

Thiosemicarbazone Metal Complexes: From Structure to Activity

Giorgio Pelosi*

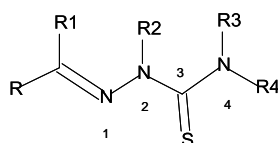
Dipartimento di Chimica Generale e Inorganica, Chimica Analitica, Chimica Fisica, viale GP Usberti 17a, 43124 Parma, Italy

Abstract: Thiosemicarbazones and their metal complexes are compounds that possess antitumor, antibacterial, antifungal and antiviral properties. For the foremost majority of cases the activity of the ligand is greatly enhanced by the presence of a metal ion. The most relevant papers recently published are reviewed with an attempt to find a relationship between common structural features and activity.

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

1. INTRODUCTION

Thiosemicarbazones (Scheme 1) are compounds that have been studied for a considerable period of time for their biological properties. Traces of interest date back to the beginning of the 20th century but the first reports on their medical applications began to appear in the Fifties as drugs against tuberculosis and leprosy [1, 2]. In the Sixties their antiviral properties were discovered and a huge amount of research was carried out that eventually led to the commercialization of methisazone, Marboran[®], to treat smallpox [3]. In this period one of the first antitumor activity results was published [4]. Recently Triapine[®] (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) has been developed as an anticancer drug and has reached clinical phase II on several cancer types [5, 6]. Presently, the areas in which thiosemicarbazones are receiving more attention can be broadly classified according to their antitumor, antiaprotzoal, antibacterial or antiviral activities and in all cases their action has been shown to involve interaction with metal ions [7, 8]. This review will be mainly focused on the most relevant papers published in the past five years since an extensive report by Beraldo *et al.* [9] covers the previous works. It is important to note that recently papers have appeared using theoretical methods to predict the specific activity of a series of thiosemicarbazones. However, to date there are no such investigations on metal complex derivatives.



Scheme 1. The drawing represents the general formula for thiosemicarbazones. R₁, R₂, R₃, R₄ = H, or any organic substituent (the conventional numbering scheme from 1 to 4 is shown).

*Address correspondence to this author at the Dipartimento di Chimica Generale e Inorganica, Chimica Analitica, Chimica Fisica, viale GP Usberti 17a, 43124 Parma, Italy; Fax: +390521905557; Tel: +390521905420; E-mail: giorgio.pelosi@unipr.it

2. BIOACTIVITY OF THIOSEMICARBAZONE METAL COMPLEXES

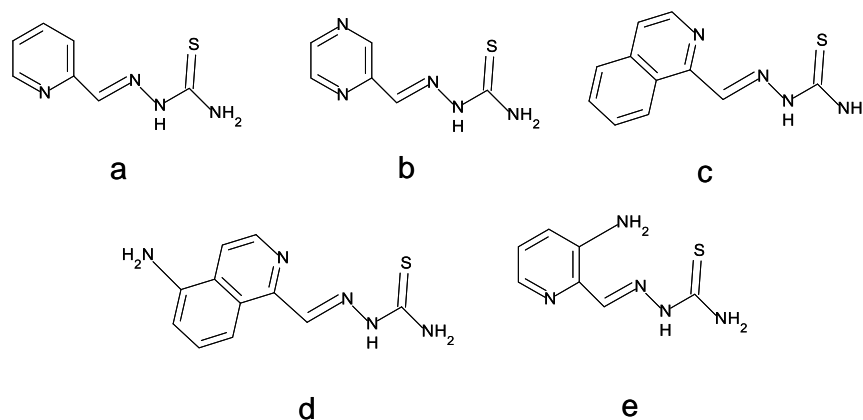
2.1. Antitumor Activity

One of the most promising areas in which thiosemicarbazone compounds are being developed is their use against cancer. Their antitumor activity is extremely differentiated and it is very much dependent on the typology of tumour cells. This characteristic renders the whole class of compounds very interesting because it implies selectivity. At the same time it makes difficult to extract from the literature general information valid for the whole class of compounds since their activity is certainly due to more than one target in the cell machinery. Nevertheless, the presence of a metal ion almost systematically increases the activity or contributes to mitigate the side effects of the organic parent compounds [10]. Presently, the main known effects related to their anticancer activity are, in order of discovery, ribonucleotide reductase (RR) inhibition [11], reactive oxygen species (ROS) production [12], topoisomerase II inhibition [13], mitochondria disruption [14], and, more recently, a multidrug resistance protein (MDR1) inhibition [15,16].

2.1.1. Ribonucleotide Reductase

The first breakthrough in the comprehension of the antitumor effect of thiosemicarbazones was obtained in the Sixties and deserves a brief résumé. The anti-leukemic effect of 2-formylpyridine thiosemicarbazone (Scheme 2a) was first reported by Brockman *et al.* [17] in 1956. Almost ten years later, in 1965, French *et al.* [10] formulated hypotheses about the mode of action of the $\alpha(N)$ -heterocyclic thiosemicarbazones: the active molecules shared a tridentate nature, that allows them to be effective chelators, and a better activity was obtained by modifying the aromatic system. Based on this principle they managed to predict the activity of pyrazine carboxaldehyde thiosemicarbazone (Scheme 2b) and 1-formylisoquinoline thiosemicarbazone (Scheme 2c).

A first hypothesis of action for these compounds, based on the high formation constant of their metal complexes, was their ability to sequester iron from the cell environment. The



Scheme 2. The drawings represent the formulas for: 2-formylpyridine thiosemicarbazone (a), pyrazine carboxaldehyde thiosemicarbazone (b), 1-formylisoquinoline thiosemicarbazone (c), 1-formyl-5-aminoisoquinoline thiosemicarbazone (Triapine®) (d), 3-amino-2-formylpyridine thiosemicarbazone (Triapine®) (e).

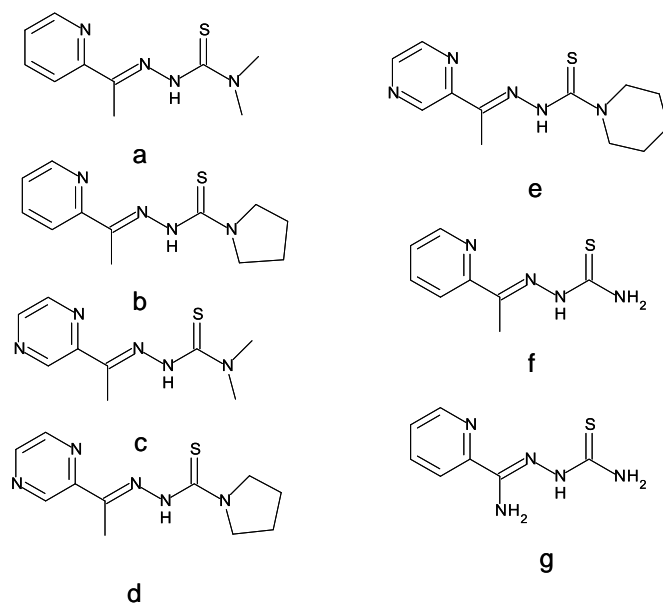
requirements for activity were identified with an NNS chelating system, which agreed with their chelating properties, but also with the presence of an aromatic fragment bound to the hydrazine moiety of thiosemicarbazide. Sartorelli *et al.* [18] observed that these compounds repressed the incorporation of ³H thymidine into the DNA and first proposed the inhibition of ribonucleotide reductase as the mechanism through which these molecules work [11,19]. Ribonucleotide reductase is an iron-dependent enzyme that promotes the reduction of ribose to deoxyribose through a free radical mechanism that is triggered by a tyrosyl radical. Inhibition of this enzyme leads to a block in the synthesis phase of the cell cycle and eventually to cell death by apoptosis. They also indirectly demonstrated that the active species was the iron(II) complex of 1-formylisoquinoline thiosemicarbazone. In fact, it was later discovered that iron and copper complexes are by far more active than the free ligands [20]. A reasonable mechanism was proposed by Thelander *et al.* [21] who proved, by exposing ribonucleotide reductase to the aforementioned molecules, that it is the tyrosyl free radical of the enzyme that is targeted by the drug and that the thiosemicarbazone complex inhibits the enzyme by destroying the radical. This mechanism requires oxygen and excludes the role of thiosemicarbazones as simple iron chelators. They also report that the reaction is reversible, and this is in agreement with the experimental observations. The fact that 1-formylisoquinoline thiosemicarbazone inhibits more strongly ribonucleotide reductase than 2-formylpyridine thiosemicarbazone gave an indirect hint about the fact that in the enzyme there must be a hydrophobic pocket or patch with which the aromatic system interacts, which could justify the fact that methylation on the aromatic ring of 2-formylpyridine thiosemicarbazone renders this compound more active. In search of an optimum bulk for the aromatic fragment Agrawal *et al.* [22] identified it with 2-formyl-4-(3-amino)phenylpyridine thiosemicarbazone that was the most active of the 3-aminophenyl derivatives. The most active compound found in the isoquinoline series was instead 1-formyl-5-aminoisoquinoline thiosemicarbazone (Scheme 2d) [23].

A recent development in the 2-formylpyridine series is the discovery of 3-amino-2-formylpyridine thiosemicarbazone (Triapine®) (Scheme 2e) [7]. This compound is a potent proliferation inhibitor of many cancer types and presents

a marked selectivity for tumour cells. Triapine is currently in phase II clinical trial on many typology of tumours [5, 12, 24, 25]. Two recent interesting papers by Kowol *et al.* [26,27] report the synthesis, characterization and biological assays of complexes of Fe(III) and Ga(III) (this latter is an ion known for inhibiting ribonucleotide reductase and for its antiproliferative properties). In the first paper 2-acetylpyridine N,N-dimethyl thiosemicarbazone (Scheme 3a), 2-acetylpyridine N-pyrrolidinyl thiosemicarbazone (Scheme 3b), acetylpyrazine N,N-dimethyl thiosemicarbazone (Scheme 3c), acetylpyrazine N-pyrrolidinyl thiosemicarbazone (Scheme 3d), and acetylpyrazine N-piperidinyl thiosemicarbazone (Scheme 3e) are studied. Those reported in the second are the complexes of 3-amino-2-formylpyridine (Triapine) (Scheme 2e), 2-formylpyridine (Scheme 2a), 2-acetylpyridine (Scheme 3f), 2-pyridine formamide thiosemicarbazones (Scheme 3g) as well as their N4-dimethylated analogues.

