α-N-Heterocyclic Thiosemicarbazone Derivatives as Potential Antitumor Agents: A Structure-Activity Relationships Approach

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Abstract: α-N-Heterocyclic thiosemicarbazones, (N)-TSCs, are potent inhibitors of ribonucleotide reductase (RR). This enzyme plays a critical role in DNA synthesis and repair, and is a well-recognized target for cancer chemotherapeutic agents. In this review the structural features of (N)-TSCs, required for maximum antitumour activity have been explored. Special attention is given to the mechanisms of action and structure-activity relationships.

Key Words: α-N-heterocyclic thiosemicarbazone, bis(thiosemicarbazone), ribonucleotide reductase inhibitors, antitumour agents, metal complexes, structure-activity relationships.

1. INTRODUCTION

The application of Inorganic Chemistry to Medicine is a rapidly developing field [1]. Advances in biocoordination chemistry are crucial for improving the design of new anti-tumor compounds [2-4]. Although cis-diamminedichloridoplatinum(II), cisplatin, is a widely used anticancer drug its clinical utility has been limited due to the frequent development of drug resistance, severe side effects and the limited spectrum of tumours against which cisplatin is active [5-7].

The majority of anticancer drugs exert their activity by inhibiting one or more processes occurring in the normal cell cycle. Interaction with any of the biomolecules whose normal function is necessary for cell division interrupts normal cellular processes resulting in cell death.

The most common target of many currently used chemotherapy drugs is DNA. There are several ways of interaction between DNA and a drug and they can be classified as: 1) formation of covalent linkage between the DNA and anticancer drug. Cisplatin, which binds to DNA by coordination of the Pt atom to the N7 positions of two guanine bases or to adenine and guanine, is the best example. 2) Non-covalent interactions achieved through hydrogen bonding, intercalation or insertion between base pairs and electrostatic interactions. 3) Inhibition processes of DNA or RNA synthesis and repair [8-10].

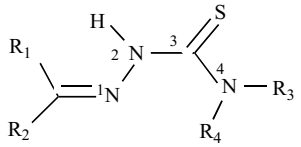
In recent years cancer chemotherapy has achieved important goals. However, several tumours still have low drug sensitivity. Novel chemotherapeutics with marked and selective antitumor activity are essential to develop, particularly those that can overcome resistance to established therapies.

Particularly, current attention is given in the design of new agents with a mechanism of action different from cisplatin. In this regard, of particular interest are the compounds targeting ribonucleotide reductase (RR), one of the most complex enzymes in the cell, from a biological, structural and regulatory point of view [11, 12].

α-(*N*)-Heterocyclic thiosemicarbazones, (N)-TSCs, are strong metal chelating agents and have been reported to be among the most effective RR inhibitors yet identified [13, 14]. Pyridine-2-carbaldehyde thiosemicarbazone was the first member of this class reported to have carcinostatic effects and since then many α-N-heterocyclic thiosemicarbazones and their metal complexes have shown anticancer activity against a wide spectrum of tumour cell lines. Currently, the 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine, Vion Pharmaceuticals, New Haven, CT) is being evaluated in human phase II clinical trials as an antineoplastic therapeutics.

2. STRUCTURAL CHARACTERISTICS OF α-(*N*)-HETEROCYCLIC THIOSEMICARBAZONES

Thiosemicarbazones are compounds of considerable interest because of their important chemical properties and potentially beneficial biological activities [15-18]. According to the IUPAC nomenclature [19], these compounds, usually obtained by condensation of an aldehyde, or a ketone with a thiosemicarbazide, may be named by adding the class name "thiosemicarbazone" after the name of the condensed aldehyde or ketone. In the same way bis(thiosemicarbazones) are derived from dicarbonyl compounds and two thiosemicarbazide moieties. The basic structure of thiosemicarbazone compounds and IUPAC numbering scheme is shown in Fig. (**1**).



R_1, R_2, R_3, R_4 = H, alkyl or aryl group

Fig. (1). Chemical structure of thiosemicarbazones.

