,20] BPYTA was found to act synergistically with the ribonucleotide

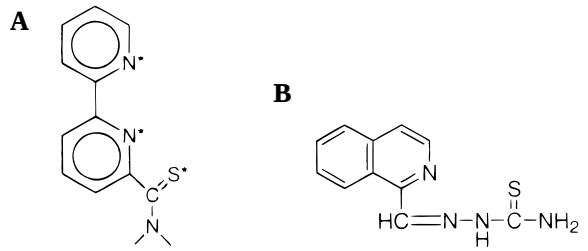

Figure 1. (A) BPYTA: 2,2′-bipyridyl-6-carbothioamide. Asterisk (∗) indicates atoms of the tridentate metal chelating system. (B) α-HCAT family: α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazone, example shown is 1-formylisoquinoline thiosemicarbazone.

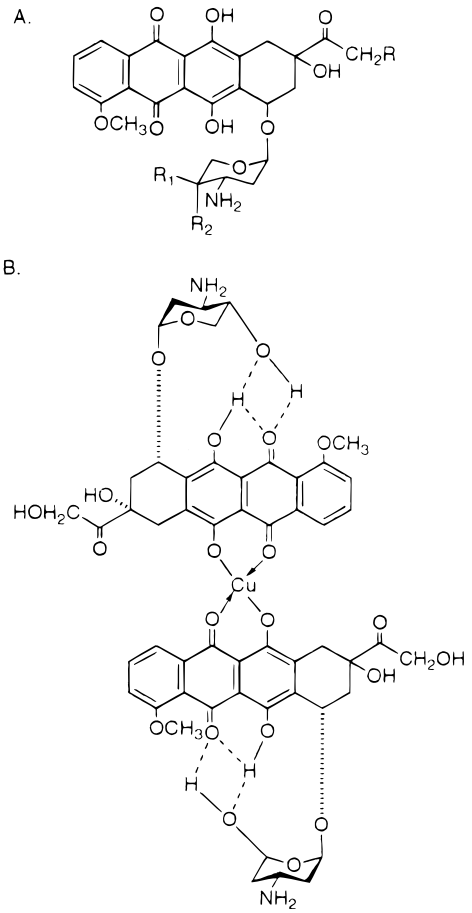

Figure 2. Anthracylines. (A) Doxorubicin: R = OH, R^1 = H, R^2 = OH. Epidoxorubicin: R = OH, R^1 = OH, R^2 = H. (B) Copper complex of epidoxorubicin.

reductase inhibitor hydroxyurea (HU), suggesting the possibility of combination therapy.

Another enzyme target for anticancer agents has been protein kinase C (PKC). PKC is a family of serine/threonine kinases that are activated by second messengers such as Ca^{2+} and phospholipid hydrolysis products (from the inositol triphosphate cascade). The nine different members of this family that have been characterized are involved in a variety of cellular activities including signal transduction, cell growth/differentiation, and hormone secretion.[21] They are also known to be receptors for phorbol esters (known chemical activators) and are also activated in response to oxidant tumor promoters.[22] Inhibition of PKC is, thus, of interest as a target to curb tumor formation. Recent evidence suggests that the regulatory domain of PKC, which holds two homologous regions of six conserved cysteines and two conserved histidines, contains nonbridged Zn^{2+} sites.[23] Antitumor agents that act on PKC include the anthracyclines (Figure 2), whose effect is mediated by coordination of transition metals. A study on purified PKC shows that the anthracycline−Cu(II) complex more efficiently inhibits PKC than either component alone and that this effect is mediated by the direct interaction between Cu(II) and PKC (based on EPR studies).[21] Anthracyclines have also been shown to exert various effects on DNA topoisomerase II,[24] but there is as yet no report of transition-metal complexes of anthracyclines in this application.

Certain selenocompounds have been found to have an inhibitory effect on PKC and demonstrate cancer-preventive activity as well.[22,25] Investigations suggest that these selenocompounds inactivate PKC by a redox mechanism, reacting with cysteines within the catalytic domain of PKC.[22] In one study, selenocompounds inhibited PKC but not two other protein kinases tested: phosphorylase kinase and protein phosphatase 2A. However, the authors acknowledge that these results do not preclude the possibility that other proteins containing clusters of cysteines may be affected.

Metallocenes are a class of non-Pt antitumor agents that appear to work in a manner distinct from the Pt drugs.[26,27] Certain metallocenes bind to terminal phosphates and/or bases of nucleotides, while others do not appear to interact with nucleotides.[27−29] In light of this, non-DNA targets have been proposed. In a work that was reviewed but not yet published, V(IV) and Mo(IV) compounds were found to inhibit PKC and topoisomerase II.[27] Nb compounds do not seem to bind to nucleotides or amino acids.[28] The mechanism of action for this class of drugs requires further investigation.

Vanadium compounds, which are best known as insulin mimetics, have also shown anticancer effects. These compounds, specifically the vanadate and peroxovanadium complexes, are competitive inhibitors of protein tyrosine phosphatases (PTP). Vanadate acts as a transition-state analogue by binding reversibly to a thiol group in the catalytic domain, while peroxovanadium complexes oxidize a critical cysteine residue in the catalytic domain.[30] In serine/threonine phosphatases, vanadate binds a hydroxyl group in the active site while the peroxovanadium complexes are inactive here, in the absence of the cysteine. Peroxovanadium complexes have demonstrated an ability to block the cell cycle at the G2-M transition (G2 = growth phase 2, a preparatory phase for mitosis; M = mitosis, a period of active cell division), and this seems to be mediated by the inhibition of a PTP whose function is essential for the progression to mitosis.[30,31] This blockage may be related to their cytotoxic effects. Serine/threonine phosphatases are also inhibited by a number of d-block transition-metal ions, the most effective being Zn^{2+}, Hg^{2+}, Cu^{2+}, Yb^{2+}, and Sc^{2+} (μM K_i).[32]

A number of other enzyme targets for anticancer agents have been investigated. Ornithine decarboxylase, inducible by phorbol esters and related to cell transformation, has been shown to be inhibited by gallium compounds.[33] Transformation is connected to the cell's transition to a cancerous state. These compounds inhibit the growth of tumors and are also likely to exert an effect on ribonucleotide reductase, as discussed earlier. Matrix metalloproteinases are involved in the remodeling of the extracellular matrix which is key to tumor invasion and metastasis in tissues. The matrix metalloproteinase human matrilysin, a zinc enzyme, has been shown to be inactivated when diluted with cadmium, which forms an inactive Cd/Zn hybrid.[34] Matrilysin is also inhibited by metal binding agents such as 1,10-phenanthroline which

Table 2. Heme-Related Applications. 1. Hyperbilirubinemia

complex	metal	enzyme inhibited	ref
ions	Sn	cytochrome P-450, ω-hydrolase	78
protoporphyrin (PP), Figure 3a	Sn, Zn	11β-hydroxylase, 21α-hydroxylase, cytochrome P-450, heme oxygenase	45−47
mesoporphyrin (MP), Figure 3b	Sn	heme oxygenase (small intestine)	49
deuteroporphyrin 2,4-bisglycol (BG), Figure 3c	Zn	heme oxygenase (rat)	53
MP	Zn	heme oxygenase (rat)	50
MP	Co	splenic heme oxygenase (rat brain membranes)	54
PP, BG, MP	Cr	heme oxygenase (rat)	55
various porphyrins	Zn, Sn, Cu	γ-aminolevulinate synthase (chick embryo liver cells), heme oxygenase	56
various porphyrins	Zn, Sn, Cr, Mg	heme oxygenase (small intestine epithelium)	48
mesoporphyrin	Sn	clinical suppression of hyperbilirubinemia	43, 44

forms an enzyme−chelate−metal complex with subsequent removal of the catalytic zinc ion.[34]

Apoptosis, which has become a prominent field of research in recent years, is a process of "programmed" cell death involved in determining structural architecture during development. Defects of apoptosis are correlated with a number of disease processes including cancer. Zn^{2+} is known to prevent apoptosis and has been shown to inhibit caspase-3, an apoptotic protease involved in the proteolysis of poly(ADP-ribose)polymerase.[35,36] A conflicting report does not observe inhibitory action of zinc on caspase-3.[37] It is suggested that the discrepancy is due to differences in the assay conditions: inhibition is observed in the case of purified recombinant protein but not for protein in the presence of *E. coli* lysate, implicating the presence of factors in the lysate that may interfere with the effects of zinc.

We note here that there are a number of agents which exert their effect by inhibiting enzymes that act upon DNA, but that this inhibition results from binding to the enyzme's site of interaction on the DNA and not direct enzyme inactivation.[24,38−40]

Given the nature of enzyme−substrate interactions, targeting of anticancer drugs to enzymes holds the promise of exquisitely specific target recognition. The advent of more sophisticated modeling techniques and more detailed information about enzyme structures has allowed rigorous characterization of drug:enzyme interactions, elucidating mechanisms of action. Most of the papers found in the cancer literature address the inhibition of a particular enzyme, frequently those involved in metabolic processes. But these investigations tend to use purified enzyme or assays that interrogate only the enzyme of interest. While it is crucial to determine inhibitory properties on the target molecule, it is still necessary to demonstrate that the compound does not act on any other enzymes. These sorts of studies are difficult in that one can only survey a reasonable number of representative enzymes, and it is easy to miss one that negates your claims of specificity. Careful consideration of the site of action of a drug should allow educated predictions for other interacting enzymes. Even with keen enzyme specificity, an additional challenge is that the enzyme targets are often present in normal cells as well. The design of an effective anticancer agent is a complicated game that must encompass not only the drug's inherent inhibitory properties but also its delivery, dosimetry, and residence time in vivo. Metal complexes overcome some of these challenges by forming strong covalent attachments to target enzymes. It will be interesting to see if the more specific design techniques at the bench yield more specific agents in the clinical setting.

B. Heme-Related Applications

1. Hyperbilirubinemia (Table 2)

Hyperbilirubinemia describes a condition in which there is an excessive accumulation of bilirubin in the blood and tissues.[41] Bilirubin is a product of heme catabolism, wherein heme is degraded by heme oxygenase (HO) to yield biliverdin, which is then reduced by biliverdin reductase to bilirubin.[42] After conjugation with glucuronic acid, by the enzyme bilirubin glucuronide transferase, to form the more soluble conjugate, bilirubin is excreted. Excessive breakdown of heme and insufficient transferase activity can both result in the accumulation of bilirubin. This buildup is known as neonatal jaundice in infants with underdeveloped liver function and premature infants who not only rapidly degrade heme (in the conversion from fetal to adult hemoglobin) but also lack adequate transferase. Hyperbilirubinemia is also seen in the genetic disease Crigler−Najjar syndrome in which there is near complete absence of transferase activity in the liver[43] and in other diseases such as thalassemia and congenital anemias.[42] Inhibition of heme breakdown at the heme oxygenase step has been investigated as a means to suppress hyperbilirubinemia.

Metalloporphyrins (Figure 3) have been extensively studied and are well-known as inhibitors of heme

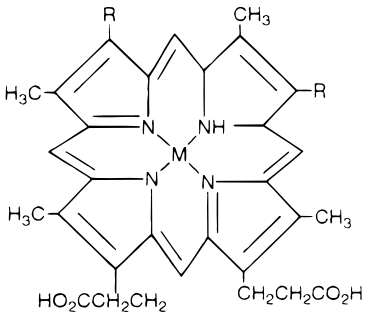

Figure 3. Porphyrins. (A) R = vinyl (CH_2=CH−): protoporphyrin. (B) R = ethyl (CH_3−CH_2−): mesoporphyrin. (C) R = 2,4-bisglycol (CH_2OH−CHOH−): deuteroporphyrin 2,5-bisglycol.

Table 3. Heme-Related Applications. 2. Nitric Oxide Signal Transduction

complex	metal	enzyme inhibited	ref
PP	Zn	soluble guanylyl cyclase (purified)	73
PP, BG	Zn, Sn	soluble guanylyl cyclase (rats)	74
sodium nitroprusside (SNP)	Fe	nitric oxide synthase (rat neutrophils)	65
SNP	Fe	NOS (cerebral cortical synaptosomes)	66
ions	Cu, Zn, Co, Ni, Fe	NOS (purified)	63
ion	Zn	NOS(purified nNOS)	68
SNP	Fe	ecto-5′-nucleotidase (renal epithelial)	72
SNP	Fe	indoleamine-2,3-dioxygenase (mononuclear phagocytes)	69
SNP	Fe	methionine synthase	60
SNP	Fe	(Na^+, K^+)-ATPase	71

oxygenase. These inhibitors bind to the catalytic site but are not effective substrates. Although it would seem that many metalloporphyrins could serve as inhibitors, some actually tend to induce heme oxygenase rather than inhibit it. The two leading inhibitors of heme oxygenase are the tin and zinc porphyrins. Some of these porphyrins have shown clinical success in suppressing bilirubin production,[43,44] and recent research, described here, delves into greater detail on the nature of the inhibition, effects on other pathways, and the biodistribution of the drugs.