These complexes, together with the parent ligands, were tested for their *in vitro* antiproliferative activity on two human cancer cell lines (41M and SK-BR-3). It has been observed that the coordination to gallium(III) increases the cytotoxicity, while the iron(III) complexes, against all odds, show reduced cytotoxic activity compared to the metal-free thiosemicarbazones. These compounds were then tested for the capacity of inhibiting ribonucleotide reductase by incorporation of ³H-cytidine into DNA in a purified protein solution and in whole cells. What emerged is that the reactivity order in inhibition between the iron complex, the gallium complex and the ligand is reversed in the two cases, phenomenon that can only be explained by admitting additional reactions or different uptake kinetics. In particular the isolation and X-ray structural determination of two forms, a cationic complex [GaL₂]⁺ (L from here on represents the monoanionic, N,N,S-coordinated thiosemicarbazone) and the neutral analogue [GaLCl₂] (see Fig. 1 and Fig. 2 for the structures of [FeL₂]⁺ and [GaLCl₂], respectively) has allowed the identification of species expected to be part of an equilibrium in solution. Since the activity is the same, it can be hypothesised that the neutral form is the one that enters the cell.

In the second series of compounds the terminal nitrogen dimethylation was found to enhance markedly the cytotoxicity of metal-free ligands and that of their iron(III) and



Scheme 3. The drawings represent the formulas for: 2-acetylpyridine N,N-dimethyl thiosemicarbazone (**a**), 2-acetylpyridine N-pyrrolidinyl thiosemicarbazone (**b**), acetylpyrazine N,N-dimethyl thiosemicarbazone (**c**), acetylpyrazine N-pyrrolidinyl thiosemicarbazone (**d**), and acetylpyrazine N-piperidinyl thiosemicarbazone (**e**), 2-acetylpyridine (**f**), 2-pyridine formamide thiosemicarbazone (**g**).

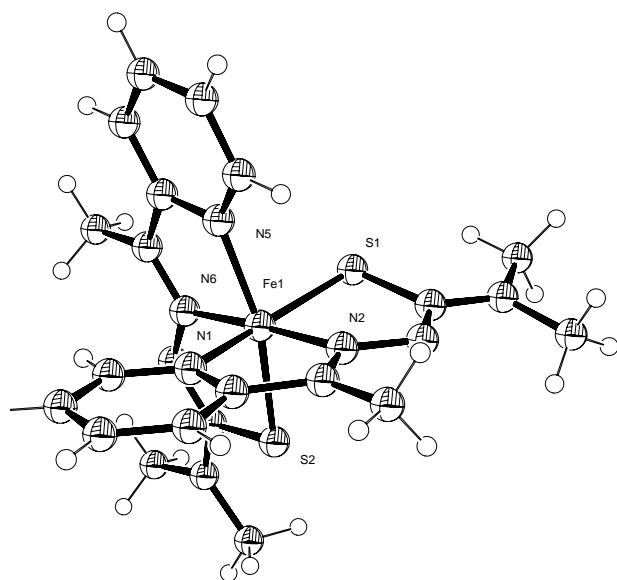


Fig. (1). Representation of the complex cation $[\text{FeL}_2]^+$ [26].

gallium(III) complexes, unless an NH_2 functionality was present anywhere on the thiosemicarbazone backbone or the aromatic part of the molecule. ^3H -Cytidine DNA incorporation assays showed that the ability of these terminally dimethylated compounds to inhibit RR can only partly explain the increased cytotoxicity.

In all these compounds it is worth noting that the metal ion, upon coordination, orients the ligands with the lipophilic and aromatic parts outwards toward the solvent (Scheme 4) and this can also account for the scarce solubility of these complexes in water. Moreover, upon complexation the acidity of the ligand is increased following a stabilization of the negative charge by the metal ion. In most structures the C-S bond is much longer than a double bond while the adjacent

C-N bond is shorter than a single bond indicating a prevalent thiolate resonance form.

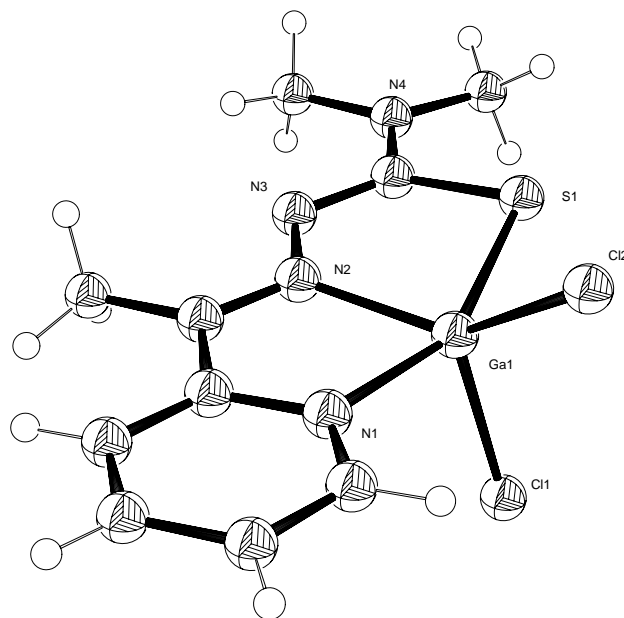
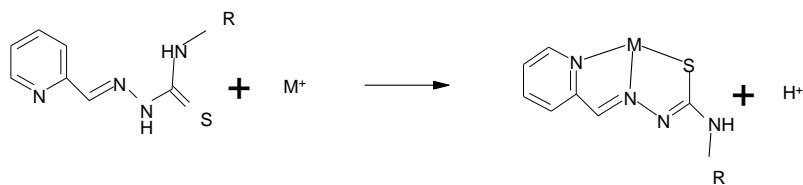


Fig. (2). Drawing of a complex obtained with stoichiometry Ga:L 1:1, $[\text{GaLCl}_2]$ [26].

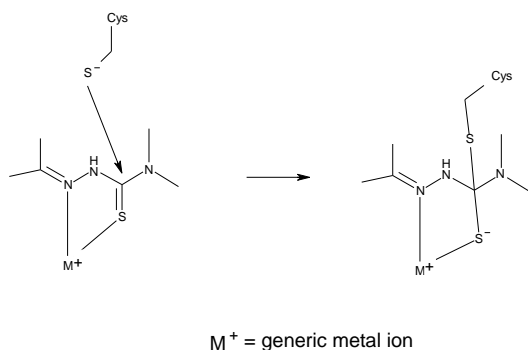
The same authors [28] have also tried to see what is the influence of a change in the chalcogen atom synthesizing 2-acetylpyridine N,N-dimethylsemicarbazone and -seleno-semicarbazones to compare with the 2-acetylpyridine N,N-dimethyl thiosemicarbazones on the same two cell lines (41M and SK-BR-3) and the outcome was that the antiproliferative activity is strongly dependent on the identity of the chalcogen atom (O, S, Se) and that it increases in the order $\text{Se} > \text{S} \gg \text{O}$. This observation could suggest a possible role



M^+ = generic metal ion

Scheme 4. The drawing shows that the metal ion, upon coordination, orients the lipophilic and aromatic parts of ligands outwards the complex molecules, toward the solvent.

of the chalcogenic element on the stabilization of a hypothetical anionic form or a radical species (this latter would then lead to the quenching of the tyrosine radical in ribonucleotide reductase). The reactivity of the thiosemicarbazone sulfur with cysteines is in fact envisaged in a paper by Trossini *et al.* [29] that proposes, as a mechanism of action, a nucleophilic attack of a cysteine sulfur on the thionyl fragment. The intermediate that forms is stabilized, in their model, by the presence of a histidine but it could be even better stabilized by a metal centre (Scheme 5). Obviously this reaction is reversible.



M^+ = generic metal ion

Scheme 5. The reactivity of the thiosemicarbazone sulfur with cysteines as envisaged in a paper by Trossini *et al.* [29]. A nucleophilic attack of a cysteine sulfur on the thionyl fragment is hypothesized.

Mendes *et al.* [30] has recently reported a study on the cytotoxic activity against malignant glioblastoma RT2 and T98 cell lines of 2-pyridineformamide thiosemicarbazone, of its N_4 -methyl and N_4 -ethyl derivatives and of their gallium(III) complexes. All these compounds were able to induce cell death by apoptosis also on cell that are resistant to gallium nitrate. The gallium(III) complexes of the 4-methyl and 4-ethyl derivatives showed IC_{50} values in the 0.81-9.57 μM range against RT2 cells and in the 3.6-11.30 μM range against T98 cells, and were 20-fold more potent than cisplatin. The interest of this paper lies on the fact that these compounds induce apoptosis independently of the presence of a p53 protein pathway and therefore ideal for gliomas which are mainly defective in this pathway to apoptosis.

An interesting insight into the distribution of Triapine in peripheral blood mononuclear cells (PBMCs) obtained from patients previously treated with the drug has been recently published by Kolesar *et al.* [31]. EPR is used to investigate what species are found *in vivo* and the findings suggest that Cu-Triapine (CuL) and Fe-Triapine (FeL₂) complexes should form in cells. The authors also notice that Fe uptake is blocked, possibly through generation of reactive oxygen species from FeL₂ or CuL that damage transferrin or transferrin receptor [32-34]. This is a hypothesis also supported

by the fact that it is known that 2-formylpyridine monothiosemicarbazonato Cu(II) inhibits cellular iron uptake as well as inhibiting ribonucleotide reductase [35]. Another site observed by EPR is heme iron, and this could suggest that reactive oxygen species could cause a release of oxidized cytochrome c and bring to the formation of the apoptosome that triggers apoptosis.

Among other recently synthesised compounds, it is noteworthy a series of di-2-pyridylketone thiosemicarbazone derivatives whose anticancer activity was assessed by using a panel of human xenografts in nude mice [36]. One of the compounds, and namely di-2-pyridylketone-4,4'-dimethyl-3-thiosemicarbazone, reduced after a 7-week treatment the net growth of a melanoma xenografted mice to only 8% of that in mice used as a reference. Even more remarkable is that no differences were observed in hematological indices between treated mice and controls which renders these compounds extremely promising for further developments as drugs. Following this discovery the authors [37] have studied the activity of Mn(II), Co(III), Ni(II), Cu(II), and Zn(II) complexes obtained from these ligands. What they observe is that all complexes undergo transmetalation upon encountering Fe(II), to form low spin ferrous complexes (Fig. 3). The assignment has been done using X-ray diffraction and EPR comparisons to Fe(III) analogous compounds. Moreover the divalent Mn(II), Ni(II), Cu(II), and Zn(II) complexes are equally active in preventing proliferation as their ligands, and the complexes could act as lipophilic carriers that simply facilitate the intracellular delivery of ligand and metal ion after dissociation.