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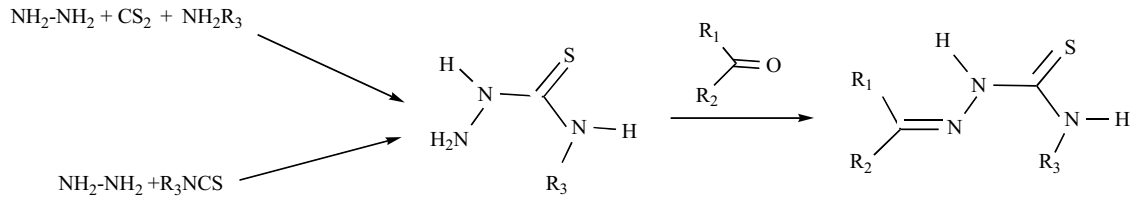


Scheme 1. Synthetic procedures of ^{4}N-substituted thiosemicarbazones.

The thiosemicarbazides used in the synthesis can be obtained by a series of synthetic procedures (Scheme **1**). For example the reaction of hydrazine hydrate with isothiocyanates or the reaction of amines with carbon disulphide followed of the addition of hydrazine hydrate [20].

In general, the synthesis of thiosemicarbazone compounds presents low cost and high atoms economy since all the atoms from the reagents (except water liberated in the condensation) are present in the final molecule.

Looking at the structural characteristics, thiosemicarbazones contain a rich set of donor atoms and are well-known chelators of metal ions. The coordination chemistry of thiosemicarbazones appear to be very interesting from the point of view of both the number of metals forming complexes with them and the stabilization of various (less common) oxidation states of metals. Moreover many of their biological activities of the thiosemicarbazones often have been attributed to their ability of chelation with endogenous metals [21, 22].

Thiosemicarbazone ligands may exist as thione/thiol tautomeric forms owing to the intramolecular proton transfer, Fig. (**2**). A review of thiosemicarbazone structures [23] shows that in solid state these molecules are almost planar, with the sulphur atom *trans* to the azomethine nitrogen atom (configuration E). Although there are several electronic and steric factors that may contribute to the adoption of this arrangement, the most important is probably that the *trans* arrangement places the amine (^{4}N) and azomethine (^{1}N) nitrogen atoms in relative positions suitable for intramolecular hydrogen bonding.

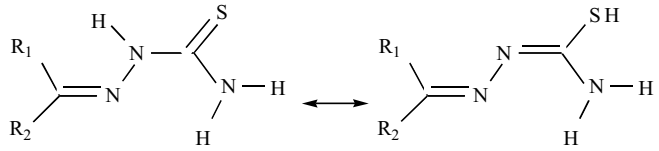


Fig. (2). Tautomeric forms of thiosemicarbazones.

However in most of the complexes the thiosemicarbazone moiety coordinates to the metal ion in the *cis* configuration through the thione/thiol atom and the azomethine nitrogen atom. The coordination capacity of thiosemicarbazones can be further increased, if the parent aldehyde or ketone contains additional functional group in position suitable for chelation. Particularly, compounds in which the thiosemicarbazone side-chain is attached in α position to a N-heterocyclic ring**,** namely α-N-heterocyclic thiosemicarbazones, have shown substantial *in vitro* activity against various human tumour lines. The (N)-TSCs possess a conjugate NNS donor set which favour the coordination to metal ions forming two five-membered chelate rings of a partially conjugate character and these particular structural characteristic seems to be essential for biological activity [24]. Moreover the aromatic ring can enter into the π-π interaction with biomolecules modifying the biological activity [25].

Therefore the modification of the structure of (N)-TSC derivatives gives the possibility of synthesizing novel compounds and exploring their biological activities. The (N)-TSC skeleton can be modified around three positions, Fig. (**3**): the heterocyclic ring, the ^{4}N-substituents on the thiosemicarbazone moiety and chelation with metal ions. A large number of different (N)-TSCs, introducing structural variation have been synthesized in order to verify if the change in the new structural motifs have positively modulated the biological activity.

