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Review

Contribution to the SAR field of metallated and coordination complexes Studies of the palladium and platinum derivatives with selected thiosemicarbazones as antitumoral drugs

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Contents

Abbreviations: **1**, **2**, **3**, **4**, **5** and **6**, thiosemicarbazone ligands (**a** and **b**: tetranuclear palladium complexes, **c**: tetranuclear platinum complexes, **d** and **e**: dinuclear palladium and platinum complexes respectively, **f** and **g**: mononuclear palladium and platinum complexes respectively, **h**: dinuclear acetate palladium complex, **I**: mononuclear lutidine palladium complex, **j**: tetranuclear not cyclometallated palladium complex); 5-GMP, 5′-guanosine monophosphate (dianion); [b.TSCN-NH2], phenylacetaldehyde thiosemicarbazone; CLs, cross-links; DC:DNA compounds, dinuclear complexes:DNA compounds; FAB Mass Spectra, fast atom bombardment mass spectra; IR, infra red; MC:DNA compounds, mononuclear complexes:DNA compounds; NMR, nuclear magnetic resonance; [*p*-is.TSCN-NR], *p*-isopropylbenzaldehyde thiosemicarbazone protected in the NH_2 group; [*p*-is.TSCN-NH2], *p*-isopropylbenzaldehyde thiosemicarbazone; SAR, structure–activity relationships; SGH, glutathione; TC:DNA compounds, tetranuclear complexes:DNA compounds; TSCN, thiosemicarbazone

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Abstract

This article reviews the chemical and antitumoral properties of thiosemicarbazone complex-based drugs containing platinum(II) or palladium(II). Tetranuclear orthometallated complexes are the preferentially formed by most of the selected TSCNs. The core of these complexes consists of a flexible eight-member ring of alternating M–S atoms, causing in some cases, formation of two different geometric isomers. The different isomers display different cytotoxic activity, which was significant especially with platinum derivatives. Moreover, they display cytotoxic activity in tumor cell lines resistant to *cis*-DDP; the analysis of the interaction with DNA indicates interhelical cross-link formation. Binuclear complexes of thiosemicarbazones are chloro-bridged complexes where no orthometallation occurs, but their cytotoxic testing shows activity against resistant cell lines. Mononuclear complexes, which show an analogous structure to cisplatin, have shown promising "in vitro" antitumor properties. In addition, the interaction with DNA indicates that they have an enhanced capacity to form DNA interstrand cross-links, by comparison with *cis*-DDP. The final purpose is to evaluate the coordination chemistry of selected thiosemicarbazone complexes of palladium and platinum in order to provide guidance and determine structure and antitumor activity relationships for continuing studies of these systems.

Keywords: SAR; Thiosemicarbazone; Metallated

1. Introduction

Thiosemicarbazones (TSCNs) are very promising molecules in coordination chemistry because of their pharmacological properties [1] which include notably their antiparasital [2], antibacterial [3] and antitumor activities [4,5]. Some thiosemicarbazones increase their antitumor activity by their ability to form chelates with specific metallic ions [6]. These ligands are formed by the condensation of a thiosemicarbazide and an aldehyde, and both of them can create another coordination site [7]. The thiosemicarbazone ligand usually coordinates with a metal through the imine nitrogen and the sulfur atom [8,9]. The ligands feature more than two covalent sites, the number of which depends on the aldehyde [10], and on the tautomeric equilibrium of the thiosemicarbazone [11,12], although the most common way to coordinate is through the thiolic form [7–13]. Evidence of this feature can be found in the tremendous volume of transition metal chemistry that has been published for these ligands, in some cases involving tridentate coordination [9,14,15].

