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Review article

Metal chelators as potential antiviral agents

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Summary

Metal-chelating compounds can inhibit virus-induced enzymes in infected cells by coordinating with metal ions at their active sites. Consideration of the coordinating properties of ligands can explain the antiviral activity of these compounds. The antiviral actions of a number of compounds (e.g., thiosemicarbazones, pyrophosphate analogues, β-diketones, cyclic polyethers and flavanoids) are discussed in the light of their metal-chelating properties.

antiviral; chelation; coordination; enzyme; inhibition; metals

Introduction

The recent development of nucleoside analogues such as acyclovir (9-(2-hydroxyethoxymethyl)guanine) [1], or (*E*)-5-(2-bromovinyl)-2′-deoxyuridine [2] which inhibit specific processes in herpes virus-infected cells has encouraged the study of compounds which might possess antiviral properties by virtue of their interaction with enzymes in virus-infected cells. Metal-chelating agents are one class of compound which might be expected to have antiviral properties as many enzymes in both infected and uninfected cells require metal ions for activity [3]. However, care must be taken to distinguish between compounds which show some specificity of action and those which are toxic to both virus and host. Problems of toxicity, specificity of action, etc., might be overcome once a better understanding of the properties of potential ligands has been achieved as it should then be possible to design compounds which would chelate with metal ions in specific environments, e.g., in a particular enzyme or in particular regions of a cell. In this way, specificity of action against virus-induced enzymes would be achieved. Many different metal ions are required to support the

metabolism of animal cells. For example, the suggestion has been made that all nucleotidyl transferases are metalloenzymes [4] and the involvement of zinc ions in both DNA and RNA polymerases is well documented [5]. Some metal ions, e.g., sodium and magnesium, are of such widespread occurrence that they do not seem promising targets for the design of specific antiviral compounds. On the other hand, zinc, iron and copper ions occur less widely in cells and appear to be more promising targets.

The concept of viral chemotherapy with metal-chelating agents is not new and this subject has been reviewed by others [6,7] who have surveyed the literature up to 1979. It is the object of the present review to summarise more recent work and to indicate areas which may be worth exploring in the future.

Aspects of complex formation

Most complexes which are formed in biological systems between a ligand and a metal ion are in dynamic equilibrium with the free metal in a more or less aqueous environment. Since a pool of free metal ions may be drawn upon by several different enzymes, the inhibition of one specific enzyme is unlikely to arise by depleting the metal ion concentration of a pool unless this enzyme is critically dependent on the concentration of the metal ion. Thus, the synthesis of polydentate ligands such as 1,2-bis(2-aminophenoxy)ethane-*N,N,N'*-tetraacetic acid (*1*) [8] which only coordinate with free and not protein-bound metal ions (in this case calcium) does not seem a promising route to antiviral compounds. A more successful approach to the development of an antiviral agent would be to investigate compounds which complex with metal ions which are already bound to an enzyme or other macromolecular ligand as stereochemical features within the enzyme could help in its selective inhibition. For example, the shape of an antiviral compound might assist its interaction with one specific enzyme and hence only this enzyme would be inhibited. Alternatively, modification of the lipophilicity of a compound might increase the likelihood of its being confined to a particular site in a virus or cell. The latter approach would be particularly useful in the design of compounds which might interfere with the uncoating of viruses.

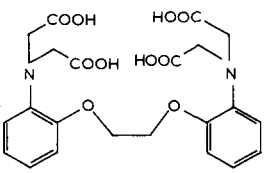
TABLE 1

Hard and soft metal ions and ligands which are relevant to biological systems

Metal ions	Ligands
Hard	*Hard*
Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Al^{3+}, Fe^{3+}	Ligands binding through O^- (e.g., P(O)-O^-, RO^-, $RCOO^-$), ROH, R_2O, Cl^-, NH_3
Intermediate	*Intermediate*
Fe^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}	Amines binding through N ranging from primary to tertiary
Soft	*Soft*
Cu^+, Cd^{2+}, Hg^{2+}	Ligands binding through S (e.g., RS^-, R_2S), CN^-, R_3P