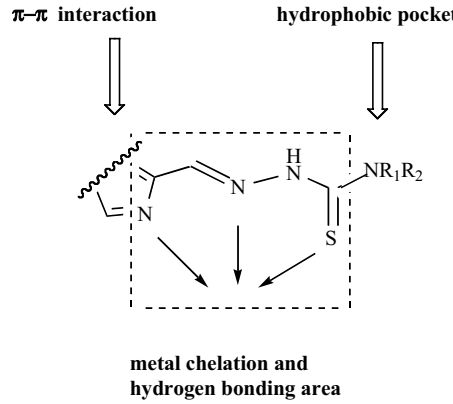
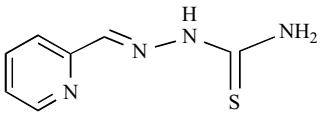


Fig. (3). Modifications of α-N-heterocyclic thiosemicarbazones.

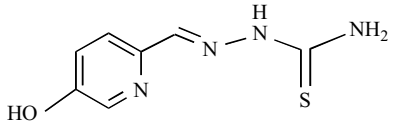
3. MODIFICATIONS OF THE HETEROCYCLIC RING SYSTEMS

Since the initial observation of the antitumour properties of pyridine-2-carboxaldehyde thiosemicarbazone, Fig. (**4a**), by Brockman *et al.* [26] a large number of different ring systems (benzene, furan, thiophene, etc) have been synthesized and evaluated for antineoplastic activity. Among them, the six-membered heterocyclic ring systems carrying the thiosemicarbazone side-chain α to the heterocyclic nitrogen have shown to be the most active antineoplastic agents. It is interesting to note that compounds in which the thiosemicarbazone moiety is affixed to the heterocyclic ring in the β- and γ-position relative to the ring nitrogen were inactive. Then, as it has been addressed above the presence of a NNS tridentate system is an essential structural requirement for the biological activity of these compounds.

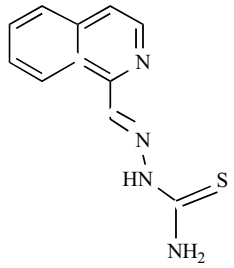
Initially, one of the most active compounds was 5-hydroxypyridine-2-carboxaldehyde thiosemicarbazone, Fig.



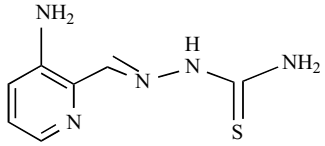
pyridine-2-carboxaldehyde thiosemicarbazone (**4a**)



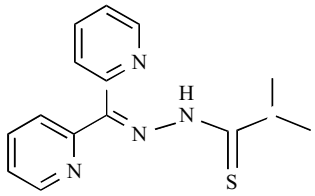
5-hydroxypyridine-2-carboxaldehyde thiosemicarbazone (**4b**)



isoquinoline-1-carboxaldehyde thiosemicarbazone (**4c**)



3-aminopyridine-2-carboxaldehyde thiosemicarbazone, Triapine (**4d**)



di-2pyrirylketone
4,4-dimethylthiosemicarbazone (**4e**)

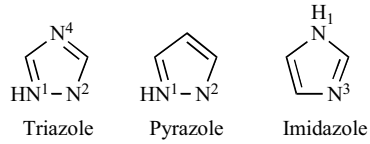
Fig. (4). Structures of some α-N-heterocyclic thiosemicarbazone derivatives.

(**4b**), which reached Phase I clinical trials, but the impressive antineoplastic activity exhibited in animal systems was not observed in man [27]. Its lack of activity was attributed to a relatively short biological half-life in humans, which was due to the rapid formation of an inactive O-glucoronide and the rapid excretion of this conjugate [28,29], accompaning by high *in vivo* toxicity. Moreover this compound exhibits poor water solubility, necessary for human formulations.

Sartorelli *et al.* in 1995 [24] first reviewed the modifications concerning to the ring systems. They studied structural modifications of the pyridine and isoquinoline series, Fig. (**4c**), in order to evaluate the effects of various substituents, on the heterocyclic rings, on antitumour activity. In an attempt to enhance water solubility, necessary for *in vivo* activity, hydrophilic groups such as amino or hydroxyl were introduced at various positions and found that the 3- and the 5- amino derivatives were the most potent compounds in the pyridine series. For the isoquinoline series, the substitution of an amino group into the 5-position have been shown to be the best option. These new derivatives resulted resistant to O-glucuronidation and they were further evaluated. Among them, the 3-aminopyridine-2-carboxaldehyde thiosemicarbazone derivative, Fig. (**4d**), appeared to be the most promising. This compound is currently being screened for antitumour effect using the National Cancer Institute panel of 60 tumour cell lines and selected for Phase II clinical trials (Triapine, Vion Pharmaceuticals, New Haven, CT) [30-33].