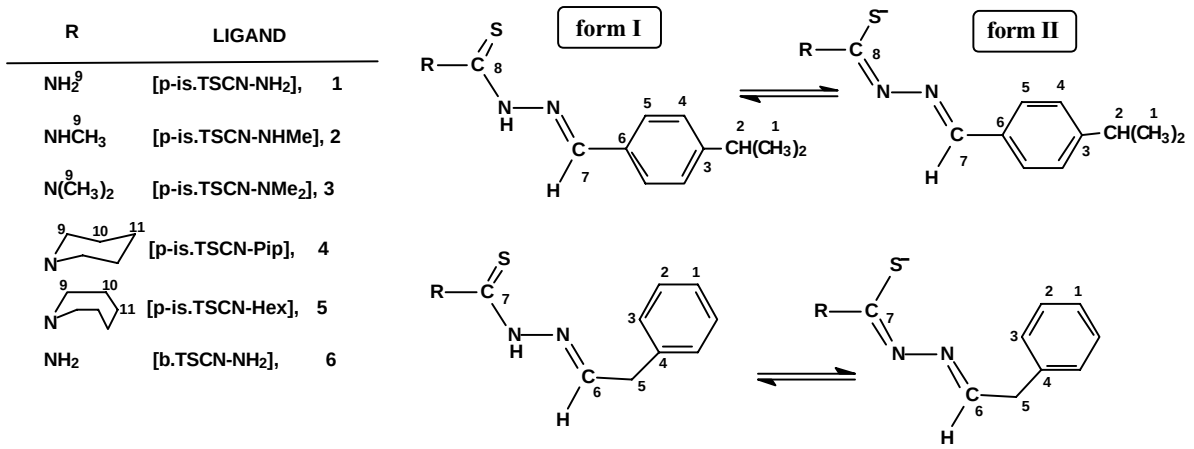
Since cisplatin emerged as the most important antitumor drug [16,17], thousands of complexes of general formulae ML_2X_2 have been synthesized and characterized [18,19] in order to study the effects of the metal, the inert group L, and the leaving group X on the structural and kinetic properties involved in the biological activity [20,21]. However, significant problems are still extant, including side effects, toxicity, cancer specificity and specially acquired resistance. Consequently, antitumor drug research has been moving toward the development of new compounds outside the usual coordination sphere, e.g. organometallic complexes, complexes with metallic elements different from platinum [22,23], or much more recently, platinum complexes with *trans* geometry [24–26].

Our interest in the cyclometallation process [27–29], and the fact that some of those cyclometallated complexes have potential antitumor activity [30,31], led us to postulate that thiosemicarbazone compounds might be suitable for the synthesis of orthometallated complexes with palladium and platinum metal ions, and that this combination might enhance the pharmacological activity of the ligand.

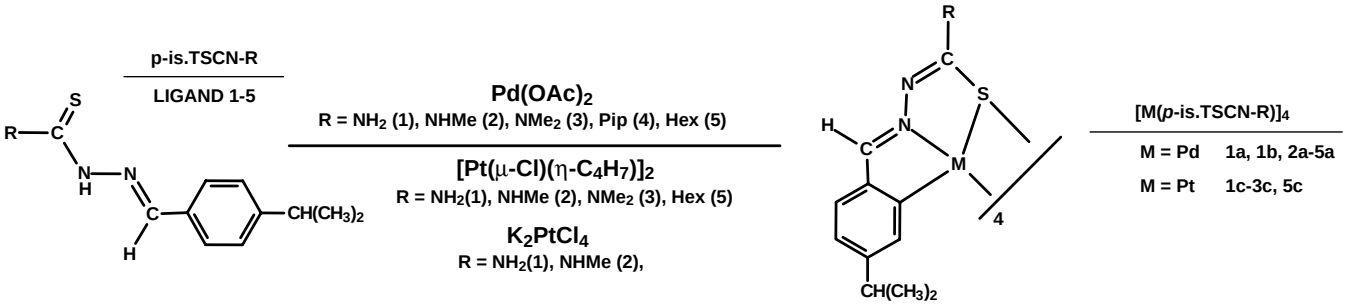
There are many studies involving thiosemicarbazones with different metal atoms [7–9], but there were only a few papers describing such palladium complexes [32] before 1998. At this time, we reported [33] several studies of the palladium and platinum chemistry of various thiosemicarbazones that we discuss here (Scheme 1). A compendium of the complexes and the references in which these ligand derivatives can be found is given in Scheme 5. Cellular testing and DNA binding assays were also performed for these complexes [33,34]. We were the first authors observing tetranuclear orthometallated complexes with thiosemicarbazone CNS donors [33], and also studying the process of the coordination [35] and orthometallation [33,36] of palladium and platinum with thiosemicarbazones. Some tridentate orthometallated complexes were reported earlier [37], but the tridentate ligand had different donor heteroatoms (CNN), and only mononuclear complexes were isolated. In the last 4 years, much transition metal chemistry has been conducted with both metals [38,39] as additional information has become available about thiosemicarbazone chemistry [40,41] and "in vivo" and preclinical studies for some thiosemicarbazone derivatives can now be found in the literature [42,43]. Semicarbazone chemistry has also been accomplished where the ligand heteroatoms are CNO [44]. The potential applications of these palladium and platinum derivatives as antitumor drugs, discussed in this article, should help in the progress of this field of research.