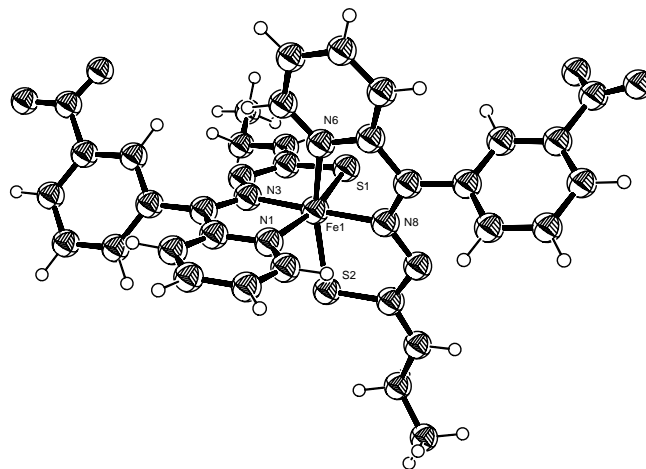


Fig. (3). Drawing of complex [Fe(II)L₂] ([37] and Ref 4 therein).

A recent paper by Opletalova *et al.* [38] presents evidence that a tridentate nature and therefore a high formation constant is a prerequisite for enhanced activity by comparing the activity of pyrazine thiosemicarbazone derivatives and an analogue derived from acetophenone. Moreover it is reported that these compounds prevent iron uptake from the serum Fe transport protein transferrin altering the iron homeostasis.

The partial conclusions that can be drawn from these experiments are that the properties of these compounds as cytotoxic compounds must satisfy two requirements. First, they must be able to cross the lipidic bilayer. The vast majority of the thiosemicarbazones reported in the literature are molecules with a polar head, the thiosemicarbazide part, and an aromatic hydrophobic part. The coordination allows the ligand to hide the thiosemicarbazone polar part around the metal and the metal complex thus exposes the hydrophobic moiety to the solvent endowing it of the features necessary to cross the cell membrane. Second, once inside the cell the thiosemicarbazone must possess the characteristics to interact with some vital enzyme, particularly those peculiar or essential to cells that must be destroyed. Inhibition of ribonucleotide reductase is certainly a target in the action of these compounds and is particularly important since it determines the rate of duplication of cells but probably it is not the sole. The cytotoxicity of thiosemicarbazone complexes often appears not to be directly proportional to the inhibition activity on ribonucleotide reductase and this suggests the existence of alternative mechanisms.

An interesting perspective has appeared, while this review was being written, in the *Journal of Medicinal Chemistry* [39]. It reports the current state of the art in the understanding of the role of thiosemicarbazones in cells.

2.1.2. ROS Generators

The action of redox metal complexes as ROS generators has long been discussed since most thiosemicarbazone complexes contain redox metal ions that potentially can activate O_2 and generate $HO\cdot$ radicals. In the literature there are data that support but also data that exclude this activity as function of the nature of the ligand and the metal centre. Recently a few more contributions on the subject have appeared in the literature. Using 2-formylpyridine thiosemicarbazone Gomez-Saiz *et al.* [40] have synthesized a few complexes and among them two were tested for biological activity on DNA. The chosen complexes were $[Cu(L)_2(pz)](ClO_4)_2$ and $\{[Cu(L)_2(dca)](ClO_4)\}_n$ where L = 2-formylpyridine thiosemicarbazone, pz=pyrazine and dca=dicyanamide. They have assayed the oxidative cleavage of DNA, in the presence of 3-mercaptopropionic acid as reducing agent, by gel electrophoresis using supercoiled pUC18. Both complexes produce single and double strand breaks. Hydroxyl scavengers such as DMSO, tert-butyl alcohol, sodium formate and potassium iodide, used as controls, inhibit the DNA cleavage and this suggests that the activity of these complexes could act as hydroxyl radical generators. Nevertheless, the complexes do not seem to interact tightly through major and minor grooves, because dystamicin and methyl green do not inhibit the DNA digestion. Almost contemporarily García *et al.* [41] have tried to demonstrate the possible interaction between this typology of complexes and nucleosides by crystallizing an adduct formed by a copper(II) ion that coordinates both a thiosemicarbazone and a nucleobase

$[CuL(cyt)](ClO_4)$ where L = pyridine-2-carbaldehyde thiosemicarbazone and cyt = cytosine (Fig. 4).

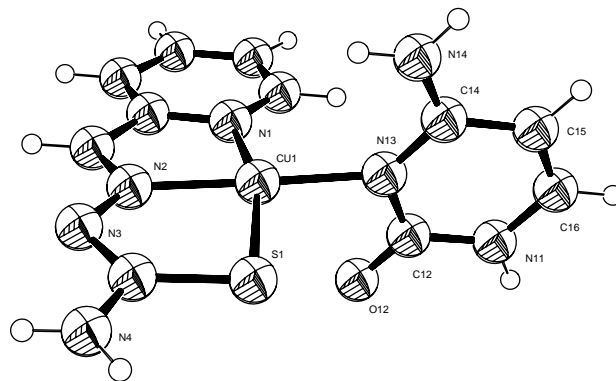
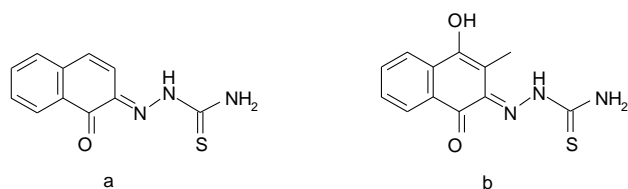


Fig. (4). Representation of the cation $[CuL(cyt)]^+$ [41].

2.1.3. Topoisomerase II and DNA Interactions

The planarity of many complexes suggests a possible intercalating behaviour towards DNA and recently another possible target has been identified in topoisomerase II. Topoisomerase II is a eukaryotic cell nuclear protein that decatenates or disentangles DNA coils, passing one helix through another to prevent supercoiling during DNA replication [42-44]. Since topoisomerase-II is necessary for DNA synthesis and cellular division, rapidly proliferating cells, such as in tumours, generally contain high levels of this enzyme and this renders it an interesting target in cancer cells. Thiosemicarbazones inhibit topoisomerase-II activity [45], acting by stabilization of the cleavable products formed by topoisomerase-II and DNA. Copper-thiosemicarbazone complexes have significantly higher growth inhibitory activity than the uncomplexed ligand and have lower IC_{50} values against tumour cells than other reported topoisomerase-II inhibitors [44]. The antitumor activity of 1,2-naphthoquinone-2-thiosemicarbazone (Scheme 6a) and that of its metal complexes of copper(II), palladium(II) and nickel(II) was investigated by Chen *et al.* [45] against MCF-7 human breast cancer cells. The results revealed that these complexes are effective antitumor chemicals in inhibiting MCF-7 cell growth. The nickel complex is the most effective among the complexes studied and, based on IC_{50} values, it is also more effective than etoposide, a commercial antitumor drug. Further data showed that 1,2-naphthoquinone-2-thiosemicarbazone copper(II), nickel(II) and platinum(II) complexes, 1,2-naphthoquinone-2-thiosemicarbazone, and naphthoquinone can only stabilize the single-strand nicked DNA, but not double-strand breakage intermediates. The metal derivatives of these ligands, but not the parent ligand molecules, exerted an antagonizing effect on topoisomerase II activity. It had been previously shown that Cu(II) derivative of 4-hydroxy-3-methyl-1,2-naphthoquinone-1-thiosemicarbazone (Scheme 6b) had the highest cytotoxicity compared to those of Fe(III), Ni(II), Pd(II), and Pt(II) metal derivatives; this was explained by the generation of Cu(I) species during intracellular enzymatic reduction or greater binding affinity of Cu(I) to an estrogen receptor protein complex [46]. Therefore, this binding would prevent the protein complex from

functioning properly during its interaction with DNA. The studies on the mechanism of action showed that metal complexes of 1,2-naphthoquinone-2-thiosemicarbazone could stabilize the cleavable complex formed by DNA and topoisomerase II. The data reported suggest that metal derivatives of 1,2-naphthoquinone-2-thiosemicarbazone have an antagonizing effect on topoisomerase II activity while 1,2-naphthoquinone and 1,2-naphthoquinone-2-thiosemicarbazone themselves do not have. This evidence indicates that these metal-thiosemicarbazone complexes can coordinate with topoisomerase II and stabilize the topoisomerase II–DNA complexes and possibly interact with DNA by forming interhelical crosslinks. These multi-inhibition mechanisms would greatly enhance cell cytotoxicity compared to that of 1,2-naphthoquinone-2-thiosemicarbazone.



Scheme 6. Structural formulas for: 1,2-naphthoquinone-2-thiosemicarbazone (**a**), 4-hydroxy-3-methyl-1,2-naphthoquinone-1-thiosemicarbazone (**b**).

2.1.4. Other Mechanisms

Kovala-Demertzi and her group have recently published their studies on 2-formyl- and 2-acetylthiosemicarbazone metal complexes. Besides the ligands also the zinc complexes have been evaluated [47] for their antiproliferative activity *in vitro* against cell lines MCF-7 (human breast cancer cell line), T24 (human bladder cancer cell line) and L-929 (mouse fibroblast). From these experiments it results apparent that also the zinc complexes display IC_{50} values in the μM range similar to or better than the usual reference of cisplatin. In a later paper [48] the acetyl derivative was modified by adding an ethyl group on the terminal nitrogen and its Pt and Pd complexes with stoichiometry $[PtL_2]$ and $[PdL_2]$ were synthesized. These complexes were tested on human tumour cell lines of different origins (breast, colon, and ovary cancers), and containing also cisplatin-refractory/resistant cell lines. The role of the metals seems not to be relevant and moreover the newly synthesized compounds are active towards cisplatin-refractory tumour cell lines. In a recent paper [49], in an effort to further understand the influence of the terminal nitrogen atom, the 2-formyl and 2-acetylpyridine thiosemicarbazones have been modified by incorporating the terminal nitrogen into an aliphatic azepane ring (Scheme 7a, Fig. 5). Reaction with platinum(II) afforded the related complexes $[Pt(L)Cl]$. Ligands and complexes have been evaluated for antiproliferative activity *in vitro* against four cancer cell lines: MCF-7 (human breast cancer cell line), T24 (human bladder cancer cell line), A-549 (human non-small cell lung carcinoma) and L-929 (murine). The 2-acetylpyridine ligand exhibited high activity as anticancer agent against all four cancer cell lines, while the 2-formylpyridine derivative exhibited selectivity against MCF-7, L-929 and its platinum complex against A-549, T-24 cancer cell lines. All four compounds were also evaluated

on leukaemia P388-bearing mice and the 2-formylpyridine thiosemicarbazone platinum complex afforded five to six cures against leukaemia P388 resulting to be a very effective chemotherapeutic antileukaemic agent.

