The Wide Pharmacological Versatility of Semicarbazones, Thiosemicarbazones and Their Metal Complexes

Heloisa Beraldo*[a] and Dinorah Gambino[b]

[a]*Departamento de Química, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte, MG, Brazil*

[b]*Cátedra de Química Inorgánica, Departamento Estrella Campos, Facultad de Química, Universidad de la República, CC 1157, Montevideo, Uruguay*

Abstract: The more significant bioactivities of a variety of semicarbazones (anti-protozoa, anticonvulsant) and thiosemicarbazones (antibacterial, antifungal, antitumoral, antiviral) and their metal complexes are reviewed together with proposed mechanisms of action and structure-activity relationships. Clinical or potential pharmacological applications of these versatile compounds are discussed.

Keywords: Semicarbazones, thiosemicarbazones, biological activity, pharmacological applications, bioactive metal complexes, mechanisms of action.

INTRODUCTION

Semicarbazones and thiosemicarbazones (Fig. (**1**)) present a wide range of bioactivities, and their chemistry and pharmacological applications have been extensively investigated. The literature contains reviews on many aspects of the chemistry of these interesting compounds, such as preparative methods, stereochemistry, bonding in metal complexes, spectral characteristics and crystal structures [1-5], but although all works mention their pharmacological properties and there are reviews on the biological activity of particular series of these versatile compounds [6-8], to our knowledge this is probably the first review entirely dedicated to their broad range of biological and therapeutic applications.

The biological properties of semicarbazones and thiosemicarbazones are often related to metal ion coordination. Firstly, lipophilicity, which controls the rate of entry into the cell, is modified by coordination [9]. Also, the metal complex can be more active than the free ligand, and some side effects may decrease upon complexation. In addition, the complex can exhibit bioactivities which are not shown by the free ligand. The mechanism of action can involve binding to a metal *in vivo* or the metal complex may be a vehicle for activation of the ligand as the cytotoxic agent. Moreover, coordination may lead to significant reduction of drug-resistance [4].

BIOACTIVITY OF SEMICARBAZONES AND THEIR METAL COMPLEXES

Semicarbazones as Anti-Protozoa Agents

A variety of 5-nitrofuryl semicarbazone (nitrofurazone) derivatives have been developed for the therapy of Chagas` disease, a major problem in Central and South America. Chagas disease or American trypanosomiasis is produced by several strains of the protozoan parasite, *Trypanosoma cruzi*, which is transmitted to humans by blood-sucking bugs (*Triatoma infestans* and *Triatoma rubroviaria*) [10]. Substituents with different electronic and steric properties have been introduced on N^4 of the nitrofurazone moiety. The compounds were tested *in vitro* against *T. cruzi* epimastigote and *in vivo* in mice infected with the parasite [11, 12]. Among the substituted derivatives the N^4-hexyl showed a good activity profile. Lipophilicity and electrochemical properties of the series of derivatives seem not to be directly related to the bio-response [13]. A theoretical study established some structural requirements to optimize the *in vitro* activity [14].

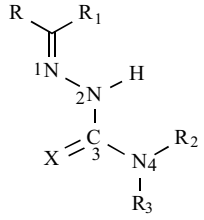

Fig. (1). General structure of semicarbazones (X = O) and thiosemicarbazones (X =S). R, R_1, R_2 and R_3 = alkyl or aryl groups.

Semicarbazones as Anticonvulsants

Today there is a need for new anti-epileptic drugs since approximately 25% of epileptic patients found that drug therapy inadequately controlled their convulsions, besides the fact that the currently used drugs cause significant side effects [15]. Recently, a series of publications reports on the anticonvulsant activity of thiosemicarbazones, semicarbazones and hydrazones derived from aromatic and unsaturated carbonyl compounds [16-24] as well as from other precursors [25]. Anticonvulsant activity was displayed by most of the compounds in the maximal electroshock (MES) screen when given orally to rats. Various compounds displayed greater potencies than mephenytoin, carbamazepine and valproate; three reference clinically used drugs. Thiosemicarbazones displayed activity but with greater neurotoxicity than the semicarbazones.

*Address correspondence to this author at the Departamento de Química, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte, MG, Brazil; Tel: 55-31-34995740; Fax: 55-31-3499-5700; E-mails: beraldo@dedalus.lcc.ufmg.br or hberaldo@ufmg.br

1389-5575/04 $45.00+.00

Studies of the mechanism of action of 4-bromobenzaldehyde semicarbazone indicated that it does not potentiate the action of gamma aminobutyric acid (GABA). Thus, although an interaction with GABA receptors occurs, it probably has a weak antagonist effect. Such a mechanism would be quite different from those of most anticonvulsants which are GABA agonists [26].

The theoretical partition coefficients (log P) and the relation between the maximum and the minimum of the molecular lipophilicity potential (MLP) were found to be significant for anticonvulsant activity. These values reflect the ability of the drugs to cross biological barriers and membranes before effecting anticonvulsant bioactivity [18].

One disadvantage of the semicarbazones is their low water solubility. As a strategy to circumvent this problem, our group prepared a novel and very effective anticonvulsant following a host-guest strategy that used hydroxypropyl-β-cyclodextrin and benzaldehyde semicarbazone to form a 1:1 inclusion compound. The minimum dose of compound necessary to produce activity in rats using the maximum electroshock (MES) screen decreased from 100 mg/Kg for the free semicarbazone to 25 mg/kg/ip (75%) and 35 mg/Kg/vo (65%) for the inclusion compound, indicating a significant increase in the bio-availability of the drug [27]. The data suggest in addition, that molecular encapsulation could be used in the preparation of new pharmaceutical formulations of anticonvulsant drugs to lower the side effects that limit their usefulness.

Other Biological Properties of Semicarbazones

In contrast to thiosemicarbazones, the literature records far fewer examples of semicarbazones presenting significant anticancer and cytotoxic activity but some nitroso, naphtopyran, and fluorene derivatives showed anti - leukemia effect in mice [8]. In an early work several N^4-substituted semicarbazone derivatives of *o*- and *p*-chlorobenzaldehyde as well as 2,6-dichlorobenzaldehyde exhibited potent anti-hypertensive effects [28]. In addition, it was recently reported that the orally administered drug naftazone (1,2-naphtoquinone semicarbazone) protects the vascular system through an inhibitory effect on nitric oxide (NO) synthesis [29]. Many other bioactivities of semicarbazones have been reported such as their antimicrobial [30], pesticide [31], herbicide [32] and hypnotic [15] properties or the ability of some of their Cu(II) complexes to mimic superoxide dismutase (SOD) activity [33].

BIOACTIVITY OF THIOSEMICARBAZONES AND THEIR METAL COMPLEXES

Thiosemicarbazones and Their Metal Complexes with Antibacterial and Antifungal Activity

Thiosemicarbazones and their metal complexes present wide anti-microbial activity. The antibacterial activity of a variety of 2-acetylpyridine thiosemicarbazones was determined in clinical isolates of bacteria. The compounds tested were able to inhibit *Neisseria gonorrhoeae, Neisseria meningitides*, *Staphylococcus faecalis, Streptococcus faecalis* and D *Enterococcus*. Poor antibacterial activity was shown toward the gram-negative bacilli, *i.e.*, *Pseudomonas*, *Klebsiella-Enterobacter*, *Shigella*, *Escherichia coli*, and *Proteus* [34]. Similar results were obtained with thiosemicarbazones of 2-acetylquinoline and 1- and 3-acetylisoquinoline [35]. Pt(II) complexes of 2-acetylpiridine thiosemicarbazone also showed a similar behavior, *i.e.* were lethal to gram-positive but inactive against gram-negative bacteria. The complexes were active against yeast [36]. In addition, a series of 2-(alpha-hydroxyacetyl)pyridine thiosemicarbazones exhibited potent inhibitory activity against penicillin-sensitive as well as penicillin-resistant *N. gonorrhoeae*, against *N. meningitides*, and *Staphylococcus aureus*. These new agents appeared to be less toxic to the host than the corresponding 2-acetylpyridine thiosemicarbazones [37]. Moreover, the *in vitro* antibacterial activity of 2-acetylpyridine-1-oxide thiosemicarbazones was tested as well against clinically significant bacterial cultures, including isolates with known antibiotic resistance. Again, the compounds were active against *N. gonorrhoeae*, *N. meningitidis*, *S. aureus* and *S. faecalis* and ineffective against the gram-negative enteric cultures and the *Pseudomonas* isolates [38].

The antimicrobial activities of 4- and 6-coordinate Ni(II) complexes of thiosemicarbazones derived from 2-acetylpyridine and 2,6-diacetylpyridine were reported. Only the labile complexes exhibited activity against *Bacillus subtilis* and *S. aureus*, suggesting that activity would correlate with ligand-replacement abilities [39].