These authors pointed out that the replacement of the six-membered heterocyclic ring with five-membered ring systems led to a decrease or complete loss of antineoplastic activity. Therefore , although a large number of thiosemicarbazone derivatives containing five-membered heterocyclic rings have been synthesized and structurally characterized, few studies including biological activity [34, 35].

However we thought that the replacement of the pyridine ring by a 1,2,4-triazole based five-membered heterocycle ring [36, 37] could be a good choice since it represents a hybrid of pyrazole and imidazole moieties with regard to the arrangement of their three donor atoms, furthermore the additional nitrogen atom in the ring was expected to exert an electron-withdrawing effect, see Fig. (**5**).



Triazole Pyrazole Imidazole

Fig. (5). Representation of triazole, pyrazole and imidazole.

The triazole heterocycle was selected not only for its symmetry, but also because the increased acidity of the N-H as a proton ionisable centre which facilitates the metal coordination. So, by condensation of the diketone 3,5-diacetyl 1,2,4-triazole with different thiosemicarbazides we have recently synthesized a series of bis(thiosemicarbazone) compounds which have shown *in vitro*, antitumour activity [38-41].

4. MODIFICATION OF THE THIOSEMICARBAZONE SIDE CHAIN

Subsequent studies have involved the preparation of a series of homologous compounds by alterations of easily

modifiable amine groups, since these modifications may cause changes in lipophilicity and structural features. It is worth emphasizing that replacement of the hydrogen attached to the ^{2}N by an alkyl group deactivates the compound as for example is observed with the 2-hydroxynaphthalene-1-carboxaldehyde ^{2}N-methyl-substituted thiosemicarbazone. This compound exhibits poor antiproliferative activity [42] which has been attributed to the absence of an ionisable proton on ^{2}N, thus the formation of the thiol tautomeric form is precluded and therefore its metal binding affinity lowered.

In general, ^{4}N-substituted thiosemicarbazones show remarkable activity in comparison with the unsubstituted ones. An enhanced inhibitory effect may be attributed to the increased lipophilicity, so they can easily cross the cell membrane.

The ^{4}N nitrogen of the thiosemicarbazone skeleton may contains: a) Two hydrogen atoms (unsubstituted thiosemicarbazones), b) one hydrogen atom and one alkyl or aryl group and c) two alkyl or aryl groups or may be a part of a cyclic ring.

Starting from the pyridine-2-carboxaldehyde thiosemicarbazone structure several ^{4}N-substituted thiosemicarbazones related, have demonstrated significant antitumour activity. Also, 2-acetylpyridine ^{4}N-substituted thiosemicarbazones have demonstrated significant activity in a series of human tumour cell lines [43-48]. Following these initial reports series of pyridine-2-carboxaldehyde thiosemicarbazone analogs have been synthesized and evaluated for antitumour activity [49-51]. Richardson *et al*. [52] have prepared thiosemicarbazone compounds derived from di-2-pyridyl ketone and 2-benzoylpyridine. These novel and patented compounds were found to have high antiproliferative activity against a number of tumour cell lines. Significantly, a number of *in vivo* studies using mouse models have confirmed the antitumor activity of the di-2-pyridylketone 4,4-dimethyl-thiosemicarbazone, Fig. (**4e**).

Also a series of α-N-heterocyclic bis(thiosemicarbazones) was also explored. These derivatives, having the two thiosemicarbazone moieties positioned in the molecule are much more powerful metal-chelating agents [53].