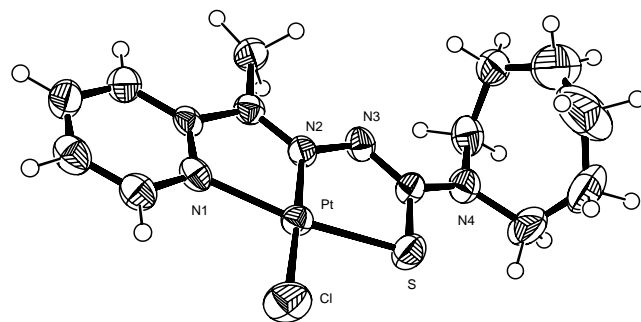
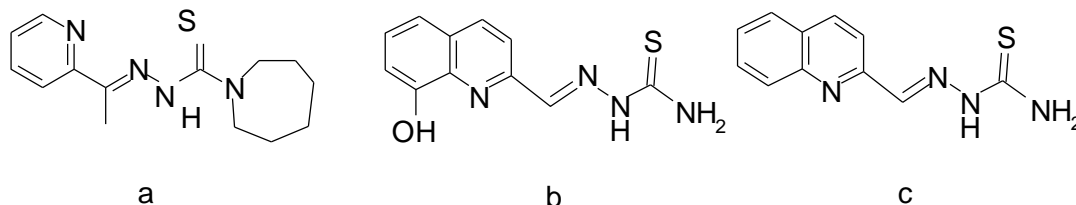


Fig. (5). Representation of $[Pt(L)Cl]$ where L is the azepane ring substituted formylpyridine thiosemicarbazone [49].

Zhang *et al.* [50] have carried out a study on 8-hydroxyquinoline-2-carboxaldehyde thiosemicarbazone (Scheme 7b), on its 4,4-dimethyl derivative and on their copper(II) complexes. The ligands chelate copper ions through an ONNS quadridentate system, and the resulting complexes are uncharged. All four compounds have been tested on SK-N-DZ (a cisplatin resistant neuroblastoma cell line). The free ligands showed no significant growth inhibition activity, whereas both metal complexes showed dose-dependent cell growth inhibition, cell cycle arrest and apoptosis induction activities. The terminal amino-substituted complex showed stronger anticancer activity than that of the unsubstituted complex. Increased expression of p53 protein molecules was detected in the SK-N-DZ cells treated with the non-methylated complex suggesting that a DNA damage is a possible cause of apoptosis.

In 2006 a simple but clever paper by Adsule *et al.* [51] reports studies about the activity of quinoline-2-carboxaldehyde thiosemicarbazone (Scheme 7c) derivatives and its copper(II) complex as proteasome inhibitors in human prostate cancer cell lines PC-3 and LNCaP. They observe that the thiosemicarbazone copper(II) complex is the most potent inhibitor among the tested compounds and also demonstrate that the observed apoptosis is triggered by inhibition of the proteasome-ubiquitin pathway and not through oxidative stress. This contribution highlights a further possible mechanism of action of thiosemicarbazone derivatives.

Also in the area of aromatic substituents on the imino nitrogen, Ferrari *et al.* [52] report a study on a dimeric copper complex of pyridoxal thiosemicarbazone $[Cu(HL)(OH_2)]_2Cl_2 \cdot 2H_2O$ (Fig. 6). The compound was used to evaluate the behaviour on different murine and human leukemic cell lines in order to compare them, *in vitro* and *in vivo*. On TS/A murine adenocarcinoma cell line *in vitro* it inhibits cell proliferation at micromolar concentrations but it has no activity *in vivo* on TLX5 lymphoma. Nevertheless, it induces apoptosis on CEM and U937 human cell lines, with IC_{50} at micromolar concentrations but it is inactive on K562. It is noteworthy that it significantly alters the cell cycle of U937 and CEM lines and decreases the telomerase activity of U937. In order to verify the role of the substituents on the



Scheme 7. Structural formula of: 2-formyl and 2-acetylpyridine thiosemicarbazones have been modified by incorporating the terminal nitrogen into an aliphatic azepane ring (a) [49], of 8-hydroxyquinoline-2-carboxaldehyde thiosemicarbazone (b) [50], and of quinoline-2-carboxaldehyde thiosemicarbazone (c) [51].

ligand, complexes with N-substituted pyridoxal thiosemicarbazones were synthesised (namely pyridoxal N1,N1-dimethylthiosemicarbazone, pyridoxal N1,N2-dimethylthiosemicarbazone, and pyridoxal N1-ethylthiosemicarbazone). Their biological activities have been tested *in vitro* on U937, CEM and K562 cell lines. The complex obtained from the N1,N1-dimethyl ligand shows weak proliferation inhibition on all three cell lines, but it does not induce apoptosis and it does not inhibit telomerase activity. The N1,N2-dimethyl derivative complex is not effective at low concentration and is toxic at higher doses whereas the third complex inhibits CEM cell growth better than the first one but it does not exert any other biological effect. These results once again demonstrate that thiosemicarbazones act in a very selective way towards different cell lines and that in this case substitutions on the terminal nitrogen reduce the biological activity of the parent compound contrarily to what observed in triapine derivatives.

In the literature are also present thiosemicarbazones with a non aromatic substituent on the imino nitrogen. Baldini *et al.* [53, 54] report the synthesis and biological evaluation of α -ketoglutaric acid thiosemicarbazone linear and cyclic derivatives and their copper and zinc complexes. In the study of the cell cycle on U937 cell line the authors notice an accumulation in phase G₂ and a depletion of the cells in phase S that would be unexpected in the hypothesis of a ribonucleotide reductase inhibition. The authors' experiment on plasmid pBR322 also excludes DNA damages due to a redox activity of the compounds and concludes that these compounds interfere with the cell cycle at different levels but none of the two envisaged mechanisms lie behind their mechanism of action.

More recently the same group has reported on a series of thiosemicarbazones derived from natural aldehydes and in particular 9-*cis*-retinal thiosemicarbazone and its cobalt(III), nickel(II) and copper (II) complexes [55]. These complexes possess neither an aromatic ring nor a donor atom on the imino side of the thiosemicarbazone but a long hydrophobic chain. DNA binding constants and spectroscopic data show an intercalative behaviour for the nickel complex while for the copper complex there are strong points in favour of an external binding mode. As expected no DNA interaction takes place for the cobalt complex. The free ligand and its Ni(II) and Cu(II) complexes have a good lipophilic degree necessary for an efficient uptake by the cells. The metal complexes exhibit a proliferation inhibition action against U937 cell line at micromolar concentration and the Cu(II) complex also induces apoptosis. Among the other natural aldehydes studied [56] a complex with particularly promising properties was the nickel complex of the *S*-citronellal thiosemicarbazone, [NiL₂] (Fig. 7), that induces an antiproliferative effect on U937 at micromolar concentrations. The X-ray diffraction analysis of these compounds reveals that the hydrophobic tails are rather free to move and possibly wrap up the polar core of the complex. This phenomenon could play an important role in the transfer through the cell membrane. Interestingly the interactions with various cellular components have been analysed and the induced apoptotic pathway has been characterised. The outcome is that the complex causes programmed cell death *via* down-regulation of Bcl-2, alteration of mitochondrial membrane potential and caspase-3 activity, regardless of p53 function. Moreover there is no activity on G₀ cells such as fresh leukocytes but is able to induce perturbation of the cell cycle on stimulated lymphocytes and U937 cells.

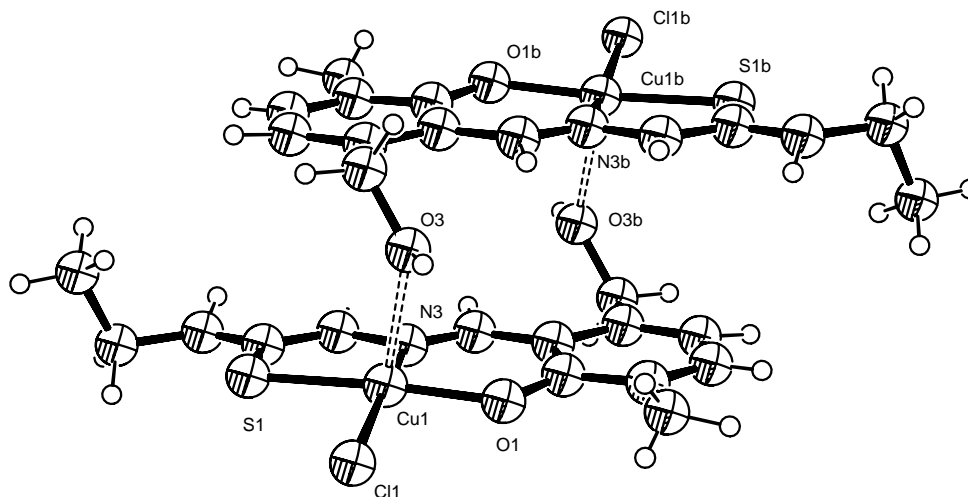


Fig. (6). Drawing of the dimeric species $[\text{Cu}(\text{HL})(\text{OH}_2)]_2^{2+}$ [52].