Semi and thiosemicarbazones derived from 2-acetylpyridine, 2-furfuraldehyde, 2-acetylnaphtalene, 2-acetylthiophene, 2-acetylfuran and their corresponding Mn(II) complexes were tested on fungi (*Macrophomina phaseolina, Fusarium oxysporum)* and bacteria (*Xanthomonas sp*., *E. coli* and *S. aureus*). The complexes were more active than the free ligands, probably due to increased lipophilicity. Moreover, the thiosemicarbazones' complexes were more active than the semicarbazone analogues. It was proposed that the ultimate action of structurally non-specific toxicants is the denaturation of cell proteins. Chelating agents are often powerful inhibitors of metalloenzymes so that the ligands may act by inhibiting the enzymes whose activity depends on metals [40].

Metallocene derivatives of titanium and zirconium with thiosemicarbazones derived from 2-acetylpyridine, 2-acetylfuran, 2-acetylthiophene and 2-acetylnaphtalene were tested against a number of pathogenic fungi (*Aspergillus niger*, *Amathia alternata* and *M. phaseolina*) and bacteria (*E. coli*, *S. aureus*, *Streptococci (+)*, *Streptococci viridans*, and *Proteus mirabilis*). All the complexes were more active against the organisms used than the ligands [41].

Many other thiosemicarbazones and metal complexes were screened for antibacterial and antifungal activity. Hence, 2-formylpyridine thiosemicarbazones and their oxovanadium(IV) complexes exhibited powerful *in vitro* antibacterial activities towards *E. coli* [42], and aryl thiosemicarbazones showed good activity against *Aeromonas hydrophilia* and *Salmonella typhimurium* [43]. A recent work reports slight antibacterial activity of thiosemicarbazones and thiocarbohydrazones of 1-adamantyl 2-pyridylketone and 1-adamantyl methyl ketone [44].

Also, N^4-substituted o-vanillin thiosemicarbazones and some of their metal complexes of the platinum group were studied for their antibacterial, antifungal and amoebicidal activity *in vitro*. The complexes exhibited significant activity against a wide spectrum of microorganisms [45].

A new drug based on cooperative effectiveness of vitamin K3 and thiosemicarbazones was synthesized along with its Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes. *In vitro* studies showed that the ligand and its complexes have strong inhibitory actions against *G(+) S. aureus*, *G(+) Hay bacillus*, and *G(-) E. coli* [46].

Base adducts of Cu(II) complexes have been prepared with N^4-phenyl salicylaldehyde thiosemicarbazone. The thiosemicarbazone and its complexes showed growth inhibitory activity against the human pathogenic bacteria *Salmonella typhi*, *Shigella dysentariae*, *non-coagulace Staphylococcus*, *Photobacterium sp*. and *S. aureus* and the complexes against plant pathogenic fungi. An increase in coordination number from 4 to 5 in the complexes led to higher activity, probably due to increase in lipophilicity [47].

Additionally, Bi(III) complexes with derivatives of tropolones and thiosemicarbazones were tested against the gastritis-causing bacteria *Helicobacter pylori*. Most of the compounds inhibited the growth of the microorganisms *in vitro* [48].

As early as 1946, Domagk reported that some thiosemicarbazones of cyclic aldehydes and ketones possessed antitubercular activity *in vitro* [49]. In some instances activity *in vivo* was also shown in animals, and some compounds have been used in the treatment of lupus and pulmonary tuberculosis with encouraging results [50].

The antitubercular activity of a series of semi and thiosemicarbazones has been tested by Hoggart *et al.* [51], namely (a) thiosemicarbazones of variously substituted benzaldehydes; (b) thiosemicarbazones of heterocyclic aldehydes; (d) aliphatic thiosemicarbazones; (e) thiosemicarbazones with substituents at N^2 and N^4 (e) ketone thiosemicarbazones; (f) benzaldehyde semicarbazones. Marked activity against acute *Mycobacterium tuberculosis* in mice was limited to the thiosemicarbazones of substituted benzaldehydes or heterocyclic aldehydes. For highest activity a *para* substituent was necessary.

The most widely studied drug in this class of compound is *p*-acetamidobenzaldehyde thiosemicarbazone, commercially available as thiacetazone. However, its predominantly bacteriostatic action *in vivo* and the rapid emergence of thiacetazone-resistant strains during therapy limit its usefulness. Though the actual mode of action of thiacetazone is unknown, it has been suggested that like ethionamide, it might inhibit the mycolic acid biosyntheses in mycobacteria since some thiacetazone-resistant strains of *M. tuberculosis* exhibit cross resistance to ethionamide [52].

F.M. Collins *et al.* report a study of structure-antimycobacterial activity in a series of N^4-substituted 2-acetylpyridine thiosemicarbazones [53]. The work reveals that antimycobacterial activity occurs for compounds having partition coefficients (log P) between 3.0 and 4.0, which suggests that the activity of these compounds depends on their possessing an optimum hydrophobicity, which in turn controls their rate of entry into the bacterial cell.

A proposal for the mechanism of antibacterial activity implicating electron transfer (ET) and/or oxidative stress for a large number of synthetic antibacterials including thiosemicarbazones has been advanced [54]. The general approach of the paper entails the active agent bound to a receptor, which then effects catalytic ET to oxygen producing toxic entities that destroy the invader.

The literature reports many works on the antifungal activity of thiosemicarbazones and their metal complexes. Early in 1960 forty thiosemicarbazones derived from aliphatic and aromatic aldehydes and ketones together with many of their metal complexes were examined for toxicity against *Chaetomium globosum* and *Aspergillus niger.* Some of the ligands showed activity while the complexes were inactive [55]. Also, metal complexes of *p*-anisaldehyde thiosemicarbazone have been screened for antifungal activity on *Alternarai sp*., *Paecilomyces sp*., and *Pestalotia sp.* In some cases the complexes were more active than the free ligand [56]. The antifungal activity of pyridine-2-aldehyde, pyridine-3-aldehyde thiosemicarbazones and their metal complexes were tested against *Candida albicans* and *Apergillus fumigatus.* The 2-derivative was found to be more active than its 3-analogue but the activity of the latter increases on coordination [57]. In addition, N^4-alkyl and N^4-dialkyl-2-acetylpyridine thiosemicarbazones showed marked inhibitory activity of *A. niger* growth, as well as some of their Cu(II) [58] and Ni(II) complexes [59, 60]. The Cu(II) complexes were more active. The relative activity against *Paecilomyces variotii* is opposite, *i.e*., the Ni(II) complexes were more effective [61]. The Zn(II) complex of the N^4-methyl derivative also showed activity against the two pathogenic fungi [62]. Moreover, dinuclear Ni(II) complexes of N^4 substituted 2-hydroxyacetophenone thiosemicarbazones presented considerable activity against *P. variotii* [63]. N^4 substituted 2-benzoylpyridine thiosemicar-bazones as well as their Cu(II) complexes also exhibited antifungal activity against *A. niger and P. variotii* [64].

Thiosemicarbazones and Their Metal Complexes with Antitumoral Activity

In 1956 Brockman *et al.* reported on the anti-leukemic effect of 2-formylpyridine thiosemicarbazone (PT) [65] but this compound was found to be cumulatively toxic. In 1963 French and col. formulated hypotheses about the mode of action of the α(N)-heterocyclic thiosemicarbazones. The first was that they were acting as tridentate ligands and the second was that modifying the ring system while retaining the ligand pattern could lead to improved activity and decreased toxicity. The electron densities, substituents and geometry could have critical effects on activity [66]. A number of predicted negative and predicted positive thiosemicarbazones were screened by these authors against transplanted rodent tumors. The first predicted active compound was pyrazine carboxaldehyde thiosemicarbazone, which exhibited greater activity on L-1210 cells than PT and was active on Lewis lung carcinoma (LLC) but was ineffective on Ehrlich carcinoma. Then 1-formylisoquinoline thiosemicarbazone (IQ-1) was predicted and found active, while its 3-formylisoquinoline analogue was predicted and

found inactive in the authors' tumor systems. IQ-1 was in fact more broadly active than the previous compounds in the series and less toxic. In all active compounds the N-N-S tridentate chelating system was present. Antineoplastic activity was only observed when the carbonyl attachment of the side chain was located at a position α to the ring nitrogen atom [67].

Other works reported that the 5-hydroxy derivative of IQ-1 exhibited pronounced antineoplastic activity [68], as well as both 3- and 5-hydroxy derivatives of PT [69].