Studies on zinc and tin protoporphyrin indicate that tin protoporphyrin (Sn−PP) is also an effective inhibitor of adrenal steroidogenesis while zinc protoporphyrin (Zn−PP) does not affect that pathway.[45,46] Steroid synthesis is mediated by a number of hemoproteins, the cytochromes P-450, and a complex interdependency exists between the pathway of heme synthesis and steroidogenesis. The concentration of the cytochromes is connected to changes in heme synthesis and degradation. In tests using the rat model, Sn−PP was found to significantly reduce the activity of steroid biosynthetic enzymes mitochondrial 11β-hydroxylase and microsomal 21α-hydroxylase, and a decrease in the content of microsomal cytochrome P-450 was observed.[45,46] This study also noted a drastic decrease in the content of HO-2, a noninducible isozyme of heme oxygenase that is far more prevalent than the inducible HO-1, and proposes that Sn−PP actually destroys HO-2 rather than inhibits it. Zn−PP, in contrast, inhibits the activity of HO but does not affect the steroid biosynthesis enzymes.[45,46]

Further investigations comparing the actions of Zn−PP and Sn−PP on HO-1 and HO-2 show that both are equally inhibitory to HO-1 without affecting protein integrity (as determined by western blot analysis) and induce an increase in the level of HO-1 transcripts. However, Sn−PP and Zn−PP differ in their activity against HO-2.[47] Although both inhibit HO-2, the degrees of inhibition differed. The degree of inhibition by Zn−PP was the same for both forms of HO, while Sn−PP exhibited greater inhibition of HO-2 than HO-1. Sn−PP appears to disrupt the integrity of the HO-2 protein (western blotting) while Zn−PP does not. The mechanism for the disparate activities of Sn−PP and Zn−PP on the HO forms is not known.

Various porphyrins have been found to inhibit intestinal heme oxygenase and reduce iron uptake from the gut, resulting in increased excretion of iron, which may be useful in treatment of cases of iron overload.[48,49] This effect is observed for all the parenterally administered porphyrins tested, but only Sn−MP, Sn−PP, and Cr−MP were effective orally (on intestinal HO, they were not found in renal, splenic, or hepatic tissues). Zn−MP has shown effectiveness in the treatment of acute porphyria (a heme deficiency disorder), and tissue distribution studies show that it is rapidly cleared from plasma and remains primarily in the liver and spleen, where the inhibition of HO persists for almost a week after a single injection.[50] Zn−MP does not appear to cross the blood-brain barrier, unlike Sn−PP which was shown to deleteriously affect HO-2 integrity and activity in the rat brain.[51] Metalloporphyrins act as photosensitizers to varying degrees[52] (they are also employed in photodynamic therapy). The Zn−PP is reported to be less photosensitizing than Sn−PP, another feature which suggests that the zinc forms may be better suited for clinical use.[53]

Co−MP and Cr−MP have also demonstrated effective inhibition of HO.[54,55] Co−MP does not appear to mediate lipid peroxidation in rat brain membranes after photoirradiation (as determined by malondialdehyde assay), indicating that it does not act as a photosensitizer.[54] Cr−MP effectively inhibited hepatic HO after oral administration but not splenic HO.[55] Zn−MP has also been found to inhibit hepatic δ-aminolevulinate synthase (ALAS), the rate-limiting enzyme in the heme biosynthesis pathway, an effect which is enhanced when heme is added with the porphyrin.[56] Iron and heme are known to feedback regulate ALAS by inhibiting mRNA transcription/transport,[57] and translation of ALAS seems to be under control of an iron responsive element.[58]

The use of metalloporphyrins for the treatment of hyperbilirubinemia is further along the developmental time line than some of the cancer agents presented in the previous section. These porphyrins have a known target, heme oxygenase, and have already shown some clinical effectiveness. However, the main target is part of a complex pathway that is highly interconnected to other processes such as steroid synthesis. These other processes should also be monitored to determine that they are not detrimentally affected.

2. Nitric Oxide Signal Transduction (Table 3)

Nitric oxide (NO) plays the role of messenger in a variety of systems. In the immune system, NO mediates the response of white blood cells to pathogens; in the cardiovascular system, NO induces smooth muscle cell relaxation in blood vessels; and

in the nervous system, NO is involved in neurotransmission and neuromodulation.[59−61] NO is generated from L-arginine by nitric oxide synthase (NOS), a heme protein which exists in multiple isoforms, and requires NADPH as an electron donor. Three isoforms of NOS exist: the constitutive isoforms found in brain (nNOS, type I) and endothelial cells (eNOS, type III) that require calcium/calmodulin and the cytokine-induced forms (iNOS, type II) that do not require calcium but have calmodulin as a tightly bound subunit.[62] Both classes exist as homodimers with each subunit containing 1 equiv of FAD, FMN, (6*R*)-5,6,7,8-tetrahydro-L-biopterin (H_4B), and protoporphyrin IX heme.[63,64] There is a long list of NOS inhibitors including selenoorganic compounds (binds to thiol groups), Zn porphyrins, and zinc (binds at heme site) which is summarized in a recent review.[64] In the signal transduction cascade, NO is known to stimulate guanylyl cyclase and induce an accumulation of the second messenger, cAMP. Many aspects of NO signaling have been elucidated by studying the ramifications of introducing NO donors to a system or by inhibiting NOS to arrest NO production. The former represents an indirect use of metal complexes as inhibitors by using a metal complex to supply NO, which subsequently inhibits the enzyme.

NO has been widely utilized as a ligand in iron−heme complexes in inorganic chemistry, and this raises the question of whether NO can autoregulate by inhibiting NOS, which is a heme protein.[62] Studies employing the NO donor, sodium nitroprusside (SNP), $Na_2Fe(CN)_5NO{\cdot}2H_2O$, implicate that NO can inhibit NOS.[65,66] In studies with rat neutrophils, lipopolysaccharide is used to induce NOS activity and this induction is inhibited by SNP.[65] Investigations using rat cortical synaptosomes demonstrate a concentration-dependent inhibition of NOS activity by SNP.[66] Both studies determine NOS activity by monitoring the formation of [^{3}H]citrulline from [^{3}H]arginine.[67] Transition metals have also been observed to inhibit NOS.[63] Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+} were all found to inhibit NOS activity when purified, inducible NOS is used, while non-heme iron increased catalytic activity. Cu^{2+} was also found to inhibit purified, neuronal NOS (the other metals were not assayed with nNOS). These results lead the authors to postulate that the non-heme iron of NOS is involved in catalysis and NOS may contain a nonheme iron/H_4B active site.

Zn^{2+} reversibly inhibits the activity of nNOS (K_i = 30 μM).[68] Both inhibition of the NADPH-dependent reduction of heme iron and the calmodulin (CaM)-dependent reduction of cytochrome *c* were observed. No inhibition of the CaM-independent reduction of cytochrome *c* reductase was noted (as measured by relative turnover of cytochrome *c*). However, even in the absence of Zn^{2+}, the CaM-independent pathway has over 10-fold lower activity than the CaM-dependent pathway and the scaling of the data as presented makes it difficult to determine if any inhibition occurs. Type II difference spectra of nNOS demonstrated a decrease in the amount of high-spin heme iron in the presence of Zn^{2+}. On the basis of these results, the authors propose that the enzyme inactivation stems from Zn^{2+} binding to sulfhydryls near the heme ligand and subsequent disturbance of the environment around the heme iron.

SNP and NO production are involved in inhibition of other enzymes. Indoleamine 2,3-dioxygenase (IDO) is a heme-containing enzyme that is part of the interferon-γ (IFNγ) induced response of mononuclear phagocytes to pathogens.[69] IDO is the rate-limiting enzyme in the tryptophan degradation pathway. Depletion of the amount of tryptophan, the least available essential amino acid, may play a role in the antipathogenic activity of mononuclear phagocytes. In studies on isolated IFNγ-primed human peripheral blood mononuclear cells and monocyte-derived macrophages, SNP was found to inhibit IDO activity.[69] IDO has also been shown to be inhibited by Zn−MP.[70] Other enzymes shown to be inhibited by SNP include methionine synthase in rat hepatocytes,[60] (N^+, K^+)−ATPase,[71] and ecto-5′-nucleotidase in renal epithelial cells.[72] Methionine synthase contains methylcobalamin as a cofactor, a vitamin B-12 derivative that bears many similarities with hemoglobin, and inhibition may occur via formation of an NO−cobalamin complex. Inhibition of the ecto enzyme is believed to take place through *S*-nitrosylation. The mechanism for inhibition of (N^+, K^+)−ATPase is unclear.

Carbon monoxide (CO) has also been implicated to be a physiological regulator with similar activities as NO, including the ability to stimulate guanylyl cyclase. CO is generated by HO in the conversion of heme to biliverdin and CO. Many of the studies on the role of CO were performed by employing HO inhibitors such as Zn−PP and Sn−PP to show that suppression of HO (and thus CO production) inhibits guanylyl cyclase. However, recent studies indicate that the metalloporphyrins themselves inhibit guanylyl cyclase, and thus, the studies utilizing these complexes to make inferences about CO may need to be reexamined.[73,74] Other studies have utilized porphyrins to examine the role of CO in spatial learning and long-term potentiation in the hippocampus.[75,76] The porphyrins were employed with the belief that they act solely to suppress CO production by HO; however, given that the action of the porphyrins can be so multifaceted, it is difficult to surmise a role for CO based solely on these experiments. These studies demonstrate some of the problems that can be encountered when the specificity of an inhibitor is not absolute and illustrate some of the complicating factors in data analysis when employing "indirect" effectors (e.g., inhibiting the enzyme that produces CO rather than directly removing CO).

C. Hypertension Applications (Table 4)

The previous section's discussion of heme-related systems is intimately connected to hypertension applications. Sodium nitroprusside is widely used as a hypotensive agent,[60,77] and heme oxygenase has been implicated in the maintenance of blood pressure.[78] $SnCl_2$ has been found to induce HO-1 transcription and, thus, inhibit cytochrome P450-arachidonic acid ω/ω-1 hydroxylase activity, resulting in a decrease in the formation of ω/ω-1 hydroxylase metabolites and lower blood pressure.[78] The hydroxy-

Table 4. Hypertension Applications

complex	metal	enzyme inhibited	ref
sulfonamide, Figure 7	Cu	carbonic anhydrase	86
heterocyclic sulfonamides, Figure 4	Zn, Cu	carbonic anhydrase	83
heterocyclic mercaptans, sulfenamides, sulfonamides	Zn, Cu	carbonic anhydrase	81
various sulfonamides, Figure 6	Ge, Sb	carbonic anhydrase	85
chlorothiazide, Figure 5	Hg, Pb	carbonic anhydrase	84
captopril, Figure 8	Zn	angiotensin converting enzyme	89
lisinopril	Cu	angiotensin converting enzyme	90

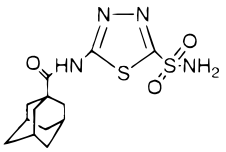

Figure 4. Sulfonamides: adamantyl derivative.

lase is a heme protein whose levels are controlled by the availability of heme. When heme oxygenase is induced, the pool of available heme is depleted, the amount of enzyme declines and, therefore, so does the amount of enzyme metabolites. The metabolites, 19[*S*]-hydroxyeicosatetraenoic acid [HETE] and 20-[HETE] act as prohypertensives by stimulating Na^+/K^+-ATPase and blood vessel constriction.

Another prime enzyme target in hypertension is carbonic anhydrase. Carbonic anhydrases (CAs) catalyze the hydration of CO_2 to bicarbonate and are involved in a wide variety of biological processes including pH homeostasis, production of ocular and cerebrovascular fluids, and secretion of electrolytes. In the eye, for example, carbonic anhydrase plays a crucial role in the maintenance of intraocular pressure. There are seven known isozymes of carbonic anhydrase and several related proteins. These isozymes are found in the cytosol (CA I, II, III, and VII), associated with cell membranes (CA IV), in mitochondria (CA V), and in a secretory form found in saliva (CA VI).[79,80] These isozymes play diverse roles and differ widely in tissue distribution, kinetic properties, and sensitivity to inhibitors. CAs are metalloenzymes, and many inhibitors of these enzymes are metal complexing anions that coordinate directly to zinc in the enzyme active site.