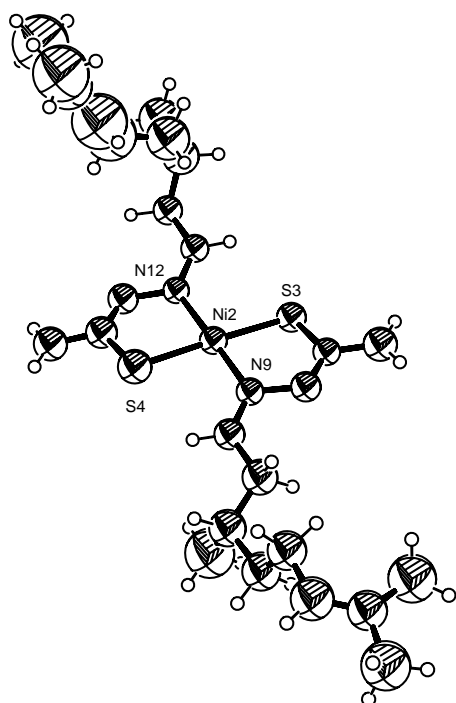
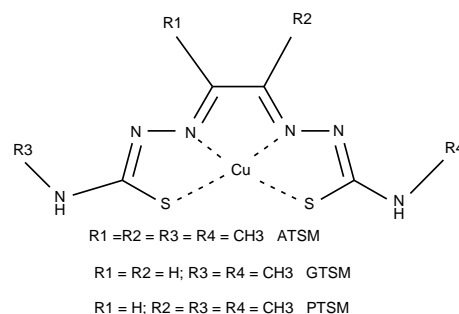


Fig. (7). Representation of $[NiL_2]$ where L is *S*-citronellal thiosemicarbazone [56].

2.1.5. Hypoxia and Multidrug Resistance

Another field in which thiosemicarbazone metal complexes are receiving a great deal of attention is their use as carrier for radiotracers such as ^{64}Cu . This technique is particularly useful in solid tumours where there is little blood perfusion. Hypoxia commonly occurs when a tumour outgrows its vascular supply and cells tend to adapt by up-regulating the production of numerous proteins that promote their survival. Usually cells in this condition slow down the rate of growth and stimulate growth of new vasculature, but what is worse is that the proteins expressed in these conditions inhibit apoptosis and cause metastatic spread. The poor blood supply and the low amount of oxygen that reaches the hypoxic cells reduce the efficacy of any treatment. This hypoxic situation has allowed researchers to develop molecules that accumulate with an inverse relationship to O_2 partial pressure, at first with the purpose to develop imaging techniques but more recently also as a mean to treat selectively cancerous cells. Among the radiotracers presently in a developing phase is a class of copper(II) thiosemicarbazones that have as a common scaffold diacetyl-bis(N4-methylthiosemicarbazone) (ATSM) (Scheme 8). ATSM is commonly used as a copper(II) carrier complex because of its various radionuclides (^{60}Cu , ^{61}Cu , ^{62}Cu , ^{64}Cu) [57-63] and taking advantage of the fact that copper ATSM complexes accumulate more actively than the ligand itself. Recently a huge amount of literature has appeared on the study of ^{64}Cu ATSM and a few reviews and papers are of fundamental importance [64-68]. Most papers are nowadays devoted to preclinical and clinical investigations being ^{64}Cu ATSM one of the two lead compounds for PET (Positron Emitting Tomography) imaging.

As already highlighted at the beginning of the paragraph, tumor hypoxia is often associated with resistance to chemo-

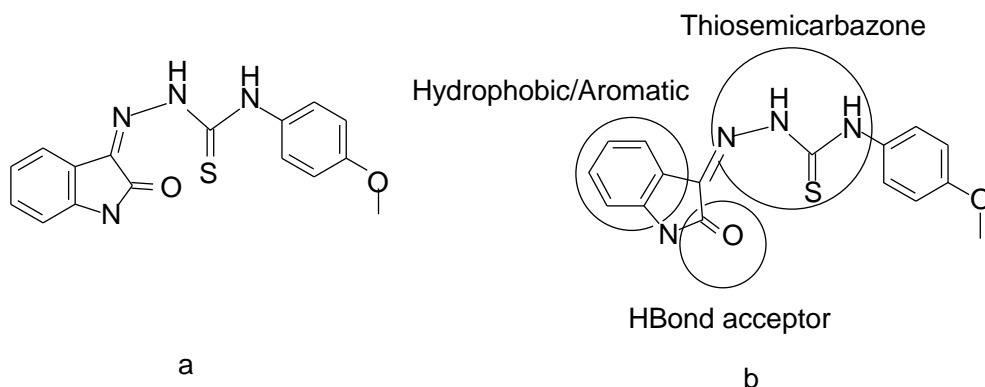


Scheme 8. Structural formulas of a class of copper(II) thiosemicarbazones radiotracers that have as a common scaffold diacetyl-bis(N4-methylthiosemicarbazone) (ATSM).

therapy. Multidrug resistance type 1 (MDR1) protein is a member of the adenosine triphosphate binding cassette (ABC) proteins, some of which are involved in the multidrug resistance (MDR) phenotype in tumours. Pyruvaldehyde bis(N(4)-methylthiosemicarbazonato)copper(II) (Cu-PTSM) [69] has been used together with CuATSM, to study the role of MDR1 proteins in the accumulation of the ^{64}Cu copper complexes within hypoxic cells.

The paper demonstrates that ^{64}Cu -ATSM and -PTSM retention is significantly increased by knocking down MDR1 expression. A further article concerning ^{64}Cu retention in hypoxic cells reports that an analogue of Cu-ATSM, glyoxal bis(N(4)-methylthiosemicarbazonato) copper(II) Cu(GTSM) [70], is non selectively trapped in all cells while CuATSM enters all cells but is washed out in normal cells and retained in the hypoxic. The authors demonstrate that the key chemical property is the redox potential: the copper contained in Cu-ATSM cannot be reduced by cellular endogenous reductants in normal cells and this is expelled while the PTSM derivative is reduced and the copper(I) is captured by intracellular sinks such as protein Ax1 and Ctr1c. One problem that arises in advanced clinical test is the delivery of the substance and Cu-ATSM is being studied for the aspects that regard possible complexes with albumin [71-73]. A further problem being faced is the scarce solubility of these molecules and an attempt aimed to obtain more water soluble compounds succeeded by functionalizing them with sugar [74] and amino acids [75]. Although ^{64}Cu -ATSM is very promising contrast agent, a strong cell dependence behaviour in the uptake of Cu-ATSM has been observed [76].

In the field of multi-drug resistance, it is noteworthy a paper by Hall *et al.* [77]. An attempt to rationalize the structural requirements necessary to endow a molecule of MDR1 inhibitory activity is reported. The authors have synthesized and tested several isatin- β -thiosemicarbazone derivatives including analogues without the thiosemicarbazide part. A number of compounds showed an improved MDR1-selective activity over the lead compound (Scheme 9a). A quantitative structure-activity relationship (QSAR) model yielded a system of requirements that effectively predicts the cytotoxicity of untested thiosemicarbazones. The key parts of the molecule (Scheme 9b) which endow it of its activity are principally due to the thiosemicarbazone part (the compounds without it are inactive), a hydrogen bond acceptor atom and a hydrophobic/aromatic moiety on the hydrazinic part of the thiosemicarbazone. Molecules lacking any one of the fea-



Scheme 9. General structural formula of isatin- β -thiosemicarbazone (a) [77], and the key parts of the molecule (b) which endow it of its activity.

tures (acceptor site, hydrophobic site, aromatic ring, or thiosemicarbazone fragment) are inactive as MDR1 inhibitors. Further requirements for MDR1-selectivity have been found on the N4 part of the thiosemicarbazone, and namely the presence of a hydrophobic substituent and possibly electron-rich fragments bound to it such as an aromatic system plus a methoxy group or a halogenide.

2.2. Antiprotozoal Activity

As part of the Drugs for Neglected Diseases Initiative a huge amount of work is being carried out by many research centres to treat diseases that hit that part of the world population that living in a state of poverty are not of primary concern for the pharmaceutical industry. These infections are numerically the foremost threat to human health around the world and in particular American and African trypanosomiasis and malaria. For trypanosomiasis, in particular, the available drugs are not very effective; the most efficient drug presently used is nifurtimox and constitutes a reference for newly synthesized compounds. Among the most promising derivatives being developed we find thiosemicarbazones, in particular their nitro derivatives, and their metal complexes. In 2006 Otero *et al.* [78] published a study on the activity of 5-nitrofuryl-3-acroleine thiosemicarbazone palladium(II) complexes but they do not report structural data. Most complexes showed higher *in vitro* growth inhibition activity against *Trypanosoma cruzi* than Nifurtimox and in many cases the activity of the ligand was increased as a result of palladium complexation. Pérez-Rebolledo *et al.* [79] report that N(4)-methyl-4-nitroacetophenone thiosemicarbazone, N4,N4-dimethyl-4-nitroacetophenonethiosemicarbazone and N4-piperidyl-4-nitroacetophenone thiosemicarbazone and their copper(II) complexes of general formula [CuL₂] were tested for their *in vitro* ability to inhibit the growth of *Trypanosoma cruzi* epimastigote forms. The N4,N4-dimethyl ligand, its copper(II) complex (Fig. 8) and the copper N4-methyl complex proved to be active as the clinical reference drugs nifurtimox. Also in this case the authors try to envisage the reason of enhancement of activity upon complexation in either to changes in lipophilicity or to redox effects involving the thiosemicarbazone and copper. Copper(II) by itself is not responsible for the studied anti-*T. cruzi* activity. The complex presents a very distorted geometry from the ideal square planar, almost tetrahedral (in Fig. 8 the two Cu-N2 and Cu-N2' are overlapped), that allows to expect an easy reduction to copper(I). The idea of a redox mechanism

is based on the fact that the mechanisms of action of nifurtimox and benzimidazole involve intracellular reduction of the nitro group. Nifurtimox acts *via* reduction of the nitro group to an unstable nitro anion radical, which produces highly toxic reactive oxygen species (ROS) while the benzimidazole causes covalent modification of biomacromolecules by nitro reduction intermediates which cause cellular damage. This could also be the mode of action of these nitro thiosemicarbazones. Given the serious side effects of nifurtimox, the compounds could constitute a new class of anti-trypanosomal drug candidates. Vieites *et al.* [80] present two series of platinum(II) complexes with 5-nitrofuryl thiosemicarbazones. Most of the complexes show IC₅₀ values in the μ M range as active as the anti-trypanosomal drug nifurtimox. In particular, the bis[4-ethyl-1-(5-nitrofurfurylidene)thiosemicarbazone] platinum(II) complex lead to a markedly activity increase with respect to the free ligand. In a later paper [81] further eight platinum(II) complexes with 3-(5-nitrofuryl)acroleine thiosemicarbazones are reported showing anti-trypanosomal activity. These complexes, together with the analogous platinum 5-nitrofuraldehyde containing thiosemicarbazones previously reported, resulted more active than the reference nifurtimox. All these papers agree suggesting that the trypanocidal mechanism of action is probably mainly due to the inhibition of parasite growth through a dual pathway involving production of toxic free radicals by thiosemicarbazone bioreduction and metal complex-DNA interaction. A further target proposed could be trypanothione reductase as reported by Otero *et al.* [78].