In an early paper Sartorelli and col. showed that PT and IQ-1 caused marked inhibition of the incorporation of ^{3}H-thymidine into the DNA of several tumor lines. The syntheses of RNA and protein were considerably less sensitive to these agents [70]. The site of metabolic lesion on the DNA biosyntheses was supposed to be the conversion of ribonucleotides to deoxiribonucleotides, catalyzed by the enzyme ribonucleoside diphosphate reductase (RDR). Studies conducted with a partially purified RDR from the Novikoff rat tumor indicated that PT and IQ-1 produced inhibition which was noncompetitive with respect to the nucleoside diphosphate substrate.

In a further work by Sartorelli and col. [71] the characterization of the biochemical mechanism of action of α(N)-heterocyclic carboxaldehyde thiosemicarbazones is described. IQ-1 is discussed as a model compound of the α(N)-heterocyclic thiosemicarbazones class and an extremely potent inhibitor of the DNA biosyntheses. Indeed, the compound was a powerful inhibitor of DNA replication in Sarcoma 180 cells *in vitro*. The authors demonstrated that IQ-1 was capable of decreasing intracellular pools of deoxyribonucleotides and that the site of blockage was RDR. Similar results had been obtained by Brockman *et al.* [72]. The data suggested that it is unlikely that the inhibitor interacts with the site on the enzyme that binds either of the nucleotides. However, a relationship was found between RDR inhibition and the concentration of the reducing substrate dithiotreitol, used as convenient substitute for the natural thioredoxin-thioredoxin reductase-NADPH system. A partially competitive relationship between IQ-1 and the dithiol substrate was demonstrated, indicating that the inhibitor binds at or near the site on the enzyme normally occupied by this substrate.

Since IQ-1 strongly chelates iron, it was conceivable that the drug acted to inhibit the enzyme by binding the iron required for catalytic activity. However, it was demonstrated that the binding of free iron by IQ-1 neither was responsible for the inhibition nor could free iron prevent inhibition by competing with the enzyme by the inhibitor. The possibility that the inhibitor might interact with an enzyme-iron complex was considered, but failure to reverse inhibition by large excess of iron would require much greater affinity of IQ-1 for the enzyme site than for free iron. Since IQ-1 has great affinity for iron, it would be more reasonable to assume that the active form of the drug is an iron complex. The complex could be visualized to bind to a site normally occupied by iron and the dithiol and thereby functions to abort electron transfer. Studies on the inhibition of RDR by the preformed Fe-chelate, [Fe(IQ-1)$_2$] showed that the active form of the drug is its Fe(II) complex.

It was also demonstrated that IQ-1 causes DNA fragmentation of sarcoma 180 and this effect is of major importance to the cytotoxic action since it creates a lesion in the genome which is reinforced by blockade of RDR activity [73].

Petering and col. showed that some heterocyclic thiosemicarbazones have the ability to remove iron from ferritin by chelation [74] and that iron can be removed from transferrin, indicating that this type of reaction could be a plausible way by which α(N)-heterocyclic thiosemicarbazones can bind iron in organisms [75].

This same group investigated the cytotoxicity of copper and iron complexes of 5-substituted-2-formylpyridine thiosemicarbazones against Ehrlich ascites tumor cells [76]. The iron complexes with the 5-methyl, 5-Cl, 5-CF_3 substituted thiosemicarbazones could completely prevent tumor growth. Copper complexes of 5-H and 5-CH_3 also successfully prevented tumor cell transplantation.

In a subsequent work, Petering and col. relate that in a series of several α(N)-heterocyclic thiosemicarbazones and their iron and copper complexes, none of the ligands was active against Ehrlich cells while some of the complexes exhibited activity. In contrast, the ligands inhibited DNA syntheses in lower concentrations than used in the cytotoxicity test. Similarly, the complexes were effective inhibitors at concentrations below those necessary to the cytotoxic action. In addition, the iron complexes of IQ-1, PT and 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone were shown to be three- to six-fold more active than their uncomplexed ligands as inhibitors of RDR to which no iron has been added, and the copper complex of PT was slightly more active than the free thiosemicarbazone [77]. Moreover, this group has shown that the copper complex of PT can inhibit tumor growth in Ehrlich cells and DNA syntheses [78].

The mechanism of inhibition of mammalian RDR by the iron complex of IQ-1 was investigated by L. Thelander and A. Graslund [79, 80]. These authors demonstrated that the tyrosine free radical of subunit M2 of the enzyme is the target of the drug and that the thiosemicarbazone inhibits the enzyme by destroying the radical. The inhibition requires oxygen, excluding that it could be due to removal of iron by the drug from the active site of the enzyme. It is suggested that the ferrous complex of the bound IQ-1 drug reacts with molecular oxygen, leading to the reversible destruction of the radical. Furthermore, the effects of the thiols on inhibition were explained by their dual functions in the reaction: they reduce Fe^{3+} to Fe^{2+} which is necessary for drug action and, in addition, in the presence of iron and oxygen, they also support the regeneration of the tyrosine free radical once it has been scavenged and thus help to counteract drug action.

Some years later our group demonstrated that the Fe(III) complex of PT can be reduced by the cellular thiol-like reducing agents dithiothreitol (a model for thioredoxin) and *N*-acetyl-L-cystein (a model for glutathione), suggesting a similar mechanism of action. The pathway could involve oxidation of the Fe(II) complex of the drug, with the release of one electron and the consequent inactivation of the tyrosine free radical, followed by reduction of the Fe(III) complex by a cellular thiol [81, 82]. More recently, based on

our results, other authors investigated the redox behavior and interaction of $[Cu(L)_2]$ (L = thiophene-2-carbaldehyde thiosemicarbazone) [83] and of $[Fe(L)_2]NO_3.0.5\ H_2O$ and $[Cu(L)(NO_3)]$, (L = PT) with reduced glutathione and 2-mercaptoethanol, together with their cytotoxic and antitumoral activities, and proposed that interaction with cellular thiols could be related to the cytotoxicity of these complexes against Friend erithroleukemia (FLC) and melanoma B16F10 cells [84].

5-hydroxy-2-formylpyridine thiosemicarbazone (5-HP) was the only member of the α(N)-heterocyclic thiosemicarbazone series to be clinically evaluated in man [85]. Conjugation to form glucuronides was the major metabolic fate of 5-HP. High concentrations of 5-HP could not be maintained because of the extremely rapid clearance of drug. In addition, 5-HP had a lower affinity for the enzyme than other agents such as IQ-1. Thus, the impressive antineoplastic activity exhibited by 5-HP in animal systems was not observed in man. Its lack of activity was attributed to its relatively low inhibitory potency for RDR and its relatively short biological life due to the rapid formation and elimination of *O*-glucuronide conjugate. These findings conducted to the development of a second generation of α(N)-heterocyclic thiosemicarbazones with greater affinity for the target.

To provide information, which would allow the design of more effective inhibitors of the enzyme, structure-activity relationships were carried out to delineate the structural requirements for optimum interaction between the enzyme and the inhibitor [86]. The need for a thiosemicarbazone side chain α to the heteroaromatic nitrogen for the inhibition of tumor growth suggested the involvement of these functions in the mode of action. The 3-, 4- and 5-methyl substituted derivatives of PT were slightly more active than the parent compound while its 6-methyl derivative was ten times less active. Within the isoquinoline series, IQ-1 was the most powerful inhibitor. The 3-methyl derivative was about six times less active, whereas 4-methyl and 5-methyl IQ-1 were only slightly less potent. The fact that IQ-1 was a considerably more potent inhibitor of RDR than PT suggested the occurrence of a hydrophobic interaction between the benzenoid portion of the molecule and the enzyme, which explains the fact that 3-, 4- and 5-methyl substitutions on the PT molecule render this compound more active. Additionally, the decreased inhibitory potencies of the 6-methyl derivative of PT and of the 3-methyl derivative of IQ-1 could be due to low bulk tolerance by the enzyme to substituted groups on position 6- of PT and 3- of IQ-1, which are analogous sites with respect to the interaction with the RDR molecule.

To enhance affinity for the target enzyme, the hydrophobic phenyl ring was introduced at various positions in the molecule of PT and the effect of these variations on the activity of RDR from the Novikoff rat tumor was tested [87]. The biodata suggested that 2-formyl-4-(*m*-amino)phenylpyridine thiosemicarbazone possessed the optimum combination of structural features and was the most active of the *m*-aminophenyl derivatives as inhibitor of both tumor growth and RDR.