All biologically important metal ions can form complexes and the number of different chemical species which can coordinate with these metal ions is very large. Nevertheless, the types of atom in these ligands are limited, being confined to those of a few elements in Groups V and VI in the Periodic Table. In a biological environment, these are usually oxygen, nitrogen and sulphur, all of which are present in proteins (see Table 1). A convenient classification for the interactions between metal ions and ligands has been put forward by Pearson [9] who introduced the concept of 'hard' and 'soft' acids and bases (HSAB). Metal ions which are 'hard' acids are usually small, highly charged and are highly polarising. Bonds formed between 'hard' metal ions and ligands have a high ionic component. 'Soft' metal ions are generally large, are not highly charged and form bonds with considerable covalent character. 'Hard' ligands are usually small and are little polarised by metal ions; on the other hand, 'soft' ligands are larger and are easily polarised. 'Hard' metal ions form their strongest complexes with 'hard' ligands (e.g., magnesium ions and the oxygen atoms in pyrophosphate). 'Soft' metal ions form their strongest complexes with 'soft' ligands (e.g., mercury ions and thiols). One feature of the HSAB classification is that some metal ions of biological interest, e.g. iron(II) and zinc ions do not fall clearly into either the 'hard' or the 'soft' categories and can form complexes with either 'hard' or 'soft' ligands. Similarly, most amino groups can coordinate with either 'hard' or 'soft' metal ions.

A further consideration which must be borne in mind during the design of antiviral agents is that the metal ion could be coordinated to a number of atoms in the protein already and that this coordination will affect the overall 'hardness' or 'softness' of the metal ion. This should be especially important for metal ions of intermediate 'hardness' or 'softness' and any differences of this nature might be important in the design of specific antiviral agents. Thus, a zinc ion which is coordinated to two oxygen atoms in a protein could well have different complexing properties compared with a zinc ion which is coordinated to two nitrogen atoms.



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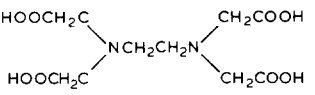

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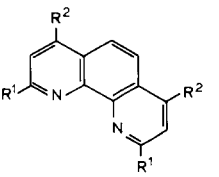


Fig. 1. Structural formulae of 1,2-bis(2-aminophenoxy)ethane-*N,N,N'*-tetraacetic acid (*1*), EDTA (*2*), and 1,10-phenanthroline (*3a*; R' = R^2 = H) and bathocuproine disulphonic acid (*3b*; R^1 = CH_3; R^2 = $-C_6H_4-SO_3H$).

Chelating agents with antiviral activity

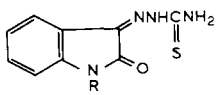
A number of chelating agents which are commonly used as complexing agents in metal analysis [10] have been shown to inhibit viral enzymes. Thus, ethylenediaminetetraacetic acid (EDTA), (*2*), which can form strong complexes with calcium and zinc ions, inhibits the neuraminidase of influenza viruses of the N1 serotype as these enzymes appear to have an absolute requirement for calcium ions [11,12]. 1,10-Phenanthroline (*3a*) and the related compound bathocuproine disulphonic acid (*3b*) which form strong complexes with copper(II), iron(II) and zinc ions, inhibit in vitro a number of viral enzymes which require zinc for activity [4], including the RNA transcriptases of influenza viruses A and B [13]. However, (*2*), (*3a*) and (*3b*) were inactive when tested against the viruses in animals [14].

(a) Thiosemicarbazones

The first report that thiosemicarbazones had chemotherapeutic activity against vaccinia virus was published in 1950 [15] and in the intervening years many compounds of this type (e.g., *4*) have been prepared and tested against viruses [16]. While antiviral activity has been observed with a number of viruses and for example (*5*) will inhibit the replication of herpes simplex viruses 1 and 2 in vitro [17], thiosemicarbazones have principally been used against pox viruses of the vaccinia family and one thiosemicarbazone (*4*, $R = CH_3$) has been used prophylactically to prevent outbreaks of smallpox in persons who had been in contact with the disease [16].

Structure–function studies have been carried out with a number of thiosemicarbazones and in general modification of the thiosemicarbazone moiety (for example, replacement of sulphur by oxygen) leads to a substantial loss of antiviral activity [13]. In the case of (*4*), the carbonyl group in the five-membered ring is essential for activity suggesting that this compound acts as a terdentate ligand. Changes in the lipophilicity of (*4*) can result in changes in antiviral activity. For example, alkylation of the ring nitrogen atom produces an increase in activity for $R = CH_3$ and C_2H_5 but larger carbon chains in this position cause a decrease in activity.