Carbonic anhydrases, with the exception of CA III, are also inhibited by sulfonamides that bind to zinc by substituting a catalytically important water molecule.[81] Sulfonamides have been in use for treatment of glaucoma for many years to lower intraocular pressure (IOP). The exact mechanism for pressure reduction is not known but is believed to be due to a reduction in the concentration of bicarbonate in the posterior chamber of the eye, with the concomitant shifts in bicarbonate ion resulting in fluid movement and a decrease of sodium.[82] Metal complexes of sulfonamides also potently inhibit carbonic anhydrase. Zn(II), Cu(II), Ni(II), Co(II), Fe(III), and Al-(III) complexes of an adamantyl sulfonamide derivative (Figure 4) all demonstrated inhibitory activity against three CA isozymes tested (hCA I, hCA II, and bCA IV) at nM K_i.[83] The Zn(II) and Cu(II) complexes, the strongest inhibitors in the series, were applied topically to the rabbit eye model and found to decrease IOP. The topically applied sulfonamide did not lower IOP, indicating that the presence of the

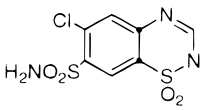

Figure 5. Sulfonamides: chlorothiazide derivatives.

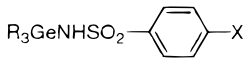

Figure 6. Sulfonamides: Ge, Sb sulfonamides.

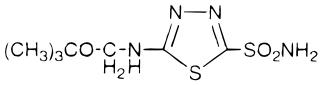

Figure 7. Sulfonamides: acetazolamide derivatives.

metal ions critically alters the properties of the inhibitor. The metal perhaps modifies the solubility of the complexes to increase availability of the drug. Hg(II) complexes of chlorothiazides (Figure 5), a diuretic in use as an antihypertensive drug, also showed strong inhibition of CA,[84] as have Ge(IV) and Sb(III) complexes of arylsulfonylamides (Figure 6)[85] and Cu(II) complexes of an acetazolamide derivative (Figure 7).[86] The metal complexes are believed to act on CA in a 2-fold manner: (i) by inhibition with the free sulfonamide and (ii) by interaction of the metal ion with a histidine in the active site.[86] Given the diversity of carbonic anhydrases, a major goal for current research is to design inhibitors that are more specific for certain isozymes.

Much of the literature about hypertensive agents is devoted to inhibitors of angiotensin-converting enzyme (ACE), a component of the renin-angiotensin system. The renin-angiotensin system is involved in blood pressure maintenance. ACE catalyzes the conversion of angiotensin I to angiotensin II, a vasoconstrictor involved in hypertension. Angiotensin I is formed from a conversion of a liver product by renin, a kidney-secreted enzyme.[87,88] There are receptor sites on many organs for angiotensin II. This system can be blocked at many levels, the familiar β-blockers block renin release, renin-substrate analogues and renin inhibitors block renin, angiotensin II receptors are blocked by salarsin, and ACE inhibitors block ACE. Most of these blockers are not metal-containing compounds, but some ACE inhibitors form metal complexes in vivo. For example, Captopril (Figure 8A), is designed to compete with angiotensin for the zinc ion in the enzyme and binds to zinc through a thiol group.[89] Lisinopril (Figure 8B), another ACE inhibitor, binds to zinc through an amino carboxylate moiety.[90] Lisinopril has been conjugated to Rh(III) and Pd(II) (iminophosphorano)phosphines, and the conjugates have slightly greater inhibitory activity than the parent compound (up to 18-fold decrease in IC_{50}, nanomolar range).[91] This derivati-

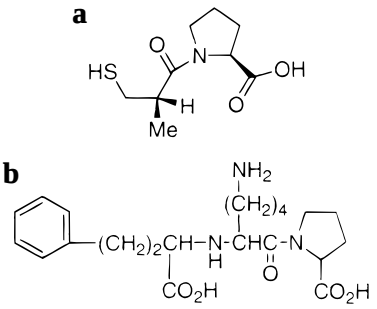

Figure 8. (A) Captopril. (B) Lisinopril.

zation is an encouraging development toward the synthesis of ^{105}Rh and ^{109}Pd radiolabeled compounds to track drug distribution in vivo.

Treatments for hypertension tend to be long term rather then single dose, and for this, the interaction stability afforded by covalent binding of metal complexes is appealing. Most of the literature in this application is populated by organic compounds, but a number of these rely on interaction with metals for their effects. As understanding of this mechanism increases, organometallic compounds may come to occupy a more prominent position in the field.

D. Serine Protease Applications (Table 5)

The serine proteases comprise a major family of proteolytic enzymes with a diverse range of functions. Serine proteases play key roles in the blood coagulation cascade, in complement activation, bacterial pathogenesis, and fibrinolysis.[92] Serine proteases have come into prominence as targets for drug design in antiviral and antibacterial research.[93] A large class of inhibitors for serine proteases are the boronic acids and peptide−boronic acids.[37,81,94,95] The boronyl groups are believed to mimic the transition state of the reaction, while the peptide portion directs specificity to a particular protease.

A series of ^{15}N and ^{1}H studies on protease-inhibitor complexes gave insight to the interactions between trypsin or α-lytic protease and various boronic acid inhibitors. In α-lytic protease and trypsin, the substrate analogues tend to form serine adducts while the nonsubstrate analogues tend to form histidine adducts,[92] although substrate analogues can sometimes form histidine adducts.[96,97] Further ^{11}B NMR studies on a histidine adduct of α-lytic protease and serine adducts of chymotrypsin and α-lytic protease reveal that the boron atom is tetrahedral in both the histidine and serine adducts.[96] Work on phenylboronic acid found that the addition of sugars (D-monosaccharides) actually increase the inhibitory effect on α-chymotrypsin.[98] This is proposed to be due to the acidification of the boron atom by self-complexation with the D-monosaccharides. This synergistic effect is pH dependent and is not observed above pH 9.5 (enzyme inhibition by the boronic acid alone is not observed). The enzyme is strongly inhibited between pH 4 and 9 in the presence of phenylboronic acid and fructose with the greatest sugar-induced enhancement of inhibition at approximately neutral pH. A variety of tripeptide boronate esters also act as serine protease inhibitors.[99]

Additional peptide−metal complexes have also been identified as serine protease inhibitors. Co(III)-ligated amino acids and dipeptides were reported to inhibit chymotrypsin and trypsin.[100,101] These studies were undertaken to examine effects of pentamminecobalt(III), which is used as a hydrophilic carboxyl protective group in enzymatic peptide synthesis. It had been previously observed that the presence of this moiety on the amino acid adjacent to the enzyme-susceptible bond blocked catalytic conversion. Inhibition of purified chymotrypsin and trypsin by the ligated peptides was competitive and reversible. Subsequent studies by the same group suggest that the ligated peptides bind to the enzymes via Coulombic interactions.[101] Cobalt(III)-ligated peptides of various lengths were tested against trypsin, chymotrypsin, and proteinase K. On the basis of modeling observations for chymotrypsin, the distance between two negatively charged Asp residues (Asp-35, Asp-64) in the S′ binding and Ser-195 in the active site corresponds with the length of the optimal-sized inhibitor peptide. The order of inhibition efficiencies metallopeptide > amide > free peptide also matches expectations considering the two negatively charged side chains in the binding pocket. In the presence of increasing concentrations of NaCl, inhibition by metallopeptides worsens in efficiency while inhibition by free peptides improves. There is no change in the inhibition by amide. These results are consistent with an electrostatic interaction between the inhibitors and the active site. In contrast, trypsin has two positively charged residues, Lys-60 and Arg-62, in the S′ binding region and, correspondingly, inhibition is more efficient for free peptides than the positively charged metallopeptides. Observations for proteinase K were not consistent with the pattern of efficiencies found in the other two enzymes, suggesting that the single positively charged Arg-218 in the S′ region does not determine binding. These studies demonstrate that the addition of a metal compound can change the binding affinity for a substrate through electrostatic effects.

Table 5. Serine Protease Inhibition

complex	metal	enzyme inhibited	ref
amino acid and peptide ligations	Co	α-chymotrypsin	100, 101
Schiff base, Figure 9	Co	thrombin, thermolysin	102−104
various boronic acids	B	trypsin	92
peptide ligated	B	α-lytic protease	96
phenylboronic acid	B	α-chymotrypsin	98
tripeptide	B	trypsin, α-lytic protease, trypsin-like proteases	99
BABIM, Figure 10	Zn	serine proteases	106−109
titanyl sulfates	Ti	trypsin	93

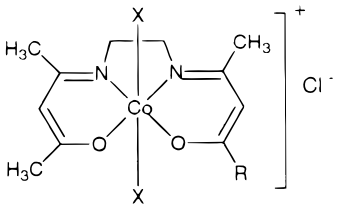

Figure 9. R = CH_3 for Co(acacen)X_2; R = COOH for Co (aciden)X_2, peptide attached through COOH group.

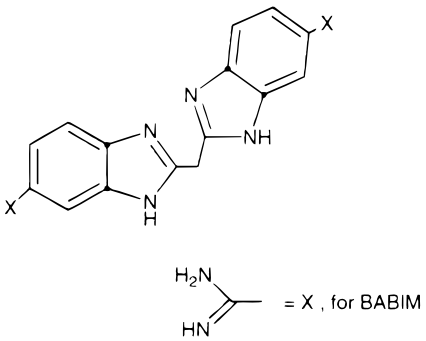

Figure 10. BABIM, bis(5-amidino-2-benzamidozolyl)-methane and derivatives.

In studies from our laboratory, Co(III) Schiff base complexes and peptide conjugates of these complexes were shown to effectively inhibit thrombin and thermolysin (Figure 9).[102−104] The peptide conjugate selectively inhibited thrombin in a mixture with two other serine proteases (K_i = μM). Selectivity is conferred by the peptide component, while inhibition is believed to occur by cobalt(III) coordination to histidines in the active site. Alternatively, the cobalt may be coordinating to histidines near but not in the active site to lock the peptide into the substrate binding pocket. We have also demonstrated the ability of these complexes to bind to histidines in a model system using a zinc finger peptide from the HIV nucleocapsid protein.[105] In those studies, ^{1}H NMR spectroscopy demonstrated direct interaction between the cobalt complexes and histidines in the zinc finger, which lead to a disruption of zinc finger structure. This offers the possibility that these complexes may inhibit zinc metalloproteases as well.

Recently, a class of zinc-mediated serine protease inhibitors has been characterized (Figure 10).[106−109] In crystallographic studies on trypsin, these inhibitors were seen to tetrahedrally coordinate an exogenous zinc ion between two chelating nitrogen atoms of the inhibitor and two residues, His57 and Ser195, in the active site.[107] The presence of zinc enhances inhibition by over 3 orders of magnitude and in the case of some inhibitors seems to promote selectivity. While this class of inhibitors falls outside the scope of this review, it represents a unique methodology in which the inhibitor itself is not a metal complex, but the involvement of a transition metal is key to inhibition.

Serine proteases are implicated in a number of disease processes, and inhibition of these enzymes is an intense field of study.[107,109] The presence of histidines in the active site and the affinity of hard acids for nitrogen make the inhibition of serine proteases by metal complexes a logical target. A new approach to inhibitor design, described above, binds organic compounds to active sites through the "bridge" of a zinc ion (borderline Lewis acid), suggesting a possible role for zinc as a regulatory element. These small molecules represent potential frameworks upon which a number of different inhibitors could be constructed, where the inhibitory portion of the molecule remains the same and the remainder of the molecule can be modified to change its specificity. This modular approach would be a valuable addition to the current repertoire of serine protease inhibitors.

E. Neurological Applications (Table 6)

One of the most well-known applications for metals in neurology has been the use of lithium as a treatment for bipolar disorder (manic-depressive illness). Lithium therapy was introduced in 1949, but the exact mechanism for its therapeutic effects was unknown. In recent years, reports have indicated that lithium affects the inositol phospholipid hydrolysis pathway (unclear if this is due to inhibition of inositol-1-phosphatase) and inhibits adenylate cyclase.[110] Lithium is the topic for another review in this issue, so we direct interested readers to that article for more information.