2. Tetranuclear complexes

Tetranuclear palladium and platinum complexes are conveniently prepared by combining the desired thiosemicarbazone (TSCN) with appropriate palladium and platinum salts. The choice of solvent depends on which salt is being used (acetic acid works well for palladium salts and MeOH



Scheme 1. The thiosemicarbazones included in this review and their respectively numbered atoms for the NMR data. The thiosemicarbazones primarily differ in two ways: in the type of substituent of the NH_2 group and in the constituent in between the nitrogen atom and the phenyl group.



Scheme 2. General synthesis for tetranuclear complexes with Pd Pt salts and the ligands from **1** to **5**.

for platinum salts). b.TSCN-NH_2 ligand **6** is problematic in these reactions; the incorporation of a CH_2 group in the TSCN moiety tends to make the isolation of the tetranuclear complex more difficult.

The complexes presented here were the first thiosemicarbazone complexes to possess a tetranuclear structure (where TSCN acts as tridentate ligand) reported in the literature (see Scheme 2). Thiosemicarbazones generally coordinate to a metal through the imine nitrogen and the sulfur atom, but these ligands feature more than two covalent sites; therefore, there will be many possibilities for coordination including the tridentate form. An understanding of how thiosemicarbazones are capable of bridging two metal atoms [45] is useful to grasp how these ligands afford tetranuclear structures. In addition, the orientation of the phenyl group and the donor atoms in these ligands appears to be appropriate for orthometallated reaction requirements [46].

2.1. *p*-Isopropylbenzaldehyde thiosemicarbazone derivatives

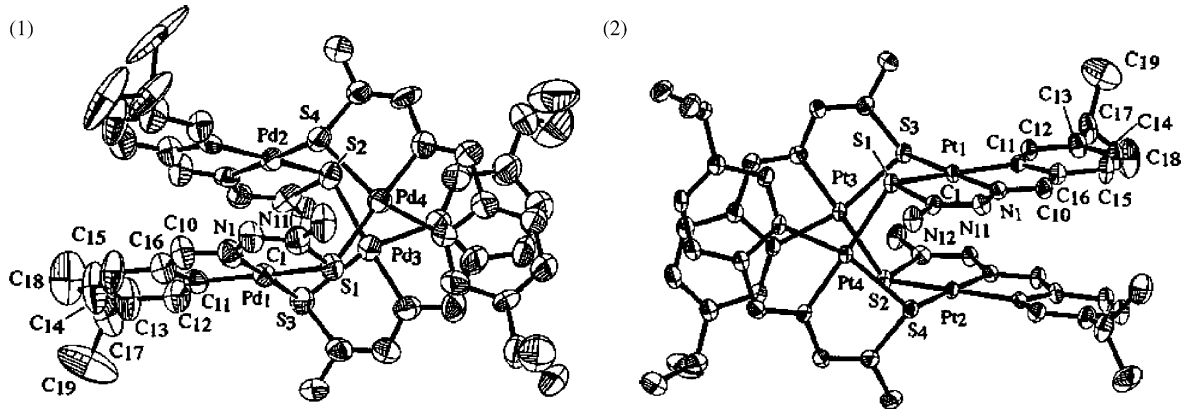
p-Isopropylbenzaldehyde thiosemicarbazone, *p*-is.TSCN-NH_2, **1** is an antiviral and antifungal compound [47], and within the large group of TSCNs this particular TSCN has not received much attention. The results obtained with the *p*-is.TSCN-NH_2 ligand are discussed in this review and demonstrate its wide spectrum of coordination possibilities. The tetranuclear compounds derived from *p*-is.TSCN-NH_2, are the first orthometallated compounds isolated with thiosemicarbazone ligands; this C–H bond activation amplifies the coordination possibilities for TSCNs even more [33].