Within the class of α-N-heterocyclic bis(thiosemicarbazones), 2,6-diacetylpyridine bis(thiosemicarbazone) derivatives have been described as promising antitumour agents

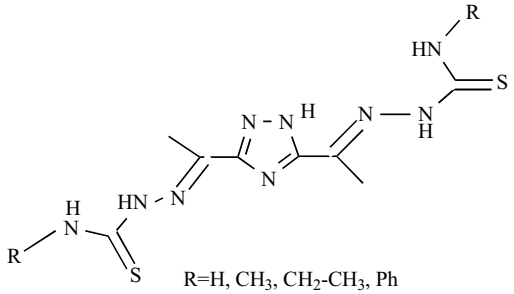


Fig. (6). Schematic representation of 3,5-diacetyl-1,2,4-triazol bis(thiosemicarbazones).

[54]. Also, a series of 3,5-diacetyl-1,2,4-triazol ^{4}N-substituted bis(thiosemicarbazone) ligands synthesized in our laboratory have showed *in vivo* antitumour activity Fig. (**6**). In this case the activity of the ligands increase with the size and shape of the hydrophobic substitution on ^{4}N of the thiosemicarbazone moieties [41].

5. METAL COMPLEXES AND STRUCTURE–ACTIVITY RELATIONSHIPS

It has been widely acknowledged that the α-N-Heterocyclic thiosemicarbazones are strong metal chelating agents. Generally react with metal ions to form 1:1 or 2:1 ligand-to-metal complexes, depending upon the coordination number of the metal. Several (N)-TSC metal complexes have shown significant biological activity, suggesting accessibility of coordination site, a fundamental requirement for biological activity by the complexes. Recently the structure trends of metal thiosemicarbazones have been reviewed [55], most of the complexes studied are mononuclear or dinuclear and with only a few complexes of higher nuclearity.

Petering and co-workers [56] first demonstrated that some iron o copper α-N-heterocyclic thiosemicarbazone chelates were more potent than the free ligands, the same effect has been observed later with other essential metals such zinc [57-59].

Recently Keppler and co-workers have reported a number of Ga(III) and Ru(II) (N)-TSCs complexes with potent antiproliferative properties [60,61]. Also, a recent publication of Beraldo *et al.* reports the cytotoxic activity of Ga(III) complexes of 2-pyridineformamide thiosemicarbazones [62].

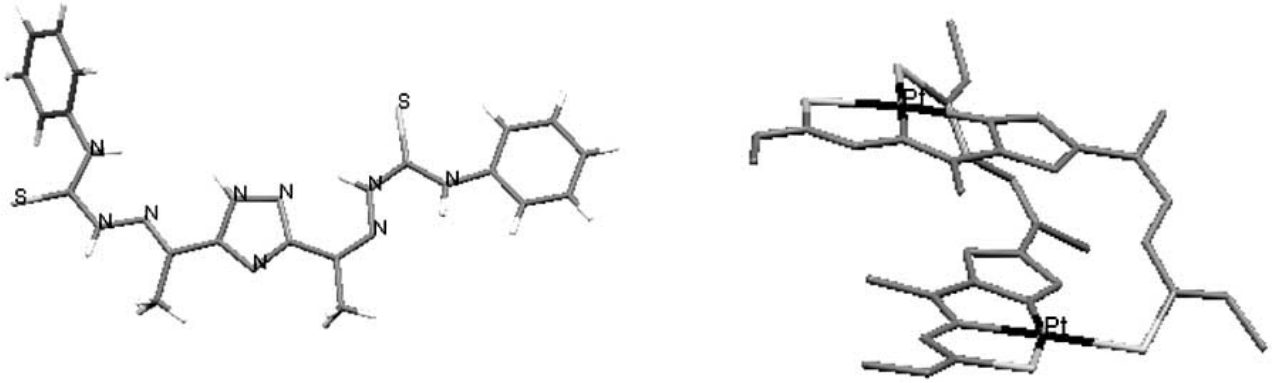


Fig. (7). Capped sticks representation of Tz(NHPh-TSC)2, **1**, and [Pt-Tz(NHMe-TSC)2]2, **11** compounds.