The role of aluminum in Alzheimer's disease has been a hotly debated subject, and there is as yet no established mechanism for its involvement in disease progression. The connection of aluminum to the disease has been implicated circumstantially by the increased levels of aluminum in the brains of Alzheimer's disease and amyotrophic lateral sclerosis patients.[111−113] Aluminum content is high in the plaques, neurofibrillary tangles, and neuritic deposits characteristic of these disorders. Aluminum has been observed to inhibit brain hexokinases[114] and noncompetitively inhibit brain mitochondrial monoamine oxidase-A (MAO-A)[115] (also observed for ileal MAO, as discussed in the section on cancer). Interestingly, aluminum seems to activate serine proteases, and the activated proteases are subsequently resistant to inhibition by other inhibitors.[111,112] Serine proteases cleave β-amyloid precursor proteins (APP) to generate β-amyloid peptides. A model is proposed in which aluminum suppresses the inhibitor domain of the proteases, thus increasing the proteolytic generation of peptides which then accumulate and initiate plaque formation.[111] This offers the intriguing idea that aluminum acts as an inhibitor of an enzyme's inhibitor domain.

Other metals have been implicated in the formation of amyloid plaques. Zn^{2+}, Fe^{2+}, and Cu^{2+} (μM) and Al^{2+} (mM) were observed to cause aggregation of physiological concentrations of β-amyloid protein.[116−121] A histidine-modified human A beta(1−40) is not aggregated by Zn^{2+}, Fe^{2+}, or Cu^{2+} suggesting that the histidines are involved in the metal-induced aggregation.[116] The metals appear to work by different mechanisms. Fe^{2+}-induced aggregation is inhibited by the presence of antioxidants, while Zn^{2+} induction of aggregation is not affected. This discrepancy implies that Fe^{2+} causes aggregation by an oxidative mechanism.[117] Oxidation is known to induce aggregation,[122] and the β-amyloids have been found

Table 6. Neurological Applications

complex	metal	enzyme inhibited	ref
ion	Li	adenylate cyclase, inositol-1-phosphate, tryptophan hydroxylase, Ca^{2+}−ATPase	110
ion	Al	serine proteases, α-chymotrypsin	111
ion	Al	monoamine oxidase-A	115
ion	Al	calpain	113
ion	Al	calpain, hexokinase, glucose-6-phosphate dehydrogenase	112
porphyrins, Figure 11	Fe, Zn	acetylcholinesterase	125
porphyrins	Cr, Zn	heme oxygenase, nitric acid synthase	75
protoporphyrin	Sn	heme oxygenase	76
cisplatin, copper sulfate	Pt, Cu	monoamine oxidase	6
ion	Cu, Cd, Pb, Mg, Zn	lysosomal, cytoplasmic proteinases, calpain I and II	127
ion	Zn	matrix metalloproteinases, secretases, cytochrome *c* oxidase, nitric oxide synthase, endonuclease	118

to have pro-oxidant properties, especially in the presence of aluminum.[123] Interestingly, nitric oxide donors such as SNP (NO is generated by oxidative stress) were found to mediate an accumulation of zinc in hippocampal cells, offering the possibility that oxidative effects are linked to zinc effects.[118] The mechanism of Zn^{2+}-induced aggregation, in contrast, does not rely on oxidation but appears to be due to direct interaction with the peptide.[116,117,120,121] In addition to its aggregation effects, Zn^{2+} inhibits a number of proteolytic enzymes involved in processing of amyloids including matrix metalloproteinases (MMPs, responsible for degrading amyloid peptides) and possibly α-secretase (responsible for cleaving the amyloid precursor protein) (see review[118]). These inhibitions of proteolysis are mediated by two different activities of the metal upon the substrate. (i) APP has a binding site for Zn^{2+} that inhibits cleavage at the α-secretase site, and (ii) aggregated amyloids are not cleaved by MMPs. Copper and cobalt (50 μM) have also been found to inhibit APP cleavage.[124] Direct interaction with the secretases was not assessed in any of the APP studies.

Besides amyloid plaques, Alzheimer brains are characterized by neurofibrillary tangles. Various factors are under investigation for the cause of this phenomenon, including abnormal transport of neurofilaments and abnormal degradation. Aluminum has been shown to inhibit a calcium-activated neutral proteinase (calpain II) which mediates the breakdown of neurofilament subunits and cytoskeletal proteins.[113] $AlCl_3$ and aluminum lactate inhibited purified calpain II, from human cerebral cortex, with IC_{50}s of 200 and 400 μM, respectively. Inhibition of calpain activity involved direct interaction with the enzyme, since preincubation of substrate [^{14}C]methylazocasein with aluminum did not affect inhibition efficiency. Aluminum inhibition also involves interaction with cytoskeletal proteins, and in some cases (neurofilament subunits NF−H and NF−M) aluminum induces the formation of urea-insoluble complexes. Thus, the inhibition of proteolysis by aluminum is a multifaceted process involving direct binding to the enzyme, binding to the substrate to interfere with proteolysis, and formation of substrate complexes that are resistant to proteolysis. Aluminum has a high affinity for phosphates and is believed to bind to phosphorylated groups on the neurofilament proteins.[113] Despite this interaction, the mechanism of calpain inhibition is not elucidated.

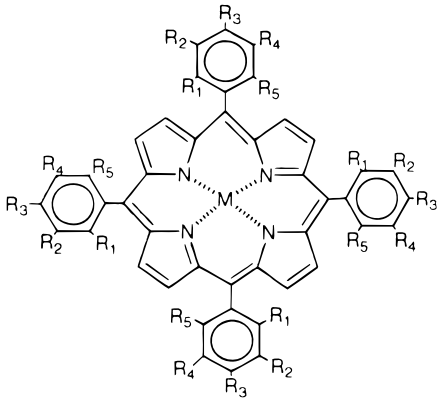

Figure 11. Tetraphenyl porphyrins.

Aluminum has also been shown to inhibit metabolic enzymes in the brain, thus producing metabolic errors. It has been suggested that the accumulation of such metabolic errors contributes to neurological disorders such as Alzheimer's disease. Hexokinase, a Mg(II)-dependent enzyme, catalyzes the first step in the glycolysis cycle, which breaks down glucose for energy. The inhibition of hexokinase seems to be mediated by binding of aluminum to the substrate; indeed, ATP has a 10^7 fold higher affinity for Al(III) than Mg(II).[112] While the majority of glucose goes through glycolysis, 15−20% goes through the hexosemonophosphate shunt. One of the key enzymes in that pathway, glucose-6-phosphate dehydrogenase (G6PD), is inhibited by Al(III) (μM range).[112] This appears to be due to direct interaction with the enzyme, and Al(III)-bound enzyme undergoes a conformation change to a more randomly coiled structure (based on circular dichroism spectroscopy).

Currently, organic acetylcholinesterase inhibitors have been used in treatment of Alzheimer's disease; however, zinc and iron porphyrins (Figure 11) also have been observed to inhibit acetylcholinesterase.[125] The number and placement of fluorine groups on the porphyrin ring had a profound effect on the inhibitory activity. Modeling studies are underway to more closely examine the contacts between the inhibitors and acetylcholinesterase to see if these interactions match configurations suggested by a recently proposed model of the enzyme active site.[126]

Given the evidence that metal ion accumulation has been observed in several diseases of the central nervous system (CNS), there is considerable interest in determining whether this accumulation plays a

role in disease pathogenesis and if so what is the biochemical nature of the involvement. In a recent study, a large number of lysosomal and cytoplasmic proteinases which are known to be found in the cerebral cortex have been screened for inhibition by metal ions.[127] Millimolar concentrations of Cu^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, and Zn^{2+} were found to inhibit all lysosomal proteinases tested. These ions also inhibited cytoplasmic proteinases to various degrees depending on the metal ions. Proline endonuclease and leucyl aminopeptidase were inhibited 50% or more by Zn^{2+} and Cu^{2+}, respectively. Alanyl-, leucyl-, and tripeptidylaminopeptidases were inhibited by 0.05 mM Fe^{2+}. Pyroglutamyl aminopeptidase was inhibited by 0.05 mM Cu^{2+}, Cd^{2+}, Mn^{2+}, Hg^{2+}, or Zn^{2+}. Alanyl- and tripeptidylaminopeptidases, proline endopeptidase, and pyroglutamyl aminopeptidase were inhibited to varying degrees by Co^{2+} and Mn^{2+}. Cd^{2+}, Hg^{2+}, Mn^{2+}, Zn^{2+}, and Al^{3+} all inhibited calpains I and II to varying degrees at 5 mM concentration. The implications of the metal ion findings remain to be determined but do indicate that the role for metal ions and enzyme inhibition in the development of CNS disease should be investigated more closely.

It is evident from these works that the role of metal ions in neurological disease is complex and varied. Metal ions demonstrate a diverse range of effects from kinetic to structural. Kinetic effects can be inhibitory or activating. Structural effects can encourage aggregation or discourage binding. These effects hint that metal ions may be intimately involved in enzyme regulation. This would be indicated by the in vivo results; however, many of these effects require millimolar concentrations of metal, and the conditions necessary to produce such concentrations in vivo must be considered before forming such conclusions. This would distinguish potential regulatory processes from toxic effects.

F. Arthritis Applications (Table 7)

Gold compounds, such as Auranofin (Figure 12), have been extensively employed in the treatment of rheumatoid arthritis, although the mechanism for their action has not been established. Many studies have demonstrated an inhibition of lysosomal enzymes,[128,129] and more recently inhibition of protein kinase C has been observed which may explain the immunosuppressive effects of gold salts.[130,131] Gold-based therapeutic agents are the topic of another review in this issue.

Copper complexes[132,133] and copper complexes of antiinflammatory drugs[134] have shown effectiveness as antiinflammatory agents. The complexes seem to affect a number of enzyme systems, and copper itself may play a role in disease pathogenesis. Arthritis

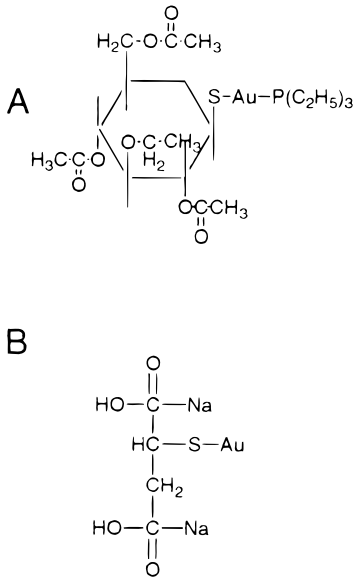

Figure 12. Gold complexes: (A) Auranofin, (B) gold sodium thiomalate.

sufferers have higher mean serum or plasma concentrations of copper in direct correlation with the severity and activity of the disease.[132] Copper was found to inhibit glutathione-*S*-transferase, and decreased GST activity is symptomatic of chronic adjuvant arthritis. A number of copper-dependent enzymes are known to be necessary for repair of inflamed tissues.

The enzymes of the proteolytic pathways are frequently implicated in the destruction of cartilage in arthritis. These are metalloproteinases, serine proteases, and cysteine proteases that are generally found in conjunction with natural inhibitors. The calpain−calpastatin system has recently received attention after the findings that calpastatin (inhibitor of calpain) is an autoantigen in almost 50% of rheumatoid arthritis patients and increasing evidence that calpain is overactivated in arthritis.[135] Calpain is a Ca(II)-dependent cysteine protease that is found in two isoforms: calpain I, which requires low concentrations of calcium for activation, and calpain II, which requires high concentrations. Aluminum ions affect the two isoforms differentially in a calcium-dependent manner.[136] In the presence of millimolar calcium, Al inhibits calpain II in a concentration-dependent manner, while calpain I is inhibited maximally within a narrow concentration range (0.1−0.5 mM Al). In the absence of calcium, aluminum activates calpain II (which is otherwise inactive). It has yet to be shown if these effects occur in vivo.