In the isoquinoline series the 5-amino derivative of IQ-1 appeared to be a candidate as a second-generation α(N)-heterocyclic thiosemicarbazone with clinical potential in cancer chemotherapy [88]. 5-amino IQ-1 exhibited similar RDR inhibition activity as IQ-1 but had the advantage over IQ-1 in that it can be rendered soluble as an acidic salt. In addition, 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone (MAIQ-1) was prepared in an effort to (a) obtain high affinity for the target RDR (b) create steric hindrance to enzymatic substitution of the 5-amino function by insertion of a bulky methyl group. MAIQ-1 was found to be an extremely powerful antineoplastic agent, twice as effective as 2-formyl-4(*m*-amino)phenylpyridine thiosemicarbazone as an inhibitor of RDR and superior to any other compound in the series [89].

Since then, new heterocyclic thiosemicarbazones have been prepared and evaluated that by virtue of their structures were resistant to *O*-glucuronidation [90]. Among them, the 3- and 5-aminopyridine-2-carboxaldehyde thiosemicarbazones showed significant antitumoral activity in mice bearing the L1210 leukemia. The 3-amino derivative appeared to be the more promising [91].

Alkylation of the amino groups on the pyridine ring results in the conversion of the primary amines to secondary amines and would lead to steric hindrance to the enzymatic acetylation of the amino function, besides the increased lipophilicity upon introduction of the alkyl group. The 5-methylamino, 5-ethylamino, and 5-allylamino derivatives of PT were good inhibitors of the reductase activity and showed significant antitumor activity *in vivo* with acceptable toxicity [90].

The only inhibitor of RDR in clinical usage is hydroxyurea, which is a relatively poor inhibitor of the enzyme and has a short serum half-live, thus being a weak anticancer agent [92]. 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, triapine) demonstrated potent inhibition of L1210 leukemia cells *in vitro*, curative capacity for mice bearing the L1210 leukemia, marked inhibition of RDR and sensitivity to hydroxyurea resistant cells. Triapine has been shown to markedly inhibit the growth of mouse M109 lung carcinoma and A2780 human ovarian carcinoma xenografts in mice. In addition, it has been demonstrated that neoplastic cells were more sensitive to DNA-syntheses inhibitory effects of triapine than normal cells. Also of interest is the fact that triapine could cross the blood-brain barrier and inhibit the growth of L1210 cells in the brain by 95%. Finally, combination of triapine with cytotoxic agents such as cisplatin and doxorubicin caused a synergistic inhibitory effect on L1210 cells in mice, due to triapine preventing repair of DNA damage induced by the cytotoxic agents [93-95]. Triapine is currently in phase II clinical trial [95].

The antitumor activity of bis(thiosemicarbazones) was widely investigated after the discovery of the strong antineoplastic activity of 3-ethoxy-2-oxobutyraldehyde bis(thiosemicarbazone) in solid animal tumors [96]. An absolute requirement for Cu(II) for its activity was demonstrated and its Cu(II) complex is a potent antitumor agent [97]. In the examination of a variety of related ligand structures, it was found that the cytotoxic effects varied markedly with structure and that some of the ligands were activated by zinc ion [98]. Also, Zn(II) complexes of 2,6-diacetylpyridine bis(thiosemicarbazones) have an inhibitory

effect on FLC cell line proliferation *in vitro* [99]. The cytotoxicity of symmetrical and unsymmetrical bis(thiosemicarbazones) and their metal complexes was investigated in murine and human tumor cells. The free bis(thiosemicarbazones) demonstrated similar cytotoxicities to previously reported thiosemicarbazones. The ligands and their Cu(II), Ni(II), Zn(II) and Cd(II) complexes showed similar activity in the suspended leukemia and suspended lymphoma cell lines. In the human solid tumors the uncomplexed bis(thiosemicarbazones) were generally less active than their metal complexes [100]. Interestingly, the Pd(II) complex of benzyl bis(thiosemicarbazone) might be endowed with important antitumor properties since it shows IC_{50} values similar to those of cisplatin and displays notable cytotoxic activity against cisplatin-resistant Pam-ras cells [101].

The antitumor properties of many other thiosemicarbazones and their metal complexes have been investigated. Thus, Cu(II) complexes of 5-formyluracil thiosemicarbazone were tested by M.B. Ferrari and *cols.* [102] on human leukemic cell lines K562 and CEM. The compounds demonstrated the ability to inhibit cell growth and one of them to induce apoptosis. Also, thiosemicarbazones derived from natural aldehydes such as citronellal, citral, octanal and octenal and their Ni(II) and Cu(II) complexes have been tested for inhibition of cell proliferation and apoptosis induction *in vitro* on human cell line U937 [103]. The same group prepared Cu(II) complexes of α-ketoglutaric acid thiosemicarbazone, the activity of which was evaluated with respect to cell proliferation, erythroid differentiation, DNA syntheses and reverse transcriptase activity on FLC and with respect to cell proliferation and cellular apoptosis induction on human leukemia cell lines U937 and K562. The Cu(II) complexes showed proliferation inhibition through an apoptosis mechanism [104]. In addition, *p*-fluorobenzaldehyde-derived thiosemicarbazones as well as the Ni(II) complex of the parent compound exhibited marked inhibitory activity on U937 cell growth but not through the apoptosis mechanism [105]. Moreover, the Zn(II) and Cd(II) complexes of 2-pyridylketone thiosemicarbazone and the Zn(II) complex of *p*-isopropylbenzaldehyde thiosemicarbazone display IC_{50} values in a range similar to cisplatin. The latter exhibits specific cytotoxic activity against cisplatin-resistant Pam-*ras* cells [106]. Finally, a structure-activity relationship study of cytotoxic Cu(II) complexes of semi and thiosemicarbazones was carried out by R. Cao and co-workers [107].

Certain organotin(IV) compounds present antitumor activity. In the case of diorganotin(IV) derivatives it is likely that the ultimate reactive groups at cellular level are the SnR_2^{2+} species [108]. With this in mind some groups investigated whether thiosemicarbazones and tin could act synergically in new complexes. It was found that dimethyltin complexes of PT and 2,6-diacetylpyridine bis(thiosemicarbazone) did not affect DMSO-induced differentiation but strongly inhibited FLC growth, suggesting that they do not have a simple specific cytotoxic effect. The ability of the complexes to bind or to interact with particular cell structures could affect the interactive mechanisms that regulate cell proliferation [109]. Additionally, the biological properties of $[SnR_2(L)]$ (R = methyl, butyl, phenyl) complexes of pyridoxal thiosemicarbazone were evaluated. While the free thiosemicarbazone had no activity, the butyl and phenyl organotin derivatives showed the lowest thresholds for the inhibition of FLC proliferation. Cu(II) complexes of the same ligand had shown to enhance DMSO-induced FLC differentiation [110]. Moreover, mixed diorganotin(IV) complexes containing 2-formylpyridine thiosemicarbazonato and diphenyldithiophosphinato ligands $[SnR_2(PyTSC)(S_2PPh_2)]$ (R = methyl, ethyl and butyl) were obtained. The thiosemicarbazone and its diphenyldithiophosphinato diorganotin(IV) complexes inhibited the proliferation of all the cell lines investigated (FLC, CEM, U937, K562 and TOM-1 leukemia cells) [111]. Additionally, thiophene-2-carboxaldehyde thiosemicarbazone and its tin complexes demonstrated cytotoxic and antifungal properties [112].

Pd(II) and Pt(II) complexes of PT were active *in vivo* against leukemia P388 cells [113] and Pd(II) complexes of N^4-alkyl-2-acetylpyridine thiosemicarbazones showed *in vitro* inhibitory activity of DNA syntheses in L1210 and P388 cell cultures [114]. Pt(II) complexes of N^4-ethyl 2-formyl and 2-acetylpyridine thiosemicarbazones showed cytotoxicity and were found to be able to overcome the cisplatin resistance of A2780/Cp8 cells [115]. Pd(II) and Pt(II) complexes of phenylacetaldehyde thiosemicarbazone [116] and binuclear chloro-bridged palladated and platinated complexes derived from *p*-isopropylbenzaldehyde thiosemicarbazone showed activity against several human and murine cell lines sensitive (HL-60, U937, HeLa and 3T3) and resistant (Pam-ras) to the clinically used cisplatin [117].

A recent publication reports that a Ga(III) complex of N^4-dimethyl 2-acetylpyridine thiosemicarbazone showed excellent antiproliferative activity in SW480, SK-BR-3 and 41-M human tumor cells [118].

Thiosemicarbazones with Antiviral Activity

The antiviral activity of thiosemicarbazones has been reviewed by Bauer [119]. This activity was first reported in 1950 by Hamre *et al.* [120], who found that benzaldehyde thiosemicarbazone derivatives were active against neurovaccinial infection in mice.