Other works have demonstrated that complexation of (N)-TSCs with metal ions like platinum(II) or palladium(II) that damage DNA produces synergetic inhibition of tumour growth [63,64]. So, the synthesis, spectral and structural characterization and antitumour activity of a vast number of transition-metal α-N-heterocyclic thiosemicarbazone complexes have been reported [65, 66].

Our recent work in this area has focused on palladium and platinum complexes derived from 3,5-diacetyl-1,2,4-triazol bis(^{4}N-substituted-thiosemicarbazones), Tz(NHR-TSC)$_2$ [41]. X-Ray data have been obtained for many of these compounds. These complexes are characterized by their dinuclear structure which results of the pairing of two mononuclear units through two thiosemicarbazone bridges, the whole molecule containing two parallel coordination planes are stabilized by the presence of π-π stacking interactions. Fig. (**7**).

The antitumor activity revealed that this series of compounds are active toward the cancer cell lines tested (A2780 and A2780*cis*R), the results obtained are shown in Table **1**.

The structure-activity relationships study revealed that in general, activity increases with the increasing size of the ^{4}N substituent but one exception was found, namely most cytotoxic ligand (^{4}N-phenyl substituted) generated a weak cytotoxic platinum complex and an inactive palladium one. Based on the monocrystal X-ray structure of the ligand (almost planar except one of the two phenyl groups) this drastic change in the activity was explained in terms of the structural changes produced upon the complexation like the presence of four bulky phenyl groups which sterically hinder interactions and also prevent direct hydrogen bonding with biological molecules.

The study of this family of compounds also points to the influence of the nature of the metal ion that appears affect to the biological activity of this series since some palladium complexes displayed slightly higher activity than platinum ones. Taking in account that palladium(II) and platinum(II) complexes have high thermodynamic stability especially with bidentate sulphur-nitrogen chelating agents but the platinum complexes are too kinetically inert (in regard to ligand exchange) whereas the palladium complexes are kinetically labile, it might be concluded that the higher kinetic lability of the palladium complexes make them more suitable for interacting with the cancer cells.

This series of compounds was active not only in the epithelian human ovarian cancer, cisplatin sensitive, A2780 cell line but also in the A2780*cis*R, cisplatin resistant, cell line. It has been shown that cellular thiols can sequester cisplatin, leading to a reduction in the levels of cisplatin-DNA damage however, some studies have demonstrated that thiosemicarbazone complexes having sulphur coordinated do not react with cellular thiols [67].

6. MECHANISMS OF ACTION

As a result of the extensive biological and chemical studies carried out on (N)-TSCs, the enzyme ribonucleotide reductase, RR, has been identified to be the principal target of the α-N-heterocyclic thiosemicarbazones[68].

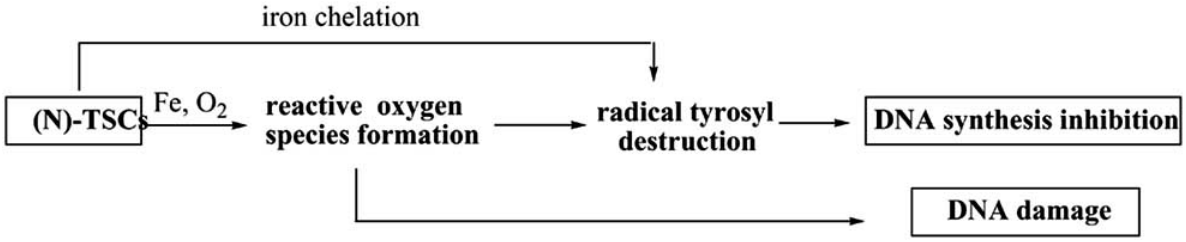
The enzyme RR which catalyzes the conversion of ribonucleotides into desoxyribonucleotides during DNA synthesis, is highly expressed in tumour cells making it a suitable and well-established target for cancer therapy.