Given the effectiveness of boranes as serine protease inhibitors, discussed in an earlier section, and

Table 7. Arthritis Applications

complex	metal	enzyme inhibited	ref
Auranofin, sodium thiomalate, Figure 12	Au	protein kinase C	130, 131
trimethylamine carboxyboranes	B, Cu, Fe	elastase, collagenase, lipoxygenase, cathepsin, cycloxygenase	137
ion	Al	calpain I	153
amine carboxyboranes and their esters	B	inosine monophosphate, de novo regulatory enzymes in lipid synthesis	138

the role of proteases in arthritis, it is not surprising that a number of amine carboxyboranes have been found to behave as antiinflammatory agents. Copper and iron complexes of trimethylamine carboxyborane inhibited lysosomal enzyme activity and proteolytic enzyme activity in macrophages,[137] as do some amine carboxyboranes themselves.[138] Inhibited enzymes include neutral cathepsin, trypsin, elastase, collagenases I and II, prostaglandin synthase, cyclo-oxygenase (induced in inflammatory arthritis[139]), and 5′-lipoxygenase. All were inhibited at micromolar concentrations.[137] The amine carboxyboranes were also shown to have a multitude of effects, including inhibition of regulatory enzymes involved in de novo lipid, DNA, and RNA synthesis. In vivo experiments also found various amine carboxyboranes to exert a diverse set of effects including lowering serum cholesterol, blocking calcium resorption (of concern in osteoporosis), and reducing inflammation.[138] Further investigation is warranted to determine the specificity of these agents and the structural features which impart the various therapeutic properties.

Arthritis is a fine example of a disease in which metal complexes have been employed heavily in therapy but have an unknown mechanism of action. As the role of proteases in disease progression becomes better characterized, new targets for therapy may be revealed which will expand the list of potentially therapeutic metal complexes.

III. Applications to Infections

A. Viral (Table 8)

Viruses use a comparatively few, unique enzymes to carry out their life cycles, making these enzymes attractive targets for antiviral drugs.[140] For example, the genome of the human immunodeficiency virus (HIV) encodes three critical enzymes: a reverse transcriptase that converts the infecting viral ssRNA to dsDNA, an integrase that inserts this dsDNA into the host genome, and a protease that trims the viral gene products to their mature forms.[141] In addition, the viral enzymes, in general, and particularly enzyme active sites tend to be more highly conserved than coat proteins, another drug target, and therefore less subject to the mutational drug resistance that is the bane of antiviral drugs. The past decade has sown many seeds of research into combatting HIV, and one of the main targets for therapy has been reverse transcriptase.

Reverse transcriptase (RT) stands out as a target for inhibitors because it is distinct from normal host cell polymerases and physically separate from the host cell replication machinery (the viral transcriptase is cytoplasmic rather than nuclear). Reverse transcriptase is a multifunctional enzyme performing as a polymerase that generates the plus and minus strands of DNA and as an RNase H that degrades the RNA portion of RNA−DNA hybrids.[142] Inhibition can target any of these functions or binding sites on the enzyme, but the currently licensed inhibitors all are directed toward the polymerase function.[143] Many nucleoside analogue and non-nucleoside inhibitors have been developed over the past years, but relatively few inhibitors have been metal complexes. Polyoxotungstates, especially a series of silicotungstates of the Keggin class, are one class of inorganic compounds that have shown antiviral activity.[144] The compounds inhibit viral replication in HIV infected cells in culture and exhibit competitive and noncompetitive inhibition of purified and poly(ethylene glycol)-precipitated reverse transcriptase. Inhibition of the RNA-dependent DNA polymerase function was achieved at 3−6-fold lower concentration than for the DNA-dependent DNA polymerase activity. Assays on other purified DNA polymerases indicate that the silicotungstate compounds are most sensitive against Muloney murine leukemia virus ($IC_{50} = 0.0035\ \mu M$), less effective against HIV-1 RT ($IC_{50} = 3\ \mu M$), and relatively ineffective against human DNA polymerase 1 ($IC_{50} = 43\ \mu M$). Curiously, although DNA polymerase α was slightly more sensitive to inhibition than HIV-1 RT, tests in culture showed antiviral activity without cytotoxicity and concentrations required to produce cytotoxicity were orders of magnitude higher than the concentration necessary to eliminate virus. Although the specificity of these compounds against RT is not established and the exact site of inhibition on RT is not known, their promising antiviral action in culture merits further investigation.

Thiosemicarbazones have existed as antiviral agents for some time and diorganotin(IV) complexes of pyridyl thiosemicarbazones (Figure 13) were recently characterized and found to inhibit RT.[145] Copper and

Table 8. Viral Applications

complex	metal	enzyme inhibited	ref
polyoxotungstates	W	HIV reverse transcriptase, RNA polymerase, DNA polymerase	144
thiosemicarbazones, Figure 13	Sn	leukemia virus reverse transcriptase	145
TSAO, Figure 15	Si	HIV reverse transcriptase	149
trifluoperazine	V, Cu, Ni, Pd, Sn	leukemia virus reverse transcriptase	147
streptonigrin, Figure 14	Zn	topoisomerase, HIV reverse transcriptase	148
ions	Fe, U, V, Ti, Pb, Cu, Pd	proteinase	152
bathocuproine disulfonic acid, Figure 16	Cu	protease, integrase	156
ions	Zn	protease	154
ions	Zn	renin, protease	153
inhibitor chelate, Figure 17	Cu	protease	157
phenanthroline, neocuproine, bathocuproine complexes, Figure 18	Cu	integrase	158
aromatic polyhydroxylates	Mn	integrase, other metal-requiring enzymes	159
porphyrins	Fe, Co, Fe	heme oxygenase	70

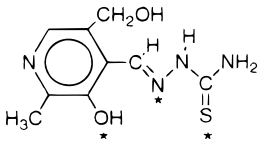

Figure 13. $SnR_2(L)$ R = Me, Et, Bu, Ph; H_2L = pyridoxal thiosemicarbazone, only ligand is shown. Asterisk (*) indicates metal binding atoms.

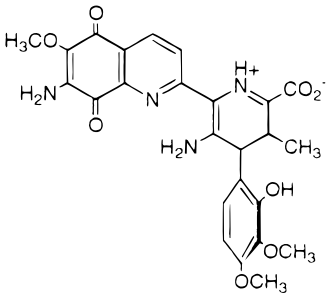

Figure 14. Streptonigrin.

cobalt complexes of the same ligand (L) have also been investigated.[146] The $Sn(Bu)_2(L)$ and $Sn(Ph)_2(L)$ complexes were the most effective against RT, but there were no tests against other polymerases to evaluate the specificity of the inhibition. Therefore, the antiviral action of the compounds cannot be solely attributed to their inhibition of RT based on these studies. Thiosemicarbazones are also known to inhibit ribonucleotide reductase, RNA-dependent DNA polymerase, and dihydrofolate reductase.[19]

Trifluoperazines (TFP), derivatives of the psychotherapeutic phenothiazine drugs, also have proven antiviral effects. Metal complexes of TFP were tested on Muloney murine leukemia virus RT, and the metals were found to enhance inhibition in the order VO(IV) > Ni(II) > Pd(II) > Cu(II) > Sn (IV) > free ligand.[147] These complexes seem to be effective against RTs of multiple retroviruses. Streptonigrin (Figure 14) is an antibiotic that has shown promise as an antitumor agent for various human cancers; however, the toxic side effects of treatment with streptonigrin led to discontinuation of clinical trials. Streptonigrin also selectively inhibits avian myeloblastosis virus (AMV) and HIV RT without inhibiting cellular polymerases.[148] The mechanism of action of streptonigrin was investigated after the clinical trials were discontinued. A review of the more recent results of these efforts, including detailed structural characterization of several metal complexes of the drug, indicates that metal complexation is key to the biological activity of the drug.[148] However, the exact mechanism for its therapeutic effect is still unknown. It will be of interest to see if metal complexation improves the antiviral activity as well as the anticancer function.

A different approach to improving inhibition of RT is represented by metal-chelating derivatives of TSAO-T, a [2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) nucleoside thymine derivative. The parent compounds, TSAO, bind to an allosteric site adjacent to the catalytic site of RT.[149] This inhibitor binding site appears to interact with a nearby Mg^{2+} binding site.[150] A new class of compounds was designed which attached the inhibitor, TSAO-T, to a metal chelating moiety, with the belief that this would form a bidentate inhibitor with stronger affinity for the enzyme (Figure 15). Several derivatives were synthesized, and those with short chain alkyls were found to be the most effective antiviral agents. A few derivatives were found to be as active as the parent inhibitor but significantly less toxic (10-fold lower concentration to produce 50% decrease in cell viability). This class of compounds is an example of inhibitors that do not themselves contain a metal but whose inhibition is achieved by formation of a metal complex.

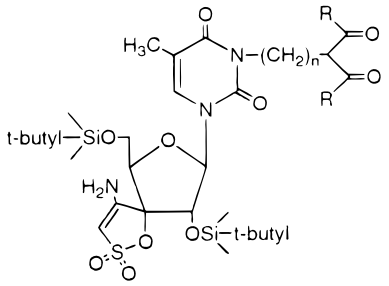

Figure 15. TSAO-T [2′,5′-bis(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathole-2″,2″-dioxide).

HIV-1 protease is an aspartyl protease that generates mature proteins from the products of the *gag* and *pol* genes. Resistance against protease inhibitors seems to be more difficult for the virus than against RT inhibitors,[151] and many peptidic inhibitors have been developed.[95] Many metal ions have been found to be inhibitors of HIV-1 protease, the best tend to be "hard" ions (Fe^{3+}, UO_2^{2+}, VO_3^-/VO_4^{3-}, $TiCp_2Cl_2$, Pb^{2+}) and the most active are tetrahedrally coordinate.[152] The metals appear to inhibit noncompetitively, implying that they may not exclude but occupy the substrate binding site simultaneously with substrate. The metals are believed to coordinate to the carboxylate side chains of active residues Asp 25 and Asp 125, but this remains to be definitively shown. These observations may lead to the design of more potent inhibitors of HIV-1 protease which contain metals that can bind to the enzyme through ionic or covalent interactions and, thus, would be expected to bind more strongly than the peptidic inhibitors.

The metal ion study described above seemingly conflicts with the results of an earlier study which showed that zinc and silver ions noncompetitively and copper ions competitively inhibit protease and renin from HIV-1.[153] The earlier study was performed on recombinant protease isolated from *E. coli*, while the later study utilized a synthetic SF2 sequence of the protease, with aminobutyric acid substituted for cysteine residues 67, 95, 167, and 195. It is unclear how closely the synthetic enzyme models the native enzyme. Also, the studies were performed at different pHs, the earlier studies at neutral pH and the later studies at pH 5. The earlier study, in fact, demonstrates that the inhibition by zinc is strongly pH dependent, estimating that zinc competes with a proton for a group with $pK_a = 7.0$. Molecular dynamic simulations performed on protease in the presence of zinc indicate that zinc ions bind to the catalytic

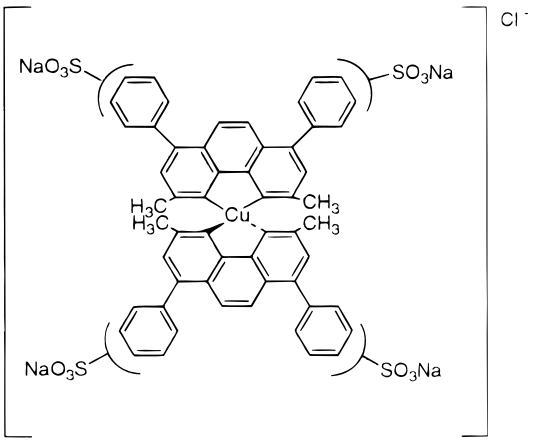

Figure 16. Bathocuproine sulfonic acid-Cu^{1+}.

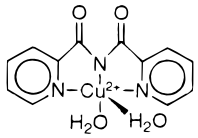

Figure 17. Diaqua[bis-(2-pyridylcarbonyl)amido]copper-(II)nitrate dihydrate.

active site Asp25 and Asp25′ residues without disrupting the structure of the enzyme.[154]

Studies have found $CuCl_2$ to be an effective inhibitor of protease but not of a mutant protease with alanines substituted for cysteines.[155] The mutant protease could be inhibited by $CuCl_2$ in the presence of copper chelators leading to investigation of the efficacy of copper chelates as inhibitors. Bathocuproine sulfonic acid Cu(I), BCSD-Cu(I) (Figure 16), was found to be an competitive inhibitor of protease.[156] BCSD alone does not inhibit the enzyme nor can inhibition by the complex be blocked by EDTA. This indicates that the metal complex is the active inhibitor and not the free ligand or free metal. The complex also inhibits the mutant protease that lacks cysteines. Other copper chelates have been observed to inhibit HIV-1 protease. On the basis of modeling studies, diaqua[bis(2-pyridylcarbonyl)amido]copper-(II) nitrate dihydrate was found to fit in the enzyme active site and experimentally characterized to be a competitive inhibitor of protease (Figure 17).[157] This study also suggests that in the active site a water molecule found between Asp25 and Asp 125 is important for the structure of the enzyme. This consideration may affect the design of inhibitors which do not contain hydroxyl substitutes.