As concerns malaria, Biot *et al.* [82] report the design, synthesis, and antimalarial activity of chimeras of thiosemicarbazones and ferroquine (Scheme 10). The authors started from the known potent antimalarial activity of thiosemicarbazones [83, 84], which were in the past abandoned for the heavy side effects. Then, they synthesize molecules in which a ferroquine, another molecule recently discovered with antimalarial properties, was inserted [85]. The compounds were tested against four strains of the malaria parasite *Plasmodium falciparum* and against the parasitic cysteine protease falcipain-2. The major contributor to the antimalarial activity seems to be the aminoquinoline thiosemicarbazone part. The most active derivatives against all strains of *P. falciparum* were the chimeras of thiosemicarbazones and ferroquine analogues but in some cases also the corresponding organic derivatives were similarly active.

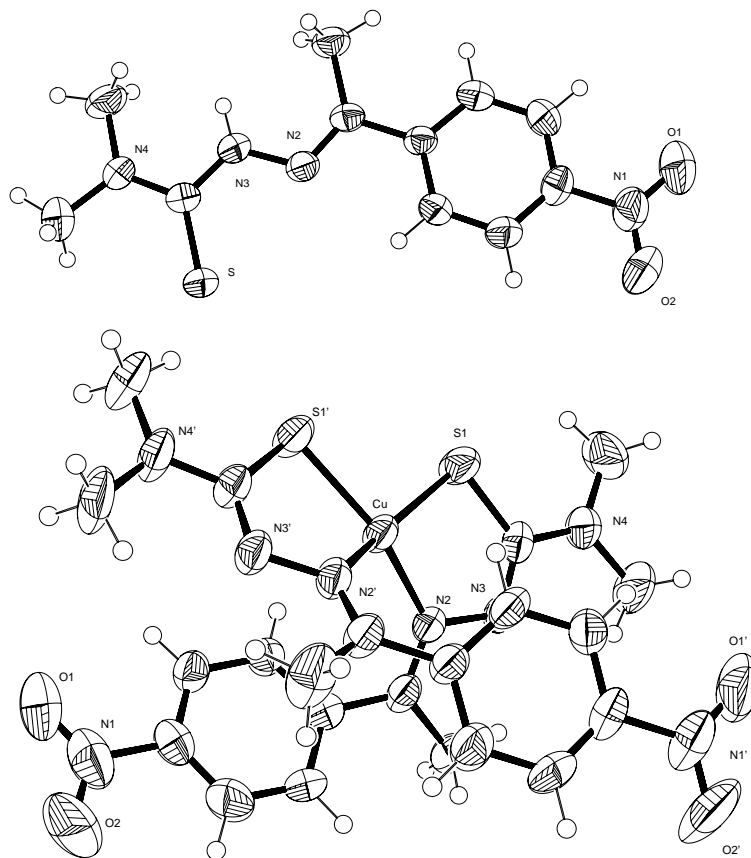
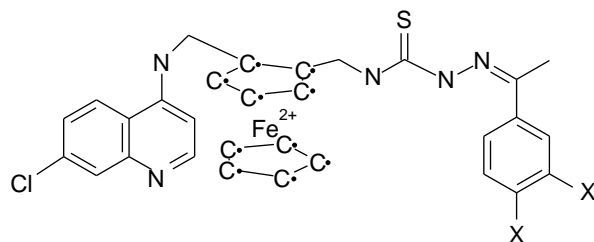


Fig. (8). Representation of N4,N4-dimethyl-4-nitroacetophenethiosemicarbazone and its copper(II) complex [79].



Scheme 10. Structural formulas of chimeras of thiosemicarbazones and ferroquine [82]. X = Cl, Br; Y = Cl, H.

3. CONCLUSIONS

As may be noticed reading through this review, our comprehension about the way thiosemicarbazone metal complexes interact with biological systems is still in its infancy notwithstanding the fact that these compounds have been studied for quite a long time. The amount of crystallographic data present in the literature to date is accordingly fairly large but the variety of biological systems on which they have been tested is also extremely variegated and heterogeneous and this renders it impossible to plan meaningful structure-activity correlation studies. Some hypotheses on their mode of action proposed in the Sixties are still valid and more have been added in the course of the years. The hypothesis of thiosemicarbazone metal complexes as inhibitors of ribonucleotide reductase has been the first to be proposed but many subsequent observations suggest that there are certainly more targets. In many cases, strong ribonucleo-

tide inhibitors *in vitro* are poor proliferation inhibitors when used on whole cells. Another point commonly accepted is that thiosemicarbazones act as strong metal ion sequestering agents and deprive cells of essential metal ions. This can be valid for tridentate ligands for their high formation constants but it is in striking contrast with the fact that the metal complexes are systematically more active than the ligands by themselves. Moreover new thiosemicarbazones have been recently discovered to be highly active even if they are bidentate and notwithstanding the fact that the metal is of the first transition series and therefore with high ligand exchange kinetics.

The grey-zone between chemistry and molecular biology still needs more light. For instance, are the complexes absorbed into the cells by simple diffusion through the membrane or is there an active transport system involved? This is not known yet. If the diffusion mechanism prevails, it cannot be excluded that the complex formation is barely a strategy by which a charged ion and a polar ligand molecule form a structure that exposes the hydrophobic part to the exterior and allows the two to enter the cell. This could explain the reason why complexes result to be more active than the parent ligands. Once inside, the metal ion and the thiosemicarbazone could act separately, the first altering the metal homeostasis within the cell and the other by interacting with enzymes. As a matter of fact thiosemicarbazones by themselves possess biological activity and there are some studies concerning their ability to interfere with enzymes containing cysteines in the active site, cruzain and ribonucleotide reduc-

tase, to quote a few [29, 77, 86-88]. This could also account for the activity of thiosemicarbazone metal complexes with relatively low formation constants such as those bidentate and for the high activity of complexes containing toxic metals such as gallium and mercury.

On the other hand we observe a high selectivity: some cell lines are very sensitive and others are totally insensitive to the very same complex. This could be evidence against the simple diffusion hypothesis. A different aspect of the same phenomenon is that certain cancer cell lines could be refractory to the action of potent antitumour compounds because the active molecules cannot reach the target tissue or the cytoplasm of cancer cells and this opens the wide chapter of drug delivery that as regards thiosemicarbazone metal complexes has not yet been explored in depth. The redox properties seem to play an important role and the presence of the metal is therefore fundamental. In the most simple hypothesis these complexes can promote the Fenton reaction and produce significant amounts of hydroxyl radicals that interfere with the cell normal functions [12]. Metal complexes can also, in certain conditions, promote the reactivity of the thiosemicarbazone to which it is bound or even to promote its decomposition to generate 1,3,4-oxadiazole derivatives, for instance [89]. Steps forward are being made in developing fluorescent thiosemicarbazones complexes that will allow to understand where they localize within a cell and this will be a first step to a rationale development in this area [90]. Recently a particularly attracting new line of action is being developed as inhibitors of multi drug resistance proteins. This target is of enormous interest since it could allow a systematic method to circumvent antibiotics or anti-cancer drug resistance. To conclude it must be recognized that, notwithstanding the amount of data concerning the activity of thiosemicarbazones, there are still many details in their mechanism of action that escape our comprehension and also many phenomena are observed that cannot be explained with our present knowledge.