The isatin-thiosemicarbazones were found to be very active and a clinical trial of *N*-methyl-isatin-β-thiosemicarbazone (methisazone) against smallpox was carried out in India [121-123]. The drug has been also used to treat patients with herpes simplex virus (HSV) [124]. Its 4',4'-diethyl derivatives have been shown to inhibit Moloney leukemia virus [125] and human immunodeficiency virus (HIV) [126]. In the latter case, a significant selective inhibition of HIV structural protein synthesis was observed. Also, the products of the reaction of isatin and its derivatives with sulphadimidine were tested for antiviral activity against HIV-1 and HIV-2 in MT-4 cells, and compared with azidothymidine (AZT) [127].

Purine-6-carboxaldehyde thiosemicarbazone was found to be active against cytomegalovirus [128] and the activity of α-heterocyclic thiosemicarbazones against HSV was demonstrated by Brockman and co-workers [129]. A series of thiosemicarbazones derived from 2-acetylpyridine, 2-acetylquinoline and 1-acetylisoquinoline was evaluated as

inhibitors of type 1 and type 2 herpes simplex virus and some of them were highly active [124]. The selective inhibition of HSV ribonucleoside diphosphate reductase by 2-acetylpyridine thiosemicarbazone derivatives was investigated and the results supported the hypothesis that the HSV-induced RDR is an important target for the design of antiviral drugs [130]. Many other works report investigations of the antiviral effect of 2-acetylpyridine-derived thiosemicarbazones [131]. More recently, a study of the electronic and structural features of thiosemicarbazones of 2-acetylpyridine, 2-acetylquinoline and 1-acetylisoquinoline demonstrating anti-HSV-1 activity was published [132]. The antiviral activity of novel formyl and acyldiazine-derived thiosemicarbazones was also shown [133].

Thiosemicarbazones' Metal Complexes in Radiopharmacy

Thiosemicarbazones´ complexes of certain radionuclides have demonstrated their potentiality as radiopharmaceuticals for diagnosis purposes as well as for radiotherapy.

^{62}Cu-ATSM (ATSM = diacetyl-bis(N^4-methylthiosemicarbazone)) is a promising PET (positron emission tomography) tracer for noninvasive hypoxic tumor imaging [134-137 and references therein]. This and other radioactive Cu(II) complexes of bis(thiosemicarbazones) achieve high level of selectivity for uptake in hypoxic cells. The selectivity of these bioreductive prodrugs clearly depends on the Cu(II)/Cu(I) redox potential of the complexes. Chemical and electronic properties underlying the observed structure-activity relationships were studied [138, 139].

Similar compounds have been tested for the radiodiagnosis of other hypoxic tissues and perfusion abnormalities. ^{62}Cu-ATSM PET studies performed in human patients with coronary artery disease showed its usefulness as a hypoxic tissue tracer in myocardial ischemia [140]. ATSM labeled with the gamma emitter ^{99m}Tc showed high myocardium uptake in regions of ischemia in LAD rat myocardium model, being a potential agent for imaging myocardium hypoxia [141]. ^{62}Cu-PTSM (PTSM = pyruvaldehyde-bis(N^4-methylthiosemicarbazone)) has been proposed as a PET agent for the myocardial perfusion imaging in the diagnosis of coronary artery disease in humans and for the assessment of blood flow changes during treatment in patients with colorectal liver metastases [142-144].

On the other hand, ^{64}Cu bis(thiosemicarbazones) complexes have been described as promising agents for radiotherapy. Systemic administration of hypoxia – selective ^{64}Cu-ATSM to hamsters bearing human GW39 colon tumors, showed a significant increase of the survival time with no acute toxicity [145]. Laparoscopic colectomy for curable colon cancer may result in the development of abdominal wall implants. ^{64}Cu-PTSM has demonstrated in hamsters its therapeutic potential in inhibiting cancer cell implantation and growth [146].

Other Biological Properties of Thiosemicarbazones and Their Metal Complexes

As an intention to find new drugs against *Entamoeba histolytica,* which show less toxicity for the host than the reference drug Metronidazole, the potential of some Ru(II) and Pd(II) thiosemicarbazones complexes has been explored [147-150]. The antiamoebic activity of thiophene-2-carboxaldehyde thiosemicarbazone derivatives and their 1,5-cyclooctadiene Ru(II) complexes was tested *in vitro* against HK-9 strain of *E. histolytica*. Although in all cases complexation enhanced the activity of the basic molecule, these complexes were less active than Metronidazole [149]. [Ru(1,5-cyclooctadiene)(2-acetylpyridine thiosemicarbazone)] was found to be more active than Metronidazole [147].

Certain arylthiosemicarbazones produced significant increases in the mean survival times of mice infected with *T. cruzi*, although no cure was observed at doses near the toxic one [151]. Some selected arylthiosemicarbazones have exhibited potent activity against cruzain, the major cysteine protease of *T. cruzi*, as well as trypanocidal activity. Extensive structure-activity relationship studies were performed [152].

Platinum(II) and Ru(III) chelates with o-vanillin thiosemicarbazone derivatives and 2-acetylpyridine thiosemicarbazone derivatives have been evaluated for their antimalarial activity in mice infected with *Plasmodium berghei,* showing significant activity [153, 45]. Finally, the superoxide dismutase (SOD)-like activity of Cu(II) complexes of thiosemicarbazones was reported [154].

ACKNOWLEDGEMENTS

Financial support from PROSUL – CNPq, Brazil, is acknowledged by the authors. HB is also indebted to FAPEMIG. DG acknowledges financial support from TWAS.

REFERENCES

[1] Campbell, M J. *Coord. Chem. Rev.,* **1975**, *15*, 279.
[2] Padhyé, S. B.; Kauffman, G.B., *Coord. Chem. Rev.*, **1985**, *63*, 127.
[3] West, D. X.; Liberta A.; Padhye, S. B.; Chikate, R. C.; Sonawane, P.B.; Kumbhar, A. S.; Yerande, R.G., *Coord. Chem. Rev.*, **1993,** *123*, 49.
[4] West, D. X.; Padhye, S. B.; Sonawane, P. B. In *Structure and Bonding*, Springer-Verlag: New York, **1991**, Vol. *76*, pp 1-49.
[5] Casas, J. S.; García-Tesende, M. S.; Sordo, J.; West, D. X.; Liberta, A.; Padhye, S. B.; Chikate, R. C.; Sonawane, P. B.; Kumbhar, A. S.; Yerande, R. G., *Coord. Chem. Rev.*, **2000,** *209*, 197.
[6] Liu, M.C.; Lin, T.S.; Sartorelli, A.C. *In Progress in Medicinal Chemistry*, *32* Ellis G.P., Luscombe, D.K., Eds.; Elsevier: Amsterdam, **1995**, 1-35.
[7] Petering, D.H. In *Metal Ions in Biological Systems*, H. Sigel Ed., Marcel Dekker, N. York, **1980**; Vol.*11*, pp. 197-227.
[8] Pandeya, S.N.; Dimmock, J.R. *Pharmazie*, **1993**, *48*, 659.
[9] Farrell, N. *Coord. Chem. Rev.*, **2002**, *232*, 1.
[10] Cerecetto, H.; González, M. *Curr. Topics Med. Chem.*, **2002**, *2*, 1185.
[11] Cerecetto, H.; Di Maio, R.; Ibarruri, G.; Seoane, G.; Denicola, A.; Quijano, C.; Peluffo, G.; Paulino, M. *Il Farmaco*, **1998**, *53*, 89.
[12] Cerecetto, H.; Di Maio, R.; González, M.; Risso, M.; Sagrera, G.; Seoane, G.; Denicola, A.; Peluffo, G. A.; Quijano, C.; Stoppani, A.O.M.; Paulino, M.; Olea-Azar, C.; Basombrio, M.A. *Eur. J. Med. Chem.*, **2000**, *35*, 343.
[13] Olea-Azar, C.; Atria, A.M.; Mendizabal, F.; Di Maio, R.; Seoane, G.; Cerecetto, H. *Spectrosc. Lett.,* **1998**, *31*, 99.
[14] Martínez, V.; Cerecetto, H. *Bioorg. Med. Chem.,* **2001**, *9*, 1025.
[15] Pandeya, S.N.; Aggarwal, N.; Jain, J.S. *Pharmazie,* **1999**, *54*(4), 300.

[16] Dimmock, J.R.; Sidhu, K.K.; Thayer, R.S.; Mack, P.; Duffy, M.J.; Reid, R.S.; Quail, J.W.; Pugazhenthi, U.; Ong, A.; Bikker, J.A.; Weaver, D.F. *J. Med. Chem.,* **1993,** *36*(16), 2244.

[17] Dimmock, J.R.; Baker, G.B. *Epilepsia,* **1994**, *35*(3), 648.

[18] Dimmock, J.R.; Sidhu, K.K.; Tumber, S.D.; Basran, S.K.; Chen, M.; Quail, J.W.; Yang, J.; Rozas, I.; Weaver, D.F. *Eur. J. Med. Chem*., **1995**, *30*, 287.