Mammalian ribonucleotide reductase (RR) is composed of two dissimilar proteins, (R1) which contains polythiols and (R2), which contains non-heme iron and a free tyrosyl radical. The only known R2 requirement for the enzymatic activity of the RR is the presence of a tyrosyl free radical,

Table 1. IC_{50}±SD (μM) and Resistance Factor (RF) Values Obtained for 3,5-diacetyl-1,2,4-triazol bis(thiosemicarbazone), Tz(NHR-TSC)$_2$, Series with Respect to Cisplatin in the Human Ovarian Carcinoma Cell-Lines Sensitive and Resistant to Cisplatin (A2780 and A2780*cis*R)

	IC_{50}(A2780)	IC_{50}(A2780*cis*R)	RF*
Tz(NHPh-TSC)$_2$ **1**	6.2±0.3	5.4±0.4	0.87
Tz(NH$_2$-TSC)$_2$ **2**	>100	>100	---
Tz(NHMe-TSC)$_2$ **3**	39±2	>100	---
Tz(NHEt-TSC)$_2$ **4**	35±2	46±2	1.31
[Pd-Tz(NHPh-TSC)$_2$]$_2$ **5**	>100	>100	---
[Pd- Tz(NH$_2$-TSC)$_2$]$_2$ **6**	>100	>100	---
[Pd- Tz(NHMe-TSC)$_2$]$_2$ **7**	15±2	18±2	1.20
[Pd-Tz(NHEt-TSC)$_2$]$_2$ **8**	25±2	10±2	0.40
[Pt –Tz(NHPh-TSC)$_2$]$_2$ **9**	40±2	>100	---
[Pt- Tz(NH$_2$-TSC)$_2$]$_2$ **10**	50±5	>100	---
[Pt-Tz(NHMe-TSC)$_2$]$_2$ **11**	40±2	56±3	1.40
[Pt-Tz(NHEt-TSC)$_2$]$_2$ **12**	63±2	66±5	1.05
Cisplatin	0.44±0.04	4.7±0.3	10.7

* RF = IC_{50}(A2780*cis*R) / IC_{50}(A2780).



Scheme 2. RR inhibition mechanisms of (N)-TSCs.

which represents the one-electron oxidized state of a tyrosine residue. Formation and stability of the radical depends on the binuclear Fe(III) center, found both in E. coli and mammalian R2 [69, 70].

The α-N-heterocyclic thiosemicarbazones are known iron chelators and as such can bind intracellular iron and/or damage the non-heme iron-stabilized tyrosyl free radical and thus inhibit the catalytical function of RR (Scheme **2**).

An important aspect that requires discussion is that (N)-TSCs are capable not only of bind Fe but also form redox-active iron complexes that are though to play an important role in their anti-proliferative efficacy. Thelander and Gräslund demonstrated for the first time that destruction of the tyrosyl radical by a (N)-TSC requires iron and oxygen [71]. Since then many authors have carried out studies on the redox active (N)-TSC iron complexes [72,73].

Recently, Richardson *et al.* have examined the mechanism of action of the di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone molecule and have shown that the antileukemic activity of this compound is mediated, at least in part, by its ability to bind cancer cell iron, forming a redox-active iron complex that generates reactive oxygen species [74].

It is assumed that, in general, complexation to metal ions increases the tumour-inhibition activity of the (N)-TSCs and this suggests that the mechanism of action of (N)-TSC complexes is not only due to the inhibition of RR but both the metal and the ligand determine the biological activity. Indeed, the metal compounds could act through dual or even multiple mechanisms of action by combining the pharmacological properties of both, the ligand and the metal.

Platinum(II) attacks DNA, but other metal ions may have different targets sites. For example, it has been demonstrated that iron and copper (N)-TSC complexes are readily reduced by thiol compounds and oxidized by oxygen or by reduced species of oxygen to produce reactive entities, including oxygen radicals which cause tissue damage [69,70]. Gallium affects intracelular iron availability due to the competitive binding of gallium(III) and iron(III) toward bioligands but also interacts directly with RR displacing iron from the R2 subunit of this enzyme [74].

In conclusion, in this review the structural features of (N)-TSCs required for maximum activity have been pointed out thus constituting a good example of how small changes in molecular structure could lead to profound differences in biological activity. Since the mechanism of action of (N)-TSCs is different from established chemotherapies, they appear to be good candidates particularly for use in combination therapy. It is important to note that several combinations of (N)-TSCs, like Triapine®, with other anticancer drugs have been reported to give additive or synergistic activity.

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