REFERENCES

- [1] Bavin EM, Rees RJW, Robson JM, Seiler M, Seymour DE, Sudaby D. The tuberculostatic activity of some thiosemicarbazones. *J Pharm Pharmacol* 1950; 2: 764-72.
- [2] Koch O, Stutgen G. Clinical and experimental studies on the effects of thiosemicarbazones. *Naunyn Schmiedebergs Arch Exp Pathol Pharmacol* 1950; 210: 409-23.
- [3] Kune GA. To-day's drugs: methisazone. *Br Med J* 1964; 2: 621.
- [4] Sartorelli AC, Booth BA. Inhibition of the growth of sarcoma 180 ascites cells by combinations of inhibitors of nucleic acid biosynthesis and the cupric chelate of kethoxal bis-(thiosemicarbazone). *Cancer Res* 1967; 27: 1614-9.
- [5] Nutting CM, van Herpen CML, Miah AB, *et al.* Phase II study of 3-AP Triapine in patients with recurrent or metastatic head and neck squamous cell carcinoma. *Ann Oncol* 2009; 20: 1275-9.
- [6] Ma B, Goh BC, Tan EH, *et al.* A multicenter phase II trial of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine) and gemcitabine in advanced non-small-cell lung cancer with pharmacokinetic evaluation using peripheral blood mononuclear cells. *Invest New Drugs* 2008; 26: 169-73.
- [7] Finch RA, Liu MC, Cory AH, Cory JG, Sartorelli AC. Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone; 3-AP): an inhibitor of ribonucleotide reductase with antineoplastic activity. *Adv Enzyme Regul* 1999; 39: 3-12.
- [8] Antholine W, Knight J, Whelan H, Petering DH. Studies of the reaction of 2-formylpyridine thiosemicarbazone and its iron and copper complexes with biological systems. *Mol Pharmacol* 1977; 13: 89-98.
- [9] Beraldo H, Gambino D. The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes. *Mini Rev Med Chem* 2004; 4: 31-9.
- [10] French FA, Blanz EJJ. The carcinostatic activity of alpha-(N) heterocyclic carboxaldehyde thiosemicarbazones. I. Isoquinoline-1-carboxaldehyde thiosemicarbazone. *Cancer Res* 1965; 25: 1454-8.
- [11] Brockman RW, Sidwell RW, Arnett G, Shaddix S. Heterocyclic thiosemicarbazones: correlation between structure, inhibition of ribonucleotide reductase, and inhibition of DNA viruses. *Proc Soc Exp Biol Med* 1970; 133: 609-14.
- [12] Shao J, Zhou B, Di Bilio AJ, *et al.* A Ferrous-Triapine complex mediates formation of reactive oxygen species that inactivate human ribonucleotide reductase. *Mol Cancer Ther* 2006; 5: 586-92.
- [13] Hall IH, Lackey CB, Kistler TD, *et al.* Cytotoxicity of copper and cobalt complexes of furfural semicarbazone and thiosemicarbazone derivatives in murine and human tumor cell lines. *Pharmazie* 2000; 55: 937-41.
- [14] Yuan J, Lovejoy DB, Richardson DR. Novel di-2-pyridyl-derived iron chelators with marked and selective antitumor activity: *in vitro* and *in vivo* assessment. *Blood* 2004; 104: 1450-8.
- [15] Ludwig JA, Szakács G, Martin SE, *et al.* Selective toxicity of NSC73306 in MDR1-positive cells as a new strategy to circumvent multidrug resistance in cancer. *Cancer Res* 2006; 66: 4808-15.
- [16] Wu C, Shukla S, Calcagno AM, Hall MD, Gottesman MM, Ambudkar SV. Evidence for dual mode of action of a thiosemicarbazone, NSC73306: a potent substrate of the multidrug resistance linked ABCG2 transporter. *Mol Cancer Ther* 2007; 6: 3287-96.
- [17] Brockman RW, Thomson JR, Bell MJ, Skipper HE. Observations on the antileukemic activity of pyridine-2-carboxaldehyde thiosemicarbazone and thiocarbohydrazone. *Cancer Res* 1956; 16: 167-70.
- [18] Sartorelli AC, Agrawal KC, Moore EC. Mechanism of inhibition of ribonucleoside diphosphate reductase by alpha-(N)-heterocyclic aldehyde thiosemicarbazones. *Biochem Pharmacol* 1971; 20: 3119-23.
- [19] Sartorelli AC, Agrawal KC, Tsiftoglou AS, Moore EC. Characterization of the biochemical mechanism of action of alpha-(N)-heterocyclic carboxaldehyde thiosemicarbazones. *Adv Enzyme Regul* 1976; 15: 117-39.
- [20] Antholine WE, Knight JM, Petering DH. Inhibition of tumor cell transplantability by iron and copper complexes of 5-substituted 2-formylpyridine thiosemicarbazones. *J Med Chem* 1976; 19: 339-41.
- [21] Thelander L, Gräslund A. Mechanism of inhibition of mammalian ribonucleotide reductase by the iron chelate of 1-formylisoquinoline thiosemicarbazone. Destruction of the tyrosine free radical of the enzyme in an oxygen-requiring reaction. *J Biol Chem* 1983; 258: 4063-6.
- [22] Agrawal KC, Lin AJ, Booth BA, Wheaton JR, Sartorelli AC. Potential antitumor agents. 9. 2-Formyl(m-amino)phenylpyridine thiosemicarbazones. *J Med Chem* 1974; 17: 631-5.
- [23] Agrawal KC, Mooney PD, Sartorelli AC. Potential antitumor agents. 13. 4-Methyl-5-amino-1-formylisoquinoline thiosemicarbazone. *J Med Chem* 1976; 19: 970-2.
- [24] Traynor AM, Lee J, Bayer GK, *et al.* A phase II trial of Triapine(R) (NSC# 663249) and gemcitabine as second line treatment of advanced non-small cell lung cancer: Eastern Cooperative Oncology Group Study 1503. *Invest New Drugs* 2009; DOI 10.1007/s10637-009-9230-z.
- [25] Mackenzie MJ, Saltman D, Hirte H, *et al.* A Phase II study of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) and gemcitabine in advanced pancreatic carcinoma. A trial of the Princess Margaret hospital Phase II consortium. *Invest New Drugs* 2007; 25: 553-8.
- [26] Kowol CR, Berger R, Eichinger R, *et al.* Gallium(III) and iron(III) complexes of alpha-N-heterocyclic thiosemicarbazones: synthesis, characterization, cytotoxicity, and interaction with ribonucleotide reductase. *J Med Chem* 2007; 50: 1254-65.
- [27] Kowol CR, Trondl R, Heffeter P, *et al.* Impact of Metal Coordination on Cytotoxicity of 3-Aminopyridine-2-carboxaldehyde Thiosemicarbazone (Triapine) and Novel Insights into Terminal Dimethylation. *J Med Chem* 2009; 52: 5032-5043.
- [28] Kowol CR, Eichinger R, Jakupec MA, Galanski M, Arion VB, Keppler BK. Effect of metal ion complexation and chalcogen donor identity on the antiproliferative activity of 2-acetylpyridine N,N-

- dimethyl(chalcogen)semicarbazones. *J Inorg Biochem* 2007; 101: 1946-57.
- [29] Trossini GHG, Guido RVC, Oliva G, Ferreira EI, Andricopulo AD. Quantitative structure-activity relationships for a series of inhibitors of cruzain from *Trypanosoma cruzi*: molecular modeling, CoMFA and CoMSIA studies. *J Mol Graph Model* 2009; 28: 3-11.
- [30] Mendes IC, Soares MA, Dos Santos RG, Pinheiro C, Beraldo H. Gallium(III) complexes of 2-pyridineformamide thiosemicarbazones: cytotoxic activity against malignant glioblastoma. *Eur J Med Chem* 2009; 44: 1870-7.
- [31] Kolesar JM, Schelman WR, Geiger PG, *et al.* Electron paramagnetic resonance study of peripheral blood mononuclear cells from patients with refractory solid tumors treated with Triapine. *J Inorg Biochem* 2008; 102: 693-8.
- [32] Richardson DR, Sharpe PC, Lovejoy DB, *et al.* Dipyriddy thiosemicarbazone chelators with potent and selective antitumor activity form iron complexes with redox activity. *J Med Chem* 2006; 49: 6510-21.
- [33] Chaston TB, Lovejoy DB, Watts RN, Richardson DR. Examination of the antiproliferative activity of iron chelators: multiple cellular targets and the different mechanism of action of triapine compared with desferrioxamine and the potent pyridoxal isonicotinoyl hydrazone analogue 311. *Clin Cancer Res* 2003; 9: 402-14.
- [34] Yu Y, Wong J, Lovejoy DB, Kalinowski DS, Richardson DR. Chelators at the cancer coalface: desferrioxamine to Triapine and beyond. *Clin Cancer Res* 2006; 12: 6876-83.
- [35] Saryan LA, Mailer K, Krishnamurti C, Antholine W, Petering DH. Interaction of 2-formylpyridine thiosemicarbazone copper (II) with Ehrlich ascites tumor cells. *Biochem Pharmacol* 1981; 30: 1595-604.
- [36] Whitnall M, Howard J, Ponka P, Richardson DR. A class of iron chelators with a wide spectrum of potent antitumor activity that overcomes resistance to chemotherapeutics. *Proc Natl Acad Sci USA* 2006; 103: 14901-6.
- [37] Bernhardt PV, Sharpe PC, Islam M, Lovejoy DB, Kalinowski DS, Richardson DR. Iron chelators of the dipyriddyketone thiosemicarbazone class: precomplexation and transmetalation effects on anticancer activity. *J Med Chem* 2009; 52: 407-15.
- [38] Opletalová V, Kalinowski DS, Vejsová M, *et al.* Identification and characterization of thiosemicarbazones with antifungal and antitumor effects: cellular iron chelation mediating cytotoxic activity. *Chem Res Toxicol* 2008; 21: 1878-89.
- [39] Yu Y, Kalinowski DS, Kovacevic Z, *et al.* Thiosemicarbazones from the old to new: iron chelators that are more than just ribonucleotide reductase inhibitors. *J Med Chem* 2009; 52: 5271-94.
- [40] Gómez-Saiz P, Gil-García R, Maestro MA, *et al.* Structure, magnetic properties and nuclease activity of pyridine-2-carbaldehyde thiosemicarbazonecopper(II) complexes. *J Inorg Biochem* 2008; 102: 1910-20.
- [41] García B, García-Tojal J, Ruiz R, *et al.* Interaction of the DNA bases and their mononucleotides with pyridine-2-carbaldehyde thiosemicarbazonecopper(II) complexes. Structure of the cytosine derivative. *J Inorg Biochem* 2008; 102: 1892-900.
- [42] Larsen AK, Skladanowski A. Cellular resistance to topoisomerase-targeted drugs: from drug uptake to cell death. *Biochim Biophys Acta* 1998; 1400: 257-74.
- [43] Larsen AK, Skladanowski A, Bojanowski K. The roles of DNA topoisomerase II during the cell cycle. *Prog Cell Cycle Res* 1996; 2: 229-39.
- [44] Easmon J, Pürstinger G, Heinisch G, *et al.* Synthesis, cytotoxicity, and antitumor activity of copper(II) and iron(II) complexes of (4)N-azabicyclo[3.2.2]nonane thiosemicarbazones derived from acyl diazines. *J Med Chem* 2001; 44: 2164-71.
- [45] Chen J, Huang Y, Liu G, *et al.* The cytotoxicity and mechanisms of 1,2-naphthoquinone thiosemicarbazone and its metal derivatives against MCF-7 human breast cancer cells. *Toxicol Appl Pharmacol* 2004; 197: 40-8.
- [46] Saha M, Saha HHT, Niskanen LK, Salmela KT, Pasternack AI. Time course of serum prolactin and sex hormones following successful renal transplantation. *Nephron* 2002; 92: 735-7.
- [47] Kovala-Demertzi D, Yadav PN, Wiecek J, Skoulika S, Varadinova T, Demertzis MA. Zinc(II) complexes derived from pyridine-2-carbaldehyde thiosemicarbazone and (1E)-1-pyridin-2-ylethan-1-one thiosemicarbazone. Synthesis, crystal structures and antiproliferative activity of zinc(II) complexes. *J Inorg Biochem* 2006; 100: 1558-67.
- [48] Kovala-Demertzi D, Boccarelli A, Demertzis MA, Coluccia M. *In vitro* antitumor activity of 2-acetyl pyridine 4n-ethyl thiosemicarbazone and its platinum(II) and palladium(II) complexes. *Chemotherapy* 2007; 53: 148-52.
- [49] Kovala-Demertzi D, Papageorgiou A, Papathanasis L, *et al.* *In vitro* and *in vivo* antitumor activity of platinum(II) complexes with thiosemicarbazones derived from 2-formyl and 2-acetyl pyridine and containing ring incorporated at N(4)-position: synthesis, spectroscopic study and crystal structure of platinum(II) complexes with thiosemicarbazones, potential anticancer agents. *Eur J Med Chem* 2009; 44: 1296-302.
- [50] Zhang H, Thomas R, Oupicky D, Peng F. Synthesis and characterization of new copper thiosemicarbazone complexes with an ONNS quadridentate system: cell growth inhibition, S-phase cell cycle arrest and proapoptotic activities on cisplatin-resistant neuroblastoma cells. *J Biol Inorg Chem* 2008; 13: 47-55.
- [51] Adsule S, Barve V, Chen D, *et al.* Novel Schiff base copper complexes of quinoline-2 carboxaldehyde as proteasome inhibitors in human prostate cancer cells. *J Med Chem* 2006; 49: 7242-6.
- [52] Belicchi Ferrari M, Bisceglie F, Pelosi G, *et al.* Synthesis, characterization and biological activity of copper complexes with pyridoxal thiosemicarbazone derivatives. X-ray crystal structure of three dimeric complexes. *J Inorg Biochem* 2004; 98: 301-12.
- [53] Baldini M, Belicchi-Ferrari M, Bisceglie F, Capacchi S, Pelosi G, Tarasconi P. Zinc complexes with cyclic derivatives of alpha-ketoglutaric acid thiosemicarbazone: Synthesis, X-ray structures and DNA interactions. *J Inorg Biochem* 2005; 99: 1504-13.
- [54] Baldini M, Belicchi-Ferrari M, Bisceglie F, *et al.* Copper(II) complexes with substituted thiosemicarbazones of alpha-ketoglutaric acid: synthesis, X-ray structures, DNA binding studies, and nuclease and biological activity. *Inorg Chem* 2004; 43: 7170-9.
- [55] Bisceglie F, Baldini M, Belicchi-Ferrari M, *et al.* Metal complexes of retinoid derivatives with antiproliferative activity: synthesis, characterization and DNA interaction studies. *Eur J Med Chem* 2007; 42: 627-34.
- [56] Buschini A, Pinelli S, Pellacani C, *et al.* Synthesis, characterization and deepening in the comprehension of the biological action mechanisms of a new nickel complex with antiproliferative activity. *J Inorg Biochem* 2009; 103: 666-77.
- [57] Laforest R, Dehdashti F, Lewis JS, Schwarz SW. Dosimetry of 60/61/62/64Cu-ATSM: a hypoxia imaging agent for PET. *Eur J Nucl Med Mol Imaging* 2005; 32: 764-70.
- [58] Dietz DW, Dehdashti F, Grigsby PW, *et al.* Tumor hypoxia detected by positron emission tomography with 60Cu-ATSM as a predictor of response and survival in patients undergoing Neoadjuvant chemoradiotherapy for rectal carcinoma: a pilot study. *Dis Colon Rectum* 2008; 51: 1641-8.
- [59] Lewis JS, Laforest R, Dehdashti F, Grigsby PW, Welch MJ, Siegel BA. An imaging comparison of 64Cu-ATSM and 60Cu-ATSM in cancer of the uterine cervix. *J Nucl Med* 2008; 49: 1177-82.
- [60] Jalilian AR, Rostampour N, Rowshanfarzad P, Shafaii K, Kamali-Dehghan M, Akhlaghi M. Preclinical studies of [61Cu]ATSM as a PET radiopharmaceutical for fibrosarcoma imaging. *Acta Pharm* 2009; 59: 45-55.
- [61] Grigsby PW, Malyapa RS, Higashikubo R, *et al.* Comparison of molecular markers of hypoxia and imaging with (60)Cu-ATSM in cancer of the uterine cervix. *Mol Imaging Biol* 2007; 9: 278-83.
- [62] Dehdashti F, Grigsby PW, Lewis JS, Laforest R, Siegel BA, Welch MJ. Assessing tumor hypoxia in cervical cancer by PET with 60Cu-labeled diacetyl-bis(N4-methylthiosemicarbazone). *J Nucl Med* 2008; 49: 201-5.
- [63] Matarrese M, Bedeschi P, Scardaoni R, *et al.* Automated production of copper radioisotopes and preparation of high specific activity [(64)Cu]Cu-ATSM for PET studies. *Appl Radiat Isot* 2010; 68: 5-13.
- [64] Krohn KA, Link JM, Mason RP. Molecular imaging of hypoxia. *J Nucl Med* 2008; 49 (Suppl 2): 129S-48S.
- [65] Vävere AL, Lewis JS. Cu-ATSM: a radiopharmaceutical for the PET imaging of hypoxia. *Dalton Trans* 2007; 43: 4893-902.
- [66] Padhani A. PET imaging of tumour hypoxia. *Cancer Imaging* 2006; 6: S117-21.
- [67] Anderson CJ, Ferdani R. Copper-64 radiopharmaceuticals for PET imaging of cancer: advances in preclinical and clinical research. *Cancer Biother Radiopharm* 2009; 24: 379-93.