[19] Dimmock, J.R.; Pandeya, S.N.; Quail, J.W.; Pugazhenthi, U.; Allen, T. M.; Kao, G.Y., Balzarini, J.; De Clerq, E. *Eur. J. Med. Chem*., **1995**, *30*, 303.

[20] Dimmock, J.R.; Sidhu, K.K.; Thayer, R.S.; Mack, P.; Duffy, M.J.; Reid, R.S.; Quail, J.W.; Pugazhenthi, U.; Ong, A.; Bikker, J.A.; Weaver, D.F. *J. Med. Chem.,* **1996**, *35*, 3984.

[21] Pandeya, S.N.; Ponnilavarasan, I.; Pandey, A.; Lahkan, R.; Stables, J.P. *Pharmazie,* **1999**, *54*, 923.

[22] Puthucode, R.N.; Pugazhenthi, U.; Quail, J.W.; Stables, J.P.; Dimmock, J.R. *Eur. J. Med. Chem.,* **1998**, *33*, 595.

[23] Dimmock, J.R.; Vashishtha, S.C.; Stables, J.P. *Eur. J. Med. Chem*., **2000**, *35*, 241.

[24] Sridhar, S.K.; Pandeya, S.N.; Stables, J.P.; Ramesh, A. *Eur. J. Med. Chem*., **2002**, *16*, 129.

[25] Taroua, M.; Ribuot, C.; Pera, M.H.; Taillandier, G.; Fatome, M.; Laval, J.D.; Demenge, P.; Leclerc, G. *Eur. J. Med. Chem*., **1996**, *31*, 589.

[26] Dimmock, J.R.; Baker, G.B. *Epilepsia*, **1994**, *35*(3), 648.

[27] Beraldo, H.; Sinisterra, R.D.; Teixeira, L.R.; Vieira, R.P.; Doretto, M.C. *Biochem. Biophys. Res. Comunn*., **2002**, *206*, 241.

[28] Warren, J.D.; Woodward, D.L.; Hargreaves, R.T. *J. Med. Chem.,* **1977**, *29*, 1520.

[29] Sogni, P.; Yang, S.; Pilette, C.; Moreau, R.; Gadano, A.; Avenard, G.; Bloy, C.; Lebrec, D. *Eur. J. Pharmacol*., **1998**, *344*, 37.

[30] Singh, A.; Dharkarey, R.; Saxena, G.C. *J. Indian Chem. Soc*., **1996**, *73*, 339.

[31] Anderson, R.J.; Cloudsdale, I.S.; Lamoreaux, R.J.; Schaefer, K.; Harr, J. **2000**, US Patent 6,110,869.

[32] Copping, L.G.; Kerry, J.C.; Watkins, T.I.; Wllis, R.J.; Bryan, H. **1983**, US Patent 4,394,387.

[33] Patole, J.; Dutta, S.; Padhye, S.; Sinn, E. *Inorg. Chim. Acta,* **2001**, *318*, 207.

[34] Dobek A.S.; Klayman D.L.; Dickson Jr., E.T.; Scovill J.P.; Tramont E.C. *Antimicrob. Agents Chemother.,* **1980,** *18*, 27.

[35] Dobek, A.S.; Klayman, D.L.; Dickson Jr., E.T.; Scovill, J.P.; Oster C.N. *Arzneimittelforschung,* **1983**, *33*, 1583.

[36] Kovala-Demertzi, D.; Demertzis, M.A.; Miller, J.R.; Papadopoulou, C.; Dodorou, C.; Filousis, G. *J. Inorg. Biochem.,* **2001**, *86*(2-3), 555.

[37] Klayman, D.L.; Lin, A.J.; Hoch, J.M.; Scovill, J.P.; Lambros, C.; Dobek, A.S. *J. Pharm. Sci.,* **1984,** *73*, 1763.

[38] Dobek, A.S.; Klayman, D.L.; Scovill, J.P.; Dickson, E.T. *J. Chemotherapy,* **1986**, *32*, 25.

[39] Kasuga, N.C.; Sekino, K.; Koumo, C.; Shimada, N.; Ishikawa, M.; Nomiya, K. *J. Inorg. Biohem*., **2000**, *84*, 55.

[40] Fahmi, N.; Singh, R.V. *J. Indian Chem. Soc*., **1996**, *7*3, 257.

[41] Singh, D.; Singh, R.V. *J. Inorg. Biochem*., **1993**, *15*, 227.

[42] Maiti, A.; Guha, A.K.; Ghosh, S. *J. Inorg. Biochem.*, **1988**, *3*3, 57.

[43] Singh, S.K.; Pandeya, S.N. *Boll. Chim. Farm.,* **2001**, *140*(4), 238.

[44] Kolocouris, A.; Dimas, K.; Pannecouque, C.; Witvrouw, M.; Boscolos, G.B.; Stamatiou, G.; Fytas, G.; Zoidis, G.; Kolocouros, N.; Andrei, G.; Snoeck, R.; De Clerq, E. *Bioorg. Med. Chem. Letters*, **2002**, *12*, 723.

[45] Offiong, O.E.; Etok, C.; Martelli, S. *Il Farmaco,* **1996,** *5*1, 801.

[46] Li, Q.; Tang, H.; Li, Y.; Wang, M.; Wang, L.; Xia, C. *J. Inorg. Biochem*., **2000**, *78*, 167.

[47] Bindu, P.; Kurup, M.R.P.; Satyakeerty, T.R. *Polyhedron,* **1999**, *18*, 321.

[48] Dittes, U.; Diemer, R.; Lenz, O.; Keppler, B.K. *J. Inorg. Biochem*., **1995**, *59*, 215.

[49] Domagk, G.; Behnish, R.; Mietzch, F.; Schmidt, H. *Naturwissenshaften*, **1946**, *33*, 315.

[50] Domagk, G. *Nordisk Medicin,* **1948**, *39*, 1322.

[51] Hoggarth, E.; Martin, A.R.; Storey, N.E.; Young, H.P. *Brit. J. Pharmacol,* **1949**, *4*, 248.

[52] Manetti, F.; Corelli, F; Biava, M.; Fioravanti, R.; Porretta, G.C.; Botta, M. *Il Farmaco*. **2000**, *55*, 484.

[53] Collins, F.M.; Klayman, D.L.; Morrison, N.E. *J. General Microbiology, **1982**, 128*, 1349.

[54] Kovacic, P.; Ames, J.D.; Ryan, M.D. *Bioelectrochemistry and Bioenergetics*, **1989**, *21*, 269, a section of *J. Electroanal. Chem*., **1989**, 275.

[55] Benns, B.G.; Gingras, B.A.; Bayley, C.H. *Appl. Microbiology*, **1960**, *8*, 353.

[56] Thimmaiah, K.N.; Chandrappa, G.T.; Rangaswami, Jayarama. *Polyhedron*, **1984**, *3*, 1237.

[57] Parwana, H.K.; Singh, G. *Inorg. Chim. Acta*, **1985**, *108*, 87.

[58] West, D.X.; Carlson, C.S.; White, A.C.; Liberta, A. *Transition Met. Chem*., **1990**, *15*, 43.

[59] West, D.X.; White, A.C.; Sharif, F.D.; Gebremedhin, H.; Liberta, A.E. *Transition Met. Chem*., **1993**, *18*, 238.

[60] West, D.X.; Carlson, C.S.; Whyte, A.C., Liberta, A.E. *Transition Met. Chem*., **1990**, *15*, 43.

[61] West, D.X.; Carlson, C.S.; Liberta, A.E.; Albert, J.N.; Daniel, C.R. *Transition Met. Chem*., **1990**, 15, 341.

[62] Bermejo, E.; Caballo, R.; Castiñeras, A.; Domínguez, R.; Liberta, A.E.; Maichle-Mössmer, C.; Salberg, M.; West, D.X. *Eur. J. Inorg. Chem*., **1999**, 965.

[63] West, D.X.; Yang, Y.; Klein, T.L.; Goldberg, K.I.; Liberta, A.E. *Polyhedron*, **1995**, *14*(20), 3051.

[64] West, D.X.; Ives, J.; Krejci, J.; Salberg, M.; Zumbahlen, T.L.; Bain, G.A.; Liberta, A.E. *Polyhedron*, **1995**, *14* (15), 2189.

[65] Brockman, R.W.; Thomson, J.R.; Bell, M.J.; Skipper, H.E. *Cancer Res*., **1956**, *16*, 167.

[66] French, F.A.; Blanz, E.J. Jr. *Cancer Res*., **1965**, *25*, 1454.