While the exact mechanism of action of thiosemicarbazones is unknown at the present time, their antiviral activity appears to be related to their metal-chelating ability. These compounds are good chelators of metal ions [18] and the sulphur atom in the thiosemicarbazone moiety is a 'soft' centre in the HSAB scale and it is not surprising that (*4*) forms stable complexes with copper(II) ions. In the case of the acetylpyridine thiosemicarbazones only the 2-substituted derivative (*5*) has significant anti-influenza activity [13]. The 3- and 4-acetylpyridine thiosemicarbazones have little antiviral activity



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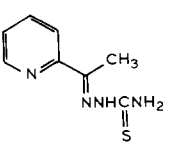


Fig. 2. Structural formulae of thiosemicarbazone compounds (*4*) and (*5*).

which supports the postulation that a metal chelate is formed between the ring nitrogen atom and the thiosemicarbazone moiety in the active compound (*5*). The thiosemicarbazone (*4*, R = H) does not interfere with early stages of viral replication but affects a late process such as virus assembly or maturation and (*4*, R = H) effectively prevents viral replication even when it is added to the system after the synthesis of viral DNA has ceased [19]. The copper(II) complex of (*4*, R = CH_3) binds to nucleic acids in vitro under conditions in which aquated copper(II) ions do not bind to a significant extent [19]. The attachment of a copper complex of (*4*) to an mRNA which is synthesised late in the infective cycle of pox viruses would affect the function of the mRNA and this may account for the antiviral activity of these compounds. As mentioned above, thiosemicarbazones are active against pox viruses and it is of interest that these viruses contain copper(II) ions in appreciable amounts [16]. The modes of action of thiosemicarbazones other than (*4*) have been less well studied and despite reports that ribonucleotide reductase may be inhibited [20], there appears to be no simple relationship between antiviral activity and metal chelation in these compounds.

(b) Pyrophosphate analogues

The discovery that phosphonoacetic acid (*6*) inhibited the replication of herpes simplex virus in animals [21] and the report that the related derivative, phosphonoformic acid (*7*) [22] also had antiviral properties in animals have stimulated research into the antiviral properties of pyrophosphate analogues [23–25]. Both compounds (*6*) and (*7*) show little toxicity to human diploid cells in culture but they are able to inhibit the replication of a variety of herpes virus (e.g., cytomegalo [26], Epstein–Barr [27], and Marek's disease [28] viruses) as well as other viruses including pseudorabies [21], infectious bovine rhinotracheitis [22] and influenza [29] viruses. In the case of the latter viruses, compound (*7*) is a much more effective inhibitor of replication than (*6*).

It was quickly recognised that (*6*) and (*7*) inhibit herpes-specific DNA polymerases [28,30] and that these compounds can inhibit the reverse transcriptases of a number of retroviruses as well as the polymerases of influenza and hepatitis B viruses [25]. Furthermore, other pyrophosphate analogues, e.g., substituted methylene bisphosphonates (*8*) are also good inhibitors of the RNA transcriptase of influenza viruses [32].

At least two mechanisms can be envisaged for the inhibition of virus-induced polymerases by pyrophosphate analogues. In the first mechanism, they could bind to

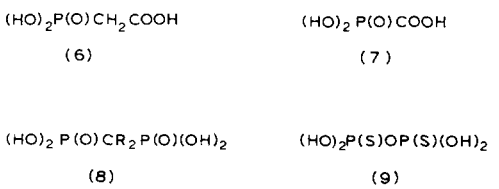


Fig. 3. Formulae of phosphonoacetic acid (*6*), phosphonoformic acid (*7*), methylene bisphosphonate (*8*; R = Cl, Br), and bisthiopyrophosphate (*9*).

the polymerases, interact with the nucleic acid and produce nucleoside triphosphate analogues. The latter could then inhibit the polymerase by acting as competitive inhibitors for the natural nucleoside triphosphate substrates. In the second mechanism, the pyrophosphate analogue could bind directly to the polymerase, possibly in place of inorganic pyrophosphate, and inhibit the polymerase by preventing the chain elongation step in the polymerase reaction.