- [68] Holland JP, Lewis JS, Dehdashti F. Assessing tumor hypoxia by positron emission tomography with Cu-ATSM. *Q J Nucl Med Mol Imaging* 2009; 53: 193-200.
- [69] Liu J, Hajibeigi A, Ren G, *et al.* Retention of the radiotracers ⁶⁴Cu-ATSM and ⁶⁴Cu-PTSM in human and murine tumors is influenced by MDR1 protein expression. *J Nucl Med* 2009; 50: 1332-9.
- [70] Xiao Z, Donnelly PS, Zimmermann M, Wedd AG. Transfer of copper between bis(thiosemicarbazone) ligands and intracellular copper-binding proteins. insights into mechanisms of copper uptake and hypoxia selectivity. *Inorg Chem* 2008; 47: 4338-47.
- [71] Basken NE, Mathias CJ, Green MA. Elucidation of the human serum albumin (HSA) binding site for the Cu-PTSM and Cu-ATSM radiopharmaceuticals. *J Pharm Sci* 2009; 98: 2170-9.
- [72] Basken NE, Green MA. Cu(II) bis(thiosemicarbazone) radiopharmaceutical binding to serum albumin: further definition of species dependence and associated substituent effects. *Nucl Med Biol* 2009; 36: 495-504.
- [73] Basken NE, Mathias CJ, Lipka AE, Green MA. Species dependence of [⁶⁴Cu]Cu-Bis(thiosemicarbazone) radiopharmaceutical binding to serum albumins. *Nucl Med Biol* 2008; 35: 281-6.
- [74] Bayly SR, King RC, Honess DJ, *et al.* *In vitro* and *in vivo* evaluations of a hydrophilic ⁶⁴Cu-bis(thiosemicarbazone)-glucose conjugate for hypoxia imaging. *J Nucl Med* 2008; 49: 1862-8.
- [75] Cowley AR, Dilworth JR, Donnelly PS, Heslop JM, Ratcliffe SJ. Bifunctional chelators for copper radiopharmaceuticals: the synthesis of [Cu(ATSM)-amino acid] and [Cu(ATSM)-octreotide] conjugates. *Dalton Trans* 2007; 2: 209-17.
- [76] Burgman P, O'Donoghue JA, Lewis JS, Welch MJ, Humm JL, Ling CC. Cell line-dependent differences in uptake and retention of the hypoxia-selective nuclear imaging agent Cu-ATSM. *Nucl Med Biol* 2005; 32: 623-30.
- [77] Hall MD, Salam NK, Hellawell JL, *et al.* Synthesis, activity, and pharmacophore development for isatin-beta-thiosemicarbazones with selective activity toward multidrug-resistant cells. *J Med Chem* 2009; 52: 3191-204.
- [78] Otero L, Vieites M, Boiani L, *et al.* Novel antitrypanosomal agents based on palladium nitrofurylthiosemicarbazone complexes: DNA and redox metabolism as potential therapeutic targets. *J Med Chem* 2006; 49: 3322-31.
- [79] Pérez-Rebolledo A, Teixeira LR, Batista AA, *et al.* 4-nitroacetophenone-derived thiosemicarbazones and their copper(II) complexes with significant *in vitro* anti-trypanosomal activity. *Eur J Med Chem* 2008; 43: 939-48.
- [80] Vieites M, Otero L, Santos D, *et al.* Platinum(II) metal complexes as potential anti-Trypanosoma cruzi agents. *J Inorg Biochem* 2008; 102: 1033-43.
- [81] Vieites M, Otero L, Santos D, *et al.* Platinum-based complexes of bioactive 3-(5-nitrofuryl)acrolein thiosemicarbazones showing anti-Trypanosoma cruzi activity. *J Inorg Biochem* 2009; 103: 411-8.
- [82] Biot C, Pradines B, Sergeant M, Gut J, Rosenthal PJ, Chibale K. Design, synthesis, and antimalarial activity of structural chimeras of thiosemicarbazone and ferroquine analogues. *Bioorg Med Chem Lett* 2007; 17: 6434-8.
- [83] Scovill JP, Klayman DL, Lambros C, Childs GE, Notsch JD. 2-Acetylpyridine thiosemicarbazones. 9. Derivatives of 2-acetylpyridine 1-oxide as potential antimalarial agents. *J Med Chem* 1984; 27: 87-91.
- [84] Klayman DL, Bartosevich JF, Griffin TS, Mason CJ, Scovill JP. 2-Acetylpyridine thiosemicarbazones. 1. A new class of potential antimalarial agents. *J Med Chem* 1979; 22: 855-62.
- [85] Biot C, Dessolin J, Ricard I, Dive D. Easily synthesized antimalarial ferrocene triazacyclononane quinoline conjugates. *J Organomet Chem* 2004; 689: 4678-82.
- [86] Xue C, Zhang L, Luo W, Xie X, Jiang L, Xiao T. 3D-QSAR and molecular docking studies of benzaldehyde thiosemicarbazone, benzaldehyde, benzoic acid, and their derivatives as phenoloxidase inhibitors. *Bioorg Med Chem* 2007; 15: 2006-15.
- [87] Krishnan K, Prathiba K, Jayaprakash V, *et al.* Synthesis and ribonucleotide reductase inhibitory activity of thiosemicarbazones. *Bioorg Med Chem Lett* 2008; 18: 6248-50.
- [88] Guido RVC, Trossini GHG, Castilho MS, Oliva G, Ferreira EI, Andricopulo AD. Structure-activity relationships for a class of selective inhibitors of the major cysteine protease from Trypanosoma cruzi. *J Enzyme Inhib Med Chem* 2008; 23: 964-73.
- [89] Gómez-Saiz P, García-Tojal J, Maestro MA, Arnaiz FJ, Rojo T. Evidence of desulfurization in the oxidative cyclization of thiosemicarbazones. Conversion to 1,3,4-oxadiazole derivatives. *Inorg Chem* 2002; 41: 1345-7.
- [90] Pascu SI, Waghorn PA, Conry TD, *et al.* Cellular confocal fluorescence studies and cytotoxic activity of new Zn(II) bis(thiosemicarbazone) complexes. *Dalton Trans* 2008; 16: 2107-10.

Received: December 08, 2009

Revised: January 07, 2010

Accepted: January 13, 2010

© Giorgio Pelosi; Licensee Bentham Open.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.