Cuprous complexes also have been found to inhibit HIV integrase (Figure 18).[158] These seem to inhibit integrase noncompetitively by some mechanism other than simple blockage of substrate binding or binding to DNA. Inhibition of C- and N-terminal deletion mutants of integrase by these complexes indicates that the complexes are active in the core region of the integrase. Selectivity of the complexes for integrase remains to be demonstrated. Integrase contains a zinc finger motif near the N-terminus and a conserved motif of aspartate and glutamate residues that may be involved in DNA binding or cleavage.[141] These may also represent sites for potential interaction with metal complexes. A large number of an-

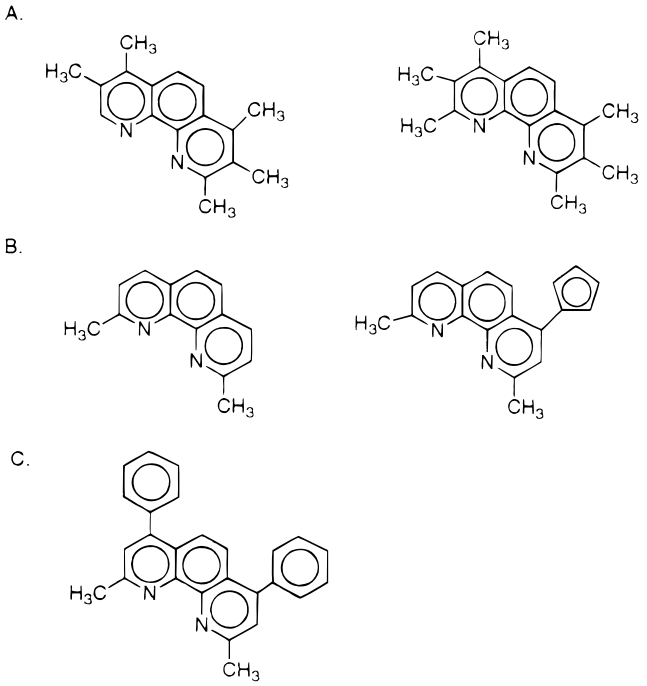

Figure 18. Integrase inhibitors. (A) 1,10-Phenanthroline and derivatives: 2,3,4,7,8-pentamethylphenanthroline, 2,3,4,7,8,9-hexamethyl phenanthroline. (B) Neocuproine (2,9-dimethyl-1,10-phenanthroline) and derivative. (C) Bathocuproine (4,7-diphenyl-2,9-dimethyl-1,10-phenanthroline).

throquinones and related polyhydroxylated phenolic molecules have been shown to inhibit integrase at sub micromolar concentrations.[159] The compounds cross-reacted with other metal-requiring enzymes tested (Muloney murine leukemia virus reverse transcriptase, MoMLV, and the restrictions enzymes Pvu II and EcoRI) but not with enzymes that do not require metals. This suggests that metals are involved in the inhibitory mechanism, and indeed, some of the compounds tested are known to be metal chelators. Furthermore, MoMLV inhibition was more efficient in the presence of Mn^{2+} than Mg^{2+} (up to 26-fold decrease in IC_{50}, depending on compound), an effect which has also been observed for integrase.[160] The catalytic domain for integrase is known to bind metals, and inhibition may involve the formation of a ternary complex between inhibitor, metal, and enzyme; however, this remains to be proven.

For the most part, the viral enzyme inhibitors presented have not demonstrated exclusive specificity. With the exception of the TSOA-T derivatives, these inhibitors have not been designed for interaction with particular enzyme sites. Many of the inhibitors were identified by screening first for inhibition of viral replication and then for activity on purified enzymes. In general, cross-reactivity with host enzymes was not vigorously addressed, and this should be pursued where lacking or a convincing case must be made for why the inhibitor should not cross-react. With the advent of detailed crystal structures and more sophisticated molecular modeling, the design of organic inhibitors has achieved a new level of precision in targeting. These advances are applicable to the design of organometallic inhibitors as

well and can be capitalized upon to accelerate advancement of the field.

B. Nonviral

1. Bacteria, Fungi, and Parasites (Table 9)

Some of the earliest applications of inorganic compounds in medicine have been as antiseptics and antimicrobials.[77,94,161] In fact, organoarsenicals were the first compounds applied for the successful treatment of syphilis (they have since been replaced by penicillin) and have been used as feed additives in livestock to prevent bacterial and parasitic infections.[94] Sulfanilimides are a large class of compounds that show antibacterial effectiveness, and several metal ions are known to act as antimicrobials.[162] Organometallic compounds are routinely screened for antimicrobial effects to identify potential drugs. In the context of this review, we limit our discussion to agents that are known to exert their effect by inhibiting enzymes. Many other agents exist for which there is yet no known mechanism; their effects may yet be revealed to be enzyme-mediated as well.[163−167]

Recent work has evaluated the inhibition of serine proteases in bacteria and fungi for the development of antimicrobial agents. Screening of a large number of metal ions against trypsin from various sources (purified and in bacterial lysates) and bovine pancreatic chymotrypsin revealed that only Ti(IV), as titanyl sulfates, inhibited trypsin while the other metals either enhanced activity (Ca^{2+}, Mn^{2+}) or had no effect.[93] This inhibition is competitive, indicating that the inhibitor binds to the active site. Chymotrypsin is not inhibited, leading the authors to propose that binding of Ti(IV) occurs at Asp-198 in the substrate binding pocket, a residue which is absent in chymotrypsin. Titanyl alcohol complexes were found to be less inhibitory (25% of inhibitory activity) than the sulfates. The five-coordinate geometry of Ti(IV) is discussed to explain the observed activity of Ti(IV) against bacteria compared to other metal ions and geometries.

Trivalent arsenical drugs such as melarsoprol (Figure 19) are still routinely utilized in the treatment of trypanosome-mediated illnesses such as African sleeping sickness. The mechanism for their activity is unknown but is believed to be due to an interaction with protein dithiols. Trivalent arsenicals are known to form stable adducts with dithiol trypanothione, and these adducts are effective competitors of trypanothione reductase.[168] Trypanothione,

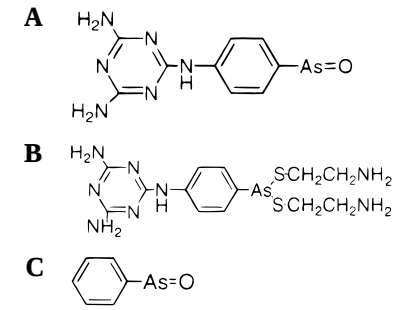

Figure 19. Arsenicals: (A) melarsen oxide, (B) cymelarsen, (C) phenylarsenoxide.

analogous to glutathione in mammals, is unique to trypanosomes. A recent study examined the influence of several arsenicals on trypanothione reductase and glutathione reductase, both of which contain catalytic sulfydryl groups.[169] Inhibition of both enzymes is much more sensitive in the presence of NADPH, indicating that the cysteine sulfydryls must be reduced to interact with the drugs. Trypanothione reductase was more sensitive to inhibition than glutathione reductase, but inhibition is abrogated by dihydrotrypanothione. While the arsenicals do inhibit the enzyme, the authors report that since dihydrotrypanothione is in vast excess of trypanothione reductase in vivo, it is unlikely that the inhibition of trypanothione reductase is the mechanism for the therapeutic effect on trypanosomiasis.

Antimonials have historical precedent as antiparasitics[94,161] and are still the drug of choice for the treatment of mucosal leishmaniasis;[170] but other metals are under investigation for their activity against leishmaniasis. Acid phosphatases (ACP) appear to be involved in the pathogenicity of leishmanias, and these phosphatases are inhibited by a number of transition metals and transition-metal complexes such as arsenate and vanadate.[171] Studies employing tartrate-sensitive acid phosphatase from human seminal fluid and tartrate-resistant phosphatase from *Leishmania donovani* demonstrate that heteropolyanionic molybdenum complexes (with Ge, Fe, As, Ce, Th) inhibit these enzymes selectively.[172] Four classes of complexes were assayed containing 4, 6−8, 12, or 18 Mo atoms. Selectivity was assessed based on the inhibition of these enzymes and not ACP from human spleen. β-glucuronidase and α-mannosidase from the spleen also showed little to no inhibition by the molybdenate heteropolyanions. Cross-competition studies between these inhibitors indicate that some of the complexes bind to the active site while others bind to another site that affects the

Table 9. Antimicrobial Applications

complex	metal	enzyme inhibited	ref
1-amino-3-(2-pyridyl)isoquinoline, ions, Figure 22	Cu	NADH oxidase, lactate dehydrogenase	176
sulfadiazene, Figure 20	Ag	phosphomannose isomerase	174
trivalent arsenicals, Figure 19	As	trypanothione reductase, glutathione reductase	169
$[X_xM_xO_oH_h]^{n-}$	Mo, V	acid phosphatases	172
sugar complexes, Figure 21	Ni	chitinase	175
trivalent antimonials	Sb	phosphofructokinase	173
Pentostam	Sb	clinical demo	170
thiosemicarbazones	Cu	dihydrofolate reductase	19
ion	V	ecto-acid phosphatase	171
titanyl sulfates	Ti	serine proteases	93

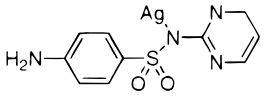

Figure 20. Silver sulfadiazine.

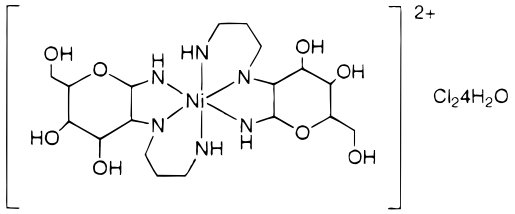

Figure 21. [Ni(D-GlcN-tn)$_2$] $Cl_2 \cdot 4H_2O$ complex cation.

active site. For example, the binding of vanadate and one of the Mo complexes was not mutually exclusive, and vanadate enhanced the inhibition afforded by the Mo complex.

Antimony is also employed as an antischistosomal agent. Recent investigations found that various antimonials inhibit phosphofructokinase (PFK) from *Schistosoma mansoni*.[173] Schistosomes rely on a high rate of glycolysis for survival, and PFK is a key enzyme, containing 20 cysteines, in the glycolytic pathway. Comparison between schistosomal PFK and mammalian PFK found a preferential inhibition of the parasitic enzyme by antimony potassium tartrate. The mechanism for this selectivity has not yet been assessed.

Metal ions and metal complexes show effectiveness as antifungal agents against the pathogenic yeast *Candida albicans*. Silver complexes (Figure 20) inhibit phosphomannose isomerase (PMI), a key enzyme in the biosynthesis of yeast cell walls, at nM K_i.[174] A mutant of PMI with Cys 150 replaced by alanine behaved in a similar manner as the native enzyme but was not inhibited by silver compounds and was a 1000-fold less sensitive to mercury. This indicated that the site of interaction for the inhibitors is Cys 150. Silver ions inhibit PMI with a 210-fold higher bimolecular rate constant for the human enzyme. The compounds reduce the specificity for the human enzyme to 1.3:1. While this does not qualify as a parasite-specific enzyme inhibitor, it suggests the potential for creating a specific drug by modifications to the organic moiety. Nickel complexes (Figure 21) inhibit Chitinase, also involved in cell wall biochemistry, from *C. albicans*.[175] These complexes contain *N*-glycosides derived from amino sugars and inhibit in a competitive manner.

Mycoplasma lack a rigid cell wall. Ionic copper is an effective inhibitor of mycoplasmal activity and has been shown to inhibit enzymes in the glycolytic pathway, NADH oxidase, and lactate dehydrogenase.[176] 2,2′-Bipyridyl type ligands (Figure 22) facilitate transport of copper across the mycoplasmal membrane, and the antimycoplasmic activity of copper is much increased in the presence of these ligands, although the presence of the ligands decreases the inhibitory effect on purified, isolated enzymes. The authors propose that this result confirms that the copper ion is the toxic species and the ligand merely serves as a carrier.

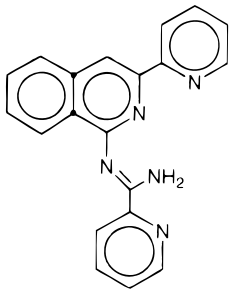

Figure 22. *N*-[3-(2-pyridyl)isoquinolin-1-yl]-2-pyridine carboxamidine.

Table 10. Ulcer Applications

complex	metal	enzyme inhibited	ref
ions	Bi	faecal mucin sulfatase	178
ions	Bi	H. pylori F ATPase	177

Echoing observations made earlier about anticancer and antiviral agents, the action of many antimicrobials is still "black box" in nature—compounds exert their effect by an unknown mechanism. More detailed isolation and structural characterization of the sites of action is necessary. Attaining a complete understanding of the effectiveness of inorganic compounds against infections may also bolster the development of anticancer agents. In both applications the therapeutic drug must combat abnormal organisms while leaving the surrounding normal tissues intact, operating as a controlled toxin.

2. Ulcer Applications (Table 10)

Bismuth salts represent the bulk of the work on inorganic compounds applied for ulcer therapy. Bismuth salts have been shown to inhibit ATPase from *H. pylori*, a bacterium involved in the development of peptic ulcers[177] and faecal mucin sulfatase, which is elevated in ulcerative colitis.[178] Since another review in this issue is devoted to medicinally relevant bismuth compounds, we will only note the possible involvement of enzyme inhibition in the therapeutic effect and defer the interested reader to the bismuth review.

IV. Toxicity Applications (Table 11)

A great deal of work has been devoted to determining the molecular mechanism for metal toxicity. The cause for lethality is likely to be multifactorial; however, some understanding of the pathways affected provides a means to combat toxicity and offers insight to potential applications that harness this toxicity for beneficial effect.

Aluminum toxicity induces anemia, and this is believed to be due to perturbation of the heme biosynthetic pathway;[179] biosynthesis was described in a previous section. Aluminum has been observed to inhibit a number of enzymes in vitro including δ-aminolevulinic acid dehydratase (aka porphobilinogen synthase),[179] hexokinase,[180] glycerol kinase,[181] and ATPase.[182] The mechanism for the inhibition differs between enzymes. δ-Aminolevulinic acid dehydratase, a metalloprotein catalyzing the second step in heme biosynthesis, binds eight zinc ions.

Table 11. Toxicity Applications

complex	metal	enzyme inhibited	ref
ions	Al	γ-aminolevulinic acid	179
ions	Al	hexokinase	180
ions	Cd, Cu, Hg, Zn, Pb	hexokinase	183
ions	Al	glycerol kinase	181
ions	Al, Cd, Pb	ATPase	182
ions	As	poly(ADP-ribose)polymerase	186
ions	As	pyruvate dehydrogenase, DNA ligase	187
ions	As	methyl transferase	185
ions	As	methyl transferase	188
arsenoglutathione	As	glutathione reductase	184
3-arsonopyruvate, Figure 23	As	phosphoenolpyruvate mutase	189
ion	Ni	DNA polymerase	190
ion	Zn, Cd	DNA ligase	193
ion	Cd	O^6-methylguanine-DNA methyltransferase, DNA polymerase β	191
cis-DDP	Pt	respiratory enzymes	10
ions	V	horse radish peroxidase, superoxide dismutase	195
ion	Ni	MutT-dGTPase	192
ion	Pb	γ-aminolevulinate dehydratase	194

Aluminum inhibits this enzyme noncompetitively, suggesting that it does not bind to the active site and may compete with some or all of the zinc ions.[179] The ionic radius for Al^{3+} is similar to that for Mg^{2+} or Fe^{3+}, and aluminum may inhibit Mg^{2+}-dependent enzymes by substituting for Mg^{2+} (for example in hexokinase[181]), although this has not been observed in all studies.[180] For glycerol kinase and hexokinase, aluminum is also postulated to compete with the MgATP substrate in the form of an aluminum−ATP chelate complex.[180,181] The kinetics of inhibition can yield some clue to the nature of the inhibition site, but structural studies will be necessary to determine the exact course of interaction.

Other studies demonstrate that Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, and Pb^{2+} inhibit hexokinase (low μM).[183] Cd^{2+} and Cu^{2+} were much more effective than the other metals. Inhibition by Cu^{2+} resulted in a concomitant decrease in reduced glutathione levels (GSH). Preincubation of the sample with GSH protected the enzyme from Cu^{2+} inhibition. GSH is known to form GSH−Cu(I) complexes and, GSH's protective effects likely are due to simple removal of Cu^{2+} from solution. A number of models are proposed for the relation between hexokinase, GSH, and heavy metal toxicity in vivo.

Arsenic toxicity appears to be redox dependent with the pentavalent As(V) being much less toxic than its trivalent form, As(III).[184,185] Arsenic is a carcinogen but does not appear to directly damage DNA. The most prominent hypothesis for its mode of action is the inhibition of enzymes by binding to sulfhydryls, and many investigations have examined arsenic's effects on DNA repair and ligation enzymes. As(III) has been found to inhibit poly(ADP-ribose)polymerase, which contains two vicinal dithiols that mediate enzyme binding to DNA strand breaks.[186] The mechanism for the inhibition remains to be determined. Pyruvate dehydrogenase is inhibited by As(III) at micromolar concentrations but not by As(V). Several other DNA repair enzymes assessed were unaffected, although some of them contain SH groups crucial to their function.[187] Arsenite could inhibit ligase activity in cultured cells but not in nuclear extracts from cultured cells, suggesting that the inhibition of ligase is not direct but occurs at some other level in repair.[187] Spectroscopic studies on As(III) inhibition of methyl transferase (an enzyme involved with modification and mismatch repair) indicate that As(III) binds to Cys223 inducing a heavy-atom effect (HAE) at a nearby Trp residue; this is confirmed by the observation that the HAE does not occur in a mutant with Cys223 replaced by Ser223.[185,188] The occurrence of an HAE was based on findings of red-shifted phosphorescence spectra and significantly decreased triplet-state lifetime for the As(III)-perturbed tryptophan. Enzyme activity is not affected by this interaction, so the observation of inhibition in the excess of arsenite is likely to be due to binding to other cysteine residues in the enzyme.

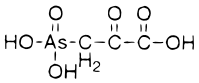

Figure 23. 3-Arsonopyruvic acid.

Inhibition of glutathione reductase by As(III) is consistent with oxidation of thiol groups in the active site of the enzyme.[184] Arsenite complexes with glutathione to form arsenoglutathione, which acts as a mixed type inhibitor for glutathione reductase. Arsenic analogues are often recognized by enzymes that act on phosphorus compounds, such as adenylate cyclase and RNA polymerase. 3-Arsonopyruvate (Figure 23) has been shown to competitively inhibit phosphoenolpyruvate mutase (PEP mutase) with $K_i = 27$ μM.[189] PEP mutase is the main enzyme in the biosynthetic pathway for the formation of C−P bonds.

Nickel, like arsenic, is a genotoxic carcinogen, but the exact mechanism for Ni toxicity is not known. Ni(II) can serve as a weak substitute for Mg(II) in some polymerases but other polymerases are strongly inhibited.[190] The effect of nickel ions on nucleotide incorporation, processivity, and misincorporation by seven different polymerases was assessed, and the primary effect was inhibition (micromolar range). However, this was dependent on a number of factors that varied in importance with each enzyme. The effects of nickel appear to be complex, and the exact mechanism for the disturbance in polymerase func-

tion is not established. Inhibition of DNA replication and repair has been strongly implicated in the mutagenicity and carcinogenicity of metal ions.[191] Ni(II) has been observed to inhibit MutT-dGTPase, which is involved in the repair of 8-oxo-dGTP lesions in DNA (millimolar K_i).[192] Cells under oxidative stress form 8-oxo-dGTP, and the presence of this nucleotide increases the mutation frequency of A to C transversions due to 8-oxo-dG-dA mispairing. Inhibition of the Mg(II)-dependent MutT repair enzyme can lead to increased mutagenesis. Modeling studies suggest that Ni(II) does not appear to bind to the Mg(II) binding site but induces conformational changes which are predicted to occur through binding to histidine residues.[192]

Zinc and cadmium have also been seen to inhibit DNA repair enzymes (see review;[191] note entire issue devoted to mechanisms of metal genotoxicity). Purified DNA ligase is inhibited by 0.8 mM $ZnCl_2$ and 0.04 mM $CdCl_2$.[193] Both metals inhibited the formation of an intermediate, the transferase activity, and the ligation activity but did not affect the misligation rate. This indicated that only the enzyme activity is inhibited and not the fidelity of the ligase. Ligases require magnesium as a cofactor, and Mg is required for all three steps of the ligation reaction that are inhibited by cadmium and zinc. Cd and Zn ions may exert their effect by substituting for the Mg ion in the metal binding site.

One of the most infamous metals, in the public eye, is lead. Lead poisoning occupied a prominent place in the public consciousness as a result of widespread incidents in involving lead in the workplace and also poisoning of children by lead-based paints.[194] One of the mechanisms for toxicity appears to be inhibition of a zinc metalloenzyme, γ-aminolevulinate dehydratase (ALAD), which (as noted earlier) is also inhibited noncompetitively by aluminum. In the case of lead, inhibition occurs through the substitution of lead for zinc. Two alleles of ALAD have been identified in humans, $ALAD^1$ and $ALAD^2$ which code for three distinct isozymes. Screening of large numbers of children (who had been exposed to low levels of lead in the environment) and lead factory workers revealed that $ALAD^2$ individuals have higher blood levels of lead after similar exposure. These individual are significantly more sensitive to lead-poisoning. It is hypothesized that $ALAD^2$ binds lead more avidly.[194]

Metal toxicity can result from oxidative reactions. Vanadate is involved in many such reactions, and recent attention has turned to its affect on antioxidant enzymes.[195] Four different enzymes were surveyed, and vanadate was found to inhibit horseradish peroxidase, but these findings do not indicate a clear relation between antioxidant enzyme inhibition and toxicity.

A large body of literature centers on another mechanism for metal ion induced "inhibition" of enzyme activity: reduction in the amount of enzyme by inhibiting transcription of the gene coding for the enzyme (see reviews in refs 196 and 197). For the most part, the collections of studies on metal ion toxicity seem to emphasize that inhibition occurs at many levels in many systems, and it is unlikely that any single event can be blamed for toxic effects.

V. Miscellaneous Enzymes (Table 12)

In this section we present metal complex-mediated inhibition of a number of enzymes that do not fit into any of the subheadings for the previous sections.

The physiological significance of diamine oxidases is unclear, due in large part to the lack of specific inhibitors for these enzymes. Recent studies investigated a series of organosilicon compounds that inhibit diamine oxidase and semicarbazide-sensitive amine oxidase at micromolar K_i.[198,199] Specificity remains to be proven, and a number of mechanisms for inhibition are proposed. Another silicon compound, ethoxysilatrane (Figure 24) has been shown to inhibit the 58k soluble 3-hydroxy-3-methylglutaryl (HMG) CoA reductase from liver microsomes.[200] Inhibition of HMG CoA reductase is a key step in the autoregulation of cholesterol biosynthesis, and HMG CoA reductase is the target for a number of antihyperlipidemic and antiatherosclerotic drugs.[201] The protein is usually membrane bound on the endoplasmic reticulum, and based on experiments

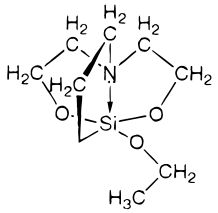

Figure 24. 1-Ethoxysilatrane.

Table 12. Miscellaneous Enzymes

complex	metal	enzyme inhibited	ref
ethoxysilatrane, Figure 24	Si	HMG CoA reductase	200
various complexes	Pd	cellobiohydrolase	202
ions	B, Mo	catechol-O-methyltransferase	204
ions	Zn, Pb, Cd	carboxypeptidase A	206
ions	Zn	caspase-3, fructose 1,6-diphosphatase, glyceraldehyde 3-phosphate dehydrogenase, aldehyde dehydrogenase, tyrosine phosphatase, yeast enolase	36
ions	Zn, Co	apoacyclase	207
dinitrosyl complexes	Fe	Na/K^+ ATPase	209
ions	Ag	Na/K^+ ATPase	208
various complexes	Si	diamine oxidase	198
organosilicon amines	Si	amine oxidase	199
ion	Mg	alkaline phosphatase	210

with soluble and microsomal preparations of the protein, the site of interaction with inhibitors appears to be on the lumenal side.

Palladium complexes inhibit cellobiohydrolase, a fungal cellulase.[202] Palladium complexes were employed to study the nature of the cellulose binding domain of the enzyme since the Pd complexes inhibit the catalytic activity of the enzyme without affecting binding. Ruthenium pentaamine modification of the enzyme, coordinating to histidine residues, did not inhibit catalytic activity, although the modified enzyme was more sensitive to inhibition by palladium. This indicates that binding to histidines is not sufficient to inhibit enzyme activity but that palladium does bind to these as well as the residues responsible for blocking activity. Interaction with disulfide bonds is likely to occur, but this remains to be proven.

Boron does not appear to be an essential trace metal for animals; however, recent evidence suggests that boron may influence steroid metabolism. Postmenopausal women taking boron supplements were found to possess elevated levels of plasma estradiol and testosterone.[203] Boron and molybdenate have been shown to inhibit catechol-*O*-methyltransferase methylation of estrogen and pyrocatechol in rat erythrocytes.[204] Inhibition appeared to be dependent on the presence of phosphate because inhibition did not occur in the presence of Tris buffer. It remains to be established if inhibition is due to direct interaction with the enzyme or due to association with hydroxyl groups on the estrogen catechol.

Carboxypeptidase A is a zinc protease that requires zinc for catalytic activity but is inhibited by zinc in the 10^{-6}−10^{-4} M range.[205] In an effort to characterize the zinc inhibitory binding sites of carboxypeptidase A, the specificity of metal ion inhibition was studied.[206] Zn^{2+}, Pb^{2+}, and Cd^{2+} competitively inhibit activity, although cadmium has a relatively high K_i (2.4×10^{-5}, 4.8×10^{-5}, and 1.1×10^{-2}, respectively). Inhibition by Zn^{2+} and Pb^{2+} is mutually exclusive, indicating that they bind to the same location. Glu 270 is believed to be the site of interaction since chemical modification of the residue with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide greatly diminishes binding. The inhibitory site seems to favor metal−monohydride complexes since the metals with the highest inhibitory activity are those that have the highest [metal−OH^+] values. [Metal−OH] levels reflect the metal's propensity for forming metal monohydride complexes. The zinc inhibitory site may be a general regulatory mechanism for a number of enzymes.

A recent study reports that caspase-3, fructose 1,6-diphosphate, glyceraldehyse 3-phosphate dehydrogenase, aldehyde dehydrogenase, tyrosine phosphatase, and yeast enolase are inhibited by zinc at nanomolar concentrations.[36] This inhibition is reversible by the removal of zinc from the enzymes by thionein (data is only shown for glyceraldehyde 3-phosphate dehydrogenase). These enzymes do not require zinc for activity, so inhibition does not involve removal of zinc from a catalytic site. The location of the zinc inhibition site has not been determined for any of these proteins.

Studies on another zinc enzyme, aminoacyclase, examine the inhibition kinetics of the zinc- and a cobalt-substituted enzyme.[207] The cobalt-substituted enzyme is catalytically similar to the zinc enzyme, and both enzymes are inhibited by Zn^{2+} but not Co^{2+}. Unlike the competitive inhibition observed for carboxypeptidase A, zinc inhibits the initial velocity noncompetitively and cooperatively and over time the enzyme activity falls to a lower level.

Thiols groups are the target for a number of metal−enzyme interactions. Investigations on Na/K−ATPase demonstrate that silver ions[208] and dinitrosyl iron complexes (DNIC)[209] inhibit enzyme activity. Silver is an extremely potent inhibitor that is effective at sub micromolar concentrations, with approximately two silver ions binding per reconstituted molecule. This inhibition is reversible with the introduction of cysteine.[208] These sulfhydryls may be involved in Na, K transport, and the discovery of the potent inhibitory effects of silver may help in elucidating the molecular mechanism behind ion transport. The inhibition by DNIC (micromolar range) is also reversible by cysteine or dithiothreitol.[209] The most effective inhibitors tested were DNIC with cysteine ligands. Inhibition is proposed to occur by the transfer of $Fe^+(NO^+)_2$ from the complex to protein thiol groups. SNP has also been reported to inhibit Na/K−ATPase, and it will be interesting to see if DNIC are formed in this case, since NO radicals do not seem to inhibit the enzyme.[71]

Studies on alkaline phosphatase from human placenta demonstrate differing responses to magnesium ions in aqueous versus micellar systems.[210] Human placental alkaline phosphatase, a membrane-anchored metalloprotein, was entrapped in reverse micellar vesicles or reconstituted in solution. These investigations observed concentration-dependent inhibition by magnesium only in the micellar system. On the basis of fluorescence assays, the authors propose that magnesium binding changes the conformation of the enzyme. The same study reports that high concentrations of zinc also inhibit the enzyme but do not produce the presumed conformation changes.

VI. Metal-Facilitated Inhibition (Table 13)

In the previous sections we have noted a number of inhibitors that do not contain metals themselves but for which the coordination to a metal was somehow key to inhibition.[95] Although they constitute an intriguing class of inhibitors, they are beyond the scope of this review, so we will only present a few additional examples here. A number of therapeutically active inhibitors exert their effect by binding to a metal in the active site of an enzyme. For example, tetracyclines, a widely utilized family of antibiotics, inhibit collagenases and several other matrix metalloproteinases presumably by binding to active site metals.[211] Biphenyl tetrazoles (Figure 25) interact directly with zinc in the active site of metallo-β-lactamase, replacing a metal-bound water molecule.[212] Sulfonilamides bind to zinc in the active

Table 13. Binding to Metal in Active Site or Metal Facilitated Binding

complex	metal	enzyme inhibited	ref
tetracyclines	Zn, Ca	collagenases	211
biphenyltetrazoles, Figure 25	Zn	metallo-β-lactamase	212
BABIM	Zn	trypsin-like proteases	106
sulfanilamide Schiff bases	Zn	carbonic anhydrase	84, 218
benzimidazoles Figures 26, 27	Pd	reverse transcriptase	216, 217
nitrogen heterocycles	Zn	angiotensin converting enzyme	89
thiol compounds	Zn	sorbitol dehydrogenase	214
phosphonate	Zn	phosphotriesterase	215

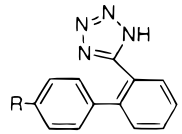

Figure 25. Biphenyltetrazoles.

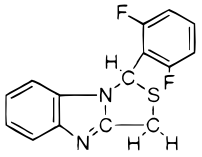

Figure 26. 1-(2′,6′-Difluorophenyl)-1*H*,3*H*-thiazolo[3,4-*a*]-benzimidazole.

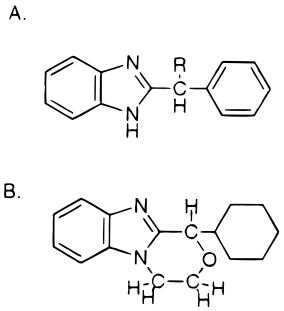

Figure 27. Inhibitors of reverse transcriptase: (A) 2-α-hydroxybenzylbenzimidazole, (B) 3,4-dihydro-1-phenyl-1*H*-[1,4]oxazino[4,3-*a*]benzamidizole.

site of carbonic anhydrase.[213] Thiol compound inhibitors of sorbitol dehydrogenase also chelate to an active site zinc.[214] Phosphonates were found to competitively inhibit phosphotriesterase from *Pseudomonas* by chelating both zinc atoms of a binuclear metal center in the enzyme's active site.[215] Less clear are the mechanism s for 1-(2′,6′-difluorophenyl)-1*H*, 3*H*-thiazolo[3,4-*a*]benzimidazole[216] (Figure 26) or 3,4-dihydro-1-phenyl-1*H*-[1,4]-oxazino[4,3-*a*]benzimidazole[217] (Figure 27). Both of these inhibitors for reverse transcriptase were found to coordinate ions readily to form metal complexes. Palladium was studied for the thiozolo− and Co(II) and Ni(II) for the oxzino−benzimidazole. Metal complexation abolishes inhibitory activity, suggesting that coordination is central to the interaction of these compounds with reverse transcriptase, but the binding site on the enzyme is yet to be determined.

VII. Conclusions and Perspectives

While the breadth and depth of applications for enzyme inhibition in biomedical research is impressive, there are a number of concerns which need to be addressed to fully realize the potential of metal complexes for use as inhibitors. Most of these concerns are not unique to metal complexes but are factors to be considered for any therapeutic agent.

(1) Specificity. Specificity is an important goal in the design of therapeutics since drugs generally exert some sort of toxic effect and nonspecific events can lead to unpleasant consequences. One of the attractions of targeting drugs to enzymes is the exquisite binding sensitivity of enzymes for their particular substrates. Many drugs are designed as substrate mimics to harness this high specificity of recognition by the target enzyme. A general observation we have made in assembling this review is that while it is relatively straightforward to demonstrate that a compound inhibits an enzyme, it is considerably more difficult to assess the specificity of the inhibition. Many inhibitors purport to be specific based on inactivity with a few other enzymes surveyed. While it is impractical, if not impossible, to screen against every enzyme known to mankind, it would be prudent to test selectivity against representatives from a number of potentially cross-reactive families of enzymes. This is especially true for metal complexes which act by coordinating to active site residues. Many of these inhibitors rely on the characteristics of the metal to interact with particular active site types but lack additional features to ensure that the complex only acts at that site. It would be serendipitous to attain specificity of activity with an inhibitor that did not incorporate specific design features targeting for a unique enzyme. Careful characterization of kinetics and enzyme/inhibitor complex structures is necessary to truly demonstrate specificity.

(2) Bioavailibility. For optimum effect, an inhibitor must be readily available at the target site. The distribution of an agent depends on the accessibility of the agent to the site of action and the fate of the agent upon arrival. The first concern addresses delivery: can the inhibitor reach the enzyme? For example, if the enzyme is cytoplasmic, the inhibitor must cross the plasma membrane to reach the enzyme. This may require a delivery vehicle if the agent itself is nonmembrane-permeant. The second concern addresses environmental effects: what happens to the inhibitor as it encounters different microenvironments? In this regard, care should be taken in comparing in vitro versus in vivo results, as a failure to duplicate results in different systems may stem from the presence of perturbing factors, for example, counterions that may cause precipitation or aggregation of complexes or metals.

(3) Compensatory effects. While an agent may specifically and efficiently inhibit a single enzyme in vitro, it can be difficult to predict its ultimate effect

in vivo. Given the complex interplay between biochemical pathways, an inhibition in one pathway may result in unanticipated changes to another pathway. For example, perturbation to the heme biosynthesis or catabolic pathways affects not only heme and bilirubin concentrations but affects all pathways utilizing heme proteins.

(4) Complex stability. Given the reputation of metals as poisons to biological systems, it is of vital importance that potential therapeutics be exceptionally stable complexes. The successes of the platinum antitumor agents and porphyrins in the treatment of jaundice have restored some public faith in the utility of metal complexes as drugs, but a wary eye is still cast upon drugs involving metals.

Almost all biosynthetic pathways involve the actions of enzymes, and using inorganic agents as synthetic regulators of such pathways holds great promise. It is evident from the diversity of therapeutic applications presented in this review that disease pathology often correlates with abnormal enzyme activity and that enzyme inhibition can be a powerful and versatile tool in the treatment of disease.

VIII. Abbreviations

ACE, angiotensin converting enzyme; ACP, acid phosphatase; ADP adenosine diphosphate; ALAD, γ-aminolevulinate dehydratase; ALAS, γ-aminolevulinate synthase; cAMP, adenosine 3′,5′-cyclic monophosphate; APP, amyloid precursor protein; BPYTA, 2,2′-bipyridyl-6-carbothioamide; CA, carbonic anhydrase; CaM, calmodulin; CNS, central nervous system; CO, carbon monoxide; CDP, cytidine diphosphate; CTP, cytidine triphosphate; DNA, deoxyribonucleic acid; DNIC, dinitrosyl iron complexes; ESR, electron spin resonance; EPR, electron paramagnetic resonance; FAD, flavin adenine dinucleotide (reduced form); FMN, flavin mononucleotide (oxidized form); GSH, reduced glutathione; GST, glutathione-*S*-transferase; α-HCAT, α-(*N*)heterocyclic carboxaldehyde thiosemicarbazones; HIV, human immunodeficiency virus; HO, heme oxygenase; IDO, indoleamine 2,3-dioxygenase; MAO, monoamine oxidase; MoMLV, Muloney murine leukemia virus; MP, mesoporphyrin; NADH, nicotinamide adenine dinucleotide (reduced form); NO, nitric oxide; NOS, mitric oxide synthase; PFK, phosphofructokinase; PKC, protein kinase C; PMI, phosphomannose isomerase; PP, protoporphyrin; RNA, ribonucleic acid; RT, reverse transcriptase; SNP, sodium nitroprusside; Tf-Ga, transferrin-gallium.

IX. Acknowledgments

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