[67] French, F.A.; Blanz, E. J. Jr. *J. Med. Chem*., **1966**, *9*, 585.

[68] Agrawal, K.C.; Booth, B.A.; Sartorelli, A.C. *J. Med. Chem.,* **1968**, *11*, 700.

[69] French, F.A.; Blanz, E. J. Jr. *Cancer Res*., **1966**, *2*6, 1638.

[70] Sartorelli, A.C.; Moore, E.C.; Agrawal, K.C. *Biochem. Pharmacol.,* **1971**, *2*0, 3119.

[71] Sartorelli, A.C.; Agrawal, K.C.; Tsiftsoglou, A.S.; Moore, E.C. *Adv. Enz. Reg.,* **1977**, *15*, 117.

[72] Brockman, R.W., Sidwell, R.W., Arnett, G.; Shaddix, S.M. *Proc. Soc. Exptl. Biol. Med*., **1970**, *133*, 609.

[73] Moore, E.C., Sartorelli, A.C. *Pharmac. Ther.,* **1984**, *24*, 439.

[74] Antholine, W.; Knight, J.; Whelan, H.; Petering, D.H. *Mol. Pharmacol*., **1977**, *13*, 89.

[75] Ankel, E.;. Petering, D.H. *Biochem. Pharmacol*., **1980**, *29*, 1833.

[76] Antholine, W.E.; Knight, J.M.; Petering, D.H. *J. Med. Chem.,* **1976**, *19*(2), 339.

[77] Saryan, L.A.; Ankel, E.; Khrishnamurti, C.; Petering, D.H. *J. Med. Chem*., **1979**, *22*, 1218.

[78] Saryan, L.A.; Mailer, K.; Khrishnamurti, C.; Antholine, W.; Petering, D.H. *Biochem. Pharmacol.,* **1981**, *30*, 1595.

[79] Thelander, L.; Graslund, A., Thelander, M., *Biochem. Biophys. Res. Commun.,* **1983**, *110*(3), 859.

[80] Thelander, L.; Graslund, A. *J. Biol. Chem.*, **1983**, *286*(7), 4063.

[81] Beraldo, H.; Fantini, E.;. Borges, R.H.U.; Rocha, M.A.; Tosi, L.; Abras, A. *Inorg. Chim. Acta*, **1990**, *172*, 113.

[82] Borges, R.H.U.; Paniago, E.; Beraldo, H. *J. Inorg. Biochem*., **1997**, *65*, 267.

[83] Garcia-Tojal, J.; García-Orad, A.; Serra, J.L.; Pizarro, J.L.; Lezama, L.; Arriortua, M.I.; Rojo, T. *J. Inorg. Biochem*., **1999**, *75*, 45.

[84] Garcia-Tojal, J.; García-Orad, A.; Alvarez-Díaz, A.; Serra, J.L.; Urtiaga, M.K.; Arriortua, M.I.; Rojo, T. *J. Inorg. Biochem*., **2001**, *84*, 271.

[85] DeConti, R.C.; Toftness, B.R.; Agrawal, K.C.; Tomchick, R.; Mead, J.A.R.; Bertino, J.R.; Sartorelli, A.C.; Creasey, W. *Cancer Res*., **1972**, *32*, 1455.

[86] Sartorelli, A. C.; Agrawal, K. C.; Moore, E C. *Biochem. Pharmacol*., **1971**, *20*, 3119.

[87] Agrawal, K.C.; Lin, A.J.; Booth, B.A.; Wheaton, J.R.; Sartorelli, A.C. *J. Med. Chem.,* **1974**, *17*, 631 and Agrawal, K.C.; Lin, A.J.; Booth, B.A.; Denuzzo, S.M.; Sartorelli, A.C. *J. Med. Chem.,* **1975**, *18*, 368.

[88] Agrawal, K.C.; Lin, A.J.; Booth, B.A.; Sartorelli, A.C. *J. Med. Chem.,* **1968**, *11*, 700.

[89] Agrawal, K.C.; Mooney, P.D.; Sartorelli, A.C. *J. Med. Chem.,* **1976**, *19*, 970.

[90] Liu, M.; Lin, T.S.; Cory, J.G.; Cory, A.H.; Sartorelli, A.C. *J. Med. Chem*., **1996**, *39*, 2586.

[91] Liu, M.C.; Lin, T.S.; Sartorelli, A.C. *J. Med. Chem.,* **1992**, *35*, 3672.

[92] Liu, M.C.; Lin, T.S.; Cory, J.G.; Cory, A.; Sartorelli, A.C. *J. Med. Chem*., **1996**, *39*, 2586.

[93] Finch, R.A.; Liu, M.C.; Grill, S.P.; Rose, W.C.; Loomis, R.; Vasquez, K.M.; Cheng, Y.; Sartorelli, A.C. *Biochem. Pharmacol.*, **2000**, *59*, 983.

[94] Finch, R.A.; Liu, M.C.; Cory, A.H.; Cory, J.G.; Sartorelli, A.C. *Advan. Enzyme Regul.,* **1999**, 39, 3.

[95] Richardson, D.R. *Critical Reviews in Oncology/Hematology*, **2002**, *42*, 267.

[96] Winkelmann, D.A.; Bermke, Y.; Petering, D.H. *Bioinorg. Chem.*, **1974**, *3*, 261.

[97] Petering, H.G.; Buskirk, H.H.; Underwood, G.E. *Cancer Res.,* **1964**, *24*, 367.

[98] Petering, H.G.; Buskirk, H.H.; Crim, J.A. *Cancer Res.,* **1967**, *27*, 1115.

[99] Rodriguez-Arguelles, M.C.; Ferrari, M.B.; Fava, G.G.; Pelizzi, C.; Tarasconi, P.; Albertini, R.; Dall'Aglio, P.; Lunghi, P.; Pinelli, S. *J. Inorg. Biochem.,* **1995**, *58*, 157.

[100] Hall, I.H.; Lackey, C.B.; Kistler, T.D.; Ives, J.S.; Beraldo, H.; Ackerman, L.; West, D.X. *Arch. Pharm. Pharm. Med. Chem.,* **2000**, *333*, 217.

[101] Matesanz, A.A.; Perez, J.M.; Navarro, P.; Moreno, J.M.; Colacio, E.; Souza, P. *J. Inorg. Biochem*., **1999**, *76*, 29.

[102] Ferrari, M.B.; Fava, G.G.; Leporati, E.; Pelosi, G.; Rossi, R.; Tarasconi, P.; Albertini, R.; Bonatti, A.; Lunghi, P.; Pinell, S. *J. Inorg. Biochem*., **1998**, *70*, 145.

[103] Ferrari, M.B.; Bisceglie, F.; Pelosi, G.; Sassi, M.; Tarasconi, P.; Cornia, M.; Capacchi, S.; Albertini, R.; Pinelli, S. *J. Inorg. Biochem*., **2002**, *90*, 113.

[104] Ferrari, M.B.; Bisceglie, F.; Fava, G.G.; Pelosi, G.; Tarasconi, P.; Albertini, R.; Pinelli, S. *J. Inorg. Biochem*., **2002**, *89*, 36.

[105] Ferrari, M.B.; Capacchi, S.; Reffo, G.; Pelosi, G.; Tarasconi, P.; Albertini, R.; Pinelli, S.; Lunghi, P. *J. Inorg. Biochem*., **2000**, *81*, 89.

[106] Perez, J.M.; Matesanz, A.I.; Martín-Ambite, A.; Navaro, P.; Alonso, C.; Souza, P. *J. Inorg. Biochem.*, **1999**, *75*, 255.

[107] Cao, R.; Garcia, A.; Castell, E. *Monatshefte für Chemie*. **1992**, *123*, 487.

[108] Penninks, A.H. *Tin Based Antitumor Drugs*, Gielen, M.; Ed., NATO ASI Series, Series H, Springer, Berlin, **1990**, *37*, 169.

[109] Casas, J.S.; García-Tasende, M.S.; Maichle-Mössmer, C.; Rodriguez-Argüelles, M.C.; Sanchez, A.; Sordo, J.; Vasquez-Lopez, A.; Pinelli, S.; Lunghi, P.; Albertini, R. *J. Inorg. Biochem*., **1996**, *62*, 41.

[110] Casas, J.S.; Rodriguez-Argüelles, M.C.; Russo, U.; Sanchez, A.; Sordo, J.; Vasquez-Lopez, A.; Pinelli, S.; Lunghi, P.; Bonati, A.; Albertini, R. *J. Inorg. Biochem*., **1998**, *69*, 283.

[111] Casas, J.S.; Castiñeras, A.; Rodriguez-Argüelles, M.C.; Sanchez, A.; Sordo, J.; Vasquez-Lopez, A.; Pinelli, S.; Lunghi, P.; Caincianaini, P.; Bonati, A.; Dall'Aglio, P.; Albertini, R. *J. Inorg. Biochem*., **1999**, 76, 277.