2.1.1. *Pd and Pt complexes with p-isopropylbenzaldehyde thiosemicarbazone p-is.TSCN-NH_2, **1***

The reaction between $Pd(AcO)_2$ and the potentially tridentate CNS donor ligand in acetic acid resulted in the formation of two orthometallated complexes, **1a** and **1b**, which were separated and purified by chromatography. The compounds differ in their physical properties, for instance solubility and color.

However, the reaction between K_2PtCl_4 and *p*-is.TSCN-NH_2, **1**, using MeOH as solvent afforded only one platinum derivative: complex **1c**. Another isomer was expected (as in the palladium reaction) but was never detected. Variations in the reaction conditions, as for example the platinum reagent,[1] solvent, longer reaction time, did not produce different isomer compounds. The existence of

[1] $[Pt(\mu\text{-}Cl)(\eta^3\text{-}C_3H_5)]_2$, and $PtCl_2(PhCN)_2$.



Figs. 1 and 2. The molecular structures of complexes **1a** and **1b**. Reproduced with permission from J. Med. Chem. 41 (1998) 1399–1408. Copyright 1998, Am. Chem. Soc.

tetranuclear structures for all complexes were checked by FAB mass spectrometry. The complexes: **1a**, **1b** and **1c** (see Scheme 2) were characterized by elemental analysis, and IR and NMR spectroscopy. The structure of complexes **1a** and **1c** has been determined by X-ray diffraction (Fig. 1 shows the tetranuclear entity of complex **1a** and Fig. 2 the same for complex **1c**). NMR spectroscopy data are consistent with the solid-state structure determined by X-ray crystallography.

In both cases, the structure consists of tetranuclear units. The core of **1a** and **1c** consists of an eight-membered ring of alternating M (Pd(II) in **1a**, Pt(II) in **1c**) and S atoms in a boat conformation. The remaining two sites of each square planar M (Pd(II) in **1a**, Pt(II) in **1c**) coordination sphere are occupied by the iminic nitrogen and the *ortho* carbon of the *p*-isopropylphenyl ring to which the imine group is attached. The two five-member chelate rings involving the metallic atom are almost coplanar, and the geometry around the metallic atom is approximately square planar. The M–M distances in both complexes indicate no direct bond between the metallic atoms, but a very weak interaction can be postulated in agreement with the reported values for polynuclear complexes with Pd [48,49] or Pt [50,51]. A similar ring disposition has been described in palladium complexes with amido [52] and in palladium and platinum complexes with 2-(1-naphthyl)benzothiazoline [53] as ligands.

Based upon all the spectroscopic data, these compounds are tetranuclear, tridentate and orthometallated. The coordination to the metallic atom through the iminic nitrogen [8,54,55] and the coordination via the sulfur atom in the thiol form [56,57] in all complexes is clearly observed in the IR spectra (see form II, Scheme 1). The NMR spectra for all the complexes are very good examples of metalated complexes in *ortho* positions [58,59]. This technique also helps to distinguish the two palladium isomers (**1a** and **1b**): there is a strong shielding effect on the protons of the phenyl group caused by through-space interactions of overlying aromatics rings in only one of the isomers [33,60]. The endo structure in both complexes [61] and the carbon where the cyclometallation has taken place [62] are also typical features which can be easily detected by the NMR technique.

Conversion is observed between the two isomers; complex **1a** in DMSO solution, after 24 h, afforded a mixture of **1a** and **1b** species in a 1:1 ratio. When compound **1b** is dissolved in $CDCl_3$, compound **1a** is formed after 24 h (see Fig. 3).

Treatment of the tetranuclear compounds **1a**, **1b** and **1c** with nucleophiles like DNA bases (5