The first mechanism does not appear to be likely for a number of reasons. Ribo- and deoxyribonucleoside triphosphate analogues have been prepared in which the terminal β,γ-phosphoryl residues have been replaced by phosphonoacetic or dichloromethylene bisphosphonic acids and these triphosphate analogues do not interact with the polymerases of herpes or influenza viruses [32,33]. Nucleoside triphosphate analogues of (*7*) cannot be prepared by standard methods and NMR evidence suggests that these analogues are very unstable [34]. Finally, when ^{3}H-labelled (*6*) is added to the polymerase system of herpes simplex or influenza viruses under conditions of limited nucleic acid synthesis, no evidence could be found for the formation of a radioactively labelled nucleoside triphosphate analogue arising from a back reaction [32,33]. The second mechanism is supported by enzyme kinetic evidence which suggests that (*6*) and (*7*) bind to the DNA polymerase of herpes viruses at the pyrophosphate binding site [28]. Enzyme kinetic analysis also shows that (*7*) is a non-competitive inhibitor of influenza RNA transcriptase activity with respect to ATP, CTP and UTP and a mixed inhibitor with respect to GTP. In the presence of (*7*), initiation of influenza mRNA synthesis occurs but elongation of the polynucleotide chain is inhibited [35]. The antiviral activity of pyrophosphate analogues, as measured by their abilities to inhibit the nucleic acid polymerases of herpes or influenza viruses, appears to be related to their abilities to bind to zinc ions under approximately physiological conditions [32].

The antiviral activities of a large number of pyrophosphate analogues have been studied and in general these have had little or no activity against cell-free polymerase systems [23,24,28,32]. The loss in activity can be correlated with a loss in ability to bind zinc ions under physiological conditions. Thus, esterification of phosphoryl or carboxyl groups generally leads to a considerable reduction in zinc binding and such simple esters have little antiviral activity. However, nucleoside esters of (*6*) and (*7*) can have antiviral activity in tissue culture [36,37]. While the mechanism of action of the esters is unknown, it is possible that they are taken up more effectively by cells than the parent compounds and that they are then hydrolysed to give relatively high concentrations of (*6*) or (*7*) within the cell. Inorganic pyrophosphate at high concentrations will inhibit the RNA transcriptase of influenza virus A and the introduction of a 'soft' chelating centre into the pyrophosphate might be expected to increase the inhibitory activity as this might increase the metal-chelating ability of the pyrophosphate for 'intermediate' and 'soft' metal ions. This has proved to be the case, and not only is bisthiopyrophosphate (*9*) a good inhibitor of the RNA transcriptase activity of influenza virus A but it also inhibits the replication of this virus in tissue culture [38].

Thus, pyrophosphate analogues appear to have potential as antiviral agents as they are product inhibitors of nucleic acid polymerases and, unlike nucleoside analogues they do not have to be metabolised before they exert their antiviral action. Moreover, they can inhibit virus replication under conditions when they are not toxic to host cells.

More work needs to be done on these compounds before they can be used systemically and one problem which must be overcome is that pyrophosphate analogues accumulate in bones and teeth although it is not known whether they have any deleterious effects [39]. Different pyrophosphate analogues have different effects on bone and it should be possible to design compounds which have antiviral activity and which for stereochemical reasons are not compatible with the hydroxyapatite matrix of bones and teeth.

Antiviral agents which may function as chelating agents

(a) β-*Diketones*

β-Diketones are good chelators of metal ions [10] and are used for the extraction of metal ions from aqueous solutions. Lipophilic β-diketones and in particular arildone, 4-[6-(2-chloro-4-methoxy)phenoxy]hexyl-3,5-heptanedione (*10*) have in vivo activity against a number of RNA and DNA viruses (e.g., picorna and herpes viruses) and this may be due to their ability to chelate metal ions [40]. This hypothesis is supported by the observation [41] that the inhibition of herpes simplex virus 2 in vitro by arildone is similar to that observed with phosphonoacetic acid and appears to involve the DNA polymerase. The most potent action of arildone is against poliovirus where it prevents the uncoating of the viral capsid [42]. It has also been observed that arildone, at low concentrations, can block herpes virus replication at an earlier stage than the polymerase possibly by preventing uncoating of the virus [40]. Since arildone has the disadvantage of being very insoluble in water, it is usually administered as a solution in dimethyl sulphoxide and the solvent properties of the latter may have a bearing on the antiviral activity of arildone.

An alternative mechanism of action for arildone may be the inactivation of an arginine group in a viral protein and β-diketones are known to react with these residues in proteins [43]. The products of the reaction between a β-diketone and a guanidinium residue are very stable and hence the inactivation of viral replication by arildone should be irreversible. Since the effect of arildone can be readily reversed in vitro by washing the cells [41,44] this mechanism does not seem to be likely and so metal chelation appears to be a plausible mechanism for the antiviral action of β-diketones.

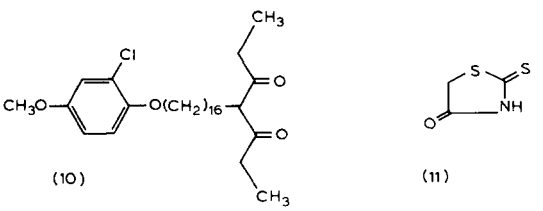


Fig. 4. Structure formulae of a β-diketone, 4-[6-(2-chloro-4-methoxy)phenoxy]hexyl-3,5-heptanedione (*10*), and rhodanine (*11*).

(b) Rhodanine

The use of rhodanine (2-thioxo-4-thiazolidinone) (*11*) as a complexing agent for the determination of metals is well established. It has recently been reported that (*11*) forms complexes with zinc, cadmium and mercury(II) ions in which the 'soft' metal ions are coordinated with the 'intermediate' nitrogen and 'soft' exocyclic sulphur atoms [45]. Rhodanine inhibits the uncoating of echovirus-12 and stabilises the virion [46]. It is tempting to propose that (*11*) acts by chelating with a metal ion at a specific site in the virus as modification of either the nitrogen or the exocyclic sulphur atom results in loss of antiviral activity [47]. The hydrophilicity of (*11*) appears to be critical as introduction of a large lipophilic group (e.g., a benzene ring) at the carbon atom in the ring resulted in a considerable loss of antiviral activity.

(c) Crown ethers

Macrocyclic polyethers (crown ethers) have the ability to form complexes with metal ions of the correct size by sequestering them within their macrocyclic rings [48]. These ethers are analogous to ionophore antibiotics, and, for example, can affect the transport of metal ions through cell membranes. [18,6]-Crown ethers, e.g. (*12*), are inhibitors of the RNA polymerase of picorna viruses at submicromolar levels [49,50]. The polymerase of poliovirus contains zinc ions and is inhibited by the chelating agents EDTA (*2*) and 1,10-phenanthroline (*3a*) [51] and hence it is likely that (*12*) chelates with zinc ions in the polymerase. On the other hand, it is unlikely from steric considerations that (*12*) can chelate with a zinc ion while the latter is coordinated to the polymerase, so an alternative possibility is that (*12*) reduces the level of zinc ions in a metabolic pool in the infected cell. Almost no information has been published on the specificity of crown ethers except that those with a smaller polyether ring than (*12*), e.g., the [15,5]-crown ether (*13*) are not effective inhibitors of the polymerase [52]. Complexes formed between [15,5]-crown ethers and sodium or potassium ions are considerably weaker than those with [18,6]-crown ethers and there is little chemical evidence for complexes formed between divalent metal ions and [15,5]-crown ethers [48]. While crown ethers are reported to be toxic [48], cytotoxic effects of (*12*) have not been reported at concentrations which are considerably higher than those required for antiviral activity [52].

(d) Chalcones and flavones

Chalcones and flavones contain a carbonyl group and a phenolic hydroxyl group in a steric arrangement which is favourable for coordination with metal ions and these compounds can form stable complexes with zinc, iron(III) and aluminium ions [53]. A flavone, 5′-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3,7′-dimethoxy-4H-benzopyran -4-one (*14*), which can be isolated from a Chinese herb, selectively inhibits rhino- and coxsackie viruses in vitro [54]. The RNA polymerase complex is not formed and (*14*) interferes with some step between uncoating of the virus and initiation of RNA synthesis. The related chalcone, 1-(4-ethoxy-2-hydroxy-6-methoxyphenyl)-3-(4-me-

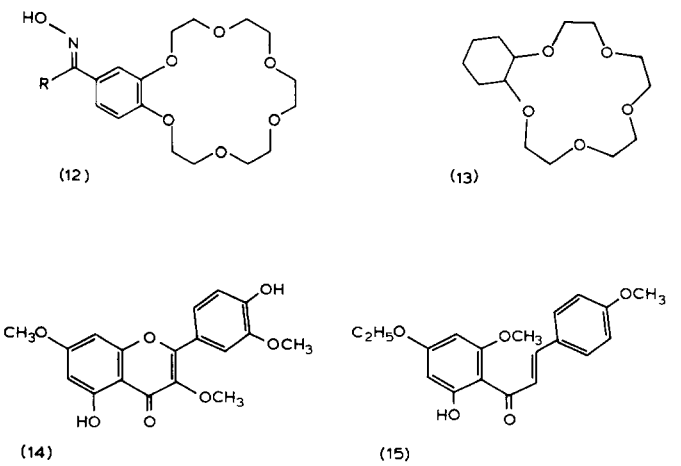


Fig. 5. Structure formulae of some macrocyclic polyesters (crown esters) (*12*), (*13*), a flavone (*14*) and a chalcone (*15*).

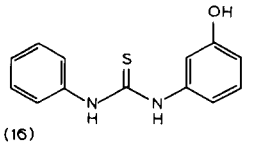
thoxyphenyl)-2-propen-1-one (*15*), also has antiviral activity in vitro, binding to human rhinovirus-2 and stabilising the virion against heat inactivation [55]. This antiviral activity may be related to the metal-chelating properties of these compounds and the binding of (*15*) to the virions appears to be non-covalent as it can be reversed by washing with chloroform. Other flavanoids have been reported to possess antiviral activity but nothing has been published as to their mode of action [56–58].

(e) Other compounds which may exert their antiviral activity through metal chelation

Numerous other compounds which are metal chelators have been reported to show antiviral activity, e.g., 8-hydroxyquinoline and isonicotinic hydrazide will inhibit the replication of Rous sarcoma virus [19]. Recent examples of antiviral agents of this type include the following.

Diphenylthioureas, e.g., *N*-phenyl-*N'*-(3-hydroxyphenyl)thiourea (*16*) which contain a nitrogen or oxygen atom in the 3-position in one aromatic ring exhibit a high antiviral activity against picornaviruses in vitro [59]. As the antiviral activity of these compounds is enhanced by the addition of copper or zinc ions to the culture medium it is suggested that these compounds might coordinate with a metal ion in the virus. In this case, however, an eight membered chelate ring would be formed and such complexes are usually rather unstable. An alternative mechanism of action might involve the thiourea–metal complex binding to a protein or nucleic acid in the virus in a manner suggested for isatin thiosemicarbazones [19].

Diaminocyclohexanes can form very stable complexes with metal ions, particularly with zinc and copper(II) ions [60], and the rigid tricyclic diamine (*17*) which has been reported to possess anti-influenza activity [61] may also form stable metal complexes with metal ions in the virus. However, no details of the site of action of (*17*) are given, and one cannot rule out the possibility that this compound may be functioning as an analogue of aminoadamantane [62].



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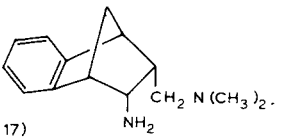

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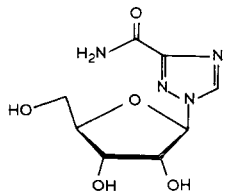

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Fig. 6. Structure formulae of a diphenylthiourea compound (*16*), diaminocyclohexane (*17*), and ribavirin (*18*).

The broad spectrum antiviral nucleoside analogue ribavirin [1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (*18*)] inhibits IMP dehydrogenase and hence is an inhibitor of RNA synthesis [63,64]. Divalent metal ions can form complexes with (*18*) which involve N(4) of the ring and the exocyclic oxygen atom [65,66]. Thus, chelation with a metal ion in IMP dehydrogenase may play a key role in the antiviral activity of (*18*).

Conclusion

A wide variety of metal-chelating compounds have been shown to possess antiviral activity in vitro or in vivo and the main enzymes to be inhibited by these compounds are those involved in uncoating of viruses and nucleic acid synthesis. Many antiviral agents appear to have been discovered fortuitously and little has been published on the systematic study of the replacement of liganding atoms, variation in lipophilicity, etc., in these compounds. Hence it is to be hoped that the recognition that metal-containing enzymes in virally infected cells are worthwhile targets for antiviral research together with a consideration of the principles involved in the formation of metal chelates will lead to the synthesis of antiviral agents with improved activity and specificity.

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