[112] Teoh, S.; Ang, S.; Fun, H.; Ong, C. *Journal of Organometallic Chemistry*, **1999**, *580*, 17.

[113] Kovala-Demertzi, D.; Miller, J.R.; Koukoumelis, N.; Hadjikakou, S.K.; Demetzis, M.A. *Polyhedron*, **1999**, *18*, 1005.

[114] Kovala-Demertzi, D.; Domopoulou, A.; Demetzis, M.A.; Papageorgiou, A.; West, D.X. *Polyhedron,* **1997**, *16*, 3625.

[115] Kovala-Demertzi, D.; Yadav, P.N.; Demetzis, M.A.; Coluccia, M. *J. Inorg. Biochem*., **2000**, *78*, 347.

[116] Quiroga, A.G.; Perez, J.M.; Montero, E.I.; Masaguer, J.R.; Alonso, C.; Navarro-Ranninguer, C. *J. Inorg. Biochem.,* **1998**, *70*, 117.

[117] Quiroga, A.G.; Perez, J.M.; Lopez-Solera, I.; Montero, E.I.; Masaguer, J.R.; Alonso, C.; Navarro-Ranninguer, C. *J. Inorg. Biochem.,* **1998**, *69*, 275.

[118] Arion, V.B.; Jakupec, M.A.; Galanski, M.; Unfried, P.; Keppler, B.K. *J. Inorg. Biochem*., **2002**, *91*, 298.

[119] Bauer, D.J. Ed. *Thiosemicarbazones in Chemotherapy of Virus Diseases,* Pergamon Press, Oxford **1972**, Vol. *1*.

[120] Hamre, D.; Bernstein, J.; Donovick, R. *Proc. Soc. Exp. Biol. Med*., **1950,** *73*, 275.

[121] Bauer, D.J. *Ann. N.Y. Acad. Sci.*, **1965**, *130*, 110.

[122] Bauer, D.J.; St. Vincent, L.; Kempe, C.H.; Downie, A.W. *Lancet*, **1963**, 494.

[123] Bauer, D.J.; St. Vincent, L.; Kempe, C.H.; Young, P.A.; Downie, A.W. *Am. J. Epidemiol*., **1969***, 90*, 130.

[124] Shipman, C.; Smith, S.H.; Drach, J.C.; Klayman, D.L. *Antiviral Research*, **1986**, *6*, 197.

[125] Ronen, D.; Nir, E.; Teitz, Y. *Antiviral Research*, **1985**, *5*, 249.

[126] Teitz, Y.; Ronen, D.; Vansover, A.; Stematsky, T.; Riggs, J.L. *Antiviral Research*, **1994**, *24*, 305.

[127] Selvam, P.; Chandramohan, M.; De Clerq, E.; Witvrouw, M.; Pannecouque, C. *Eur. J. Pharm. Sciences*, **2001**, *14*, 313.

[128] Sidwell, R.W.; Arnett, G.; Dixon, J.G.; Schabel Jr., F.M. *Proc. Soc. Exp. Biol. Med*., **1969**, *131*,1223.

[129] Brockman, R.W.; Sidwell, R.W.; Arnett, G.; Shadix, S. *Proc. Soc. Exp. Biol. Med.,* **1970**, *133*, 609.

[130] Turk, S.R.; Shipman, C.; Drach, J.C. *Biochem. Pharmacol.*, **1986**, *35*(9), 1539.

[131] Drach, J.C.; Eck, C.R.; Perrott, E.K.; Moreau, J.P.; Shipman, C. *Antiviral Research,* **1990**, *13*(1), 100.

[132] Altun, A.; Kumru, M.; Dimoglo, A. *J. Molec. Struct. (Teochem)*, **2001**, *535*, 235.

[133] Easmon, J.; Heinish, G.; Holzer, H.; Rosenwith, R. *J. Med. Chem,* **1992**, *35*, 3288.

[134] Blower, P. J.; Dilworth,J. R.; Maurer, R. I.; Mullen, G. D.; Reynolds, C. A.; Zheng, Y. *J. Inorg. Biochem.*, **2001**, *85*(1), 15.

[135] Ballinger, J. R. *Semin. Nucl. Med.*, **2001**, *31*(4), 321.

[136] Takahashi, N.; Fujibayashi, Y.; Yonekura, Y.; Welch, M. J.; Waki, A.; Tsuchida, T.; Sadato, N.; Sugimoto, K.; Itoh, H. *Ann. Nucl. Med.*, **2000**, *14*(5), 323.

[137] Chao, K. S.; Bosch, W. R.; Mutic, S.; Lewis, J. S.; Dehdashti, F.; Mintun, M. A.; Dempsey,J. F.; Perez, C. A.; Purdy, J. A.; Welch, M. J. *Int. J. Radiat. Oncol. Biol. Phys.*, **2001**, *49*(4), 1171.

[138] Cowley, A.R.; Dilworth, J.R.; Donnelly, P.S.; Labishal, E.; Sousa, A. *J. Am. Chem. Soc.*, **2002**, *124*, 5270.

[139] Maurer, R. I.; Blower, P. J.; Dilworth, J. R.; Reynolds, C. A.; Zheng, Y.; Mullen, G. E. D. *J. Med. Chem*., **2002**, *45*(7), 1420.

[140] Takahashi, N.; Fujibayashi, Y.; Yonekura, Y.; Welch, M. J.; Waki, A.; Tsuchida, T.; Sadato, N.; Sugimoto, K.; Nakano, A.; Lee, J. D.; Itoh, H. *Ann. Nucl. Med.*, **2001**, *15*(3), 293.

[141] Horiuchi, K.; Tsukamoto, T.; Saito, M.; Nakayama, M.; Fujibayashi, Y.; Saji, H. *Nucl. Med. Biol.*, **2000**, *27*(4), 391.

[142] Haynes, N. G.; Lacy, J. L.; Nayak, N.; Martin, C. S.; Dai, D.; Mathias, C. J.; Green, M. A. *J. Nucl. Med.*, **2000**, *41*(2), 309.

[143] Wallhaus, T. R.; Lacy, J.; Stewart, R.; Bianco, J.; Green, M. A.; Nayak, N.; Stone, C. K. *J. Nucl. Cardiol.,* **2001***, 8*(1), 67.

[144] Flower, M. A.; Zweit, J.; Hall, A. D.; Burke, D.; Davies, M. M.; Dworkin, M. J.; Young, H. E.; Mundy, J. Ott, R. J.; McCready, V. R.; Carnochan, P.; Allen-Mersh, T. G. *Eur. J. Nucl. Med.*, **2001**, *28*(1), 99.

[145] Lewis, J.; Laforest, R.; Buettner, T.; Song, S.; Fujibayashi, Y.; Connett, J.; Welch, M. *Proc. Natl. Acad. Sci. USA*, **2001**, 98(3), 1206.

[146] Lewis, J. S.; Connett, J. M.; Garbow, J. R.; Buettner, T. L.; Fujibayashi, Y.; Fleshman, J. W.; Welch, M. J. *Cancer Res.*, **2002**, *62*(2), 445.

[147] Bharti, N; Maurya, M. R.; Naqvi, F; Azam, A. *Bioorg. Med. Chem-Lett*., **2000**, *10(20)*, 2243.

[148] Neelam, B.; Mannar, M.; Fehmida, N.; Alok, B.; Sudha, B.; Amir, A. *Eur. J. Med. Chem.*, **2000**, *35(5*), 481.

[149] Shailendra, N. S.; Bharti, N.; Gonzalez-Garza, M. T.; Cruz-Vega, D. E.; Castro-Garza, J.; Saleem, K.; Naqvi, F.; Azam, A. *Bioorg. Med. Chem-Lett*., **2001**, *11*, 2675.

[150] Offiong, O. E.; Martelli, S. *Il Farmaco*, **1994**, *49(7-8)*, 513.

[151] Wilson, H. R.; Revankar, G. R.; Tolman, R. L. *J. Med. Chem.*, **1974**, *17(7)*, 760.

[152] Du, X.; Guo, C.; Hansell, E; Doyle, P.S.; Caffrey, C. R.; Holler, T. P.; McKerrow, J. H.; Cohen, F. E. *J. Med. Chem.*, **2002**, *45(13)*, 2695.

[153] Offiong, O. E.; Martelli, S. *Il Farmaco*, **1995**, *50(9)*, 625.

[154] Diaz, A.; Cao, R.; Fragoso, A.; Sánchez, H. *Inorg. Chem. Comm.*, **1999**, *2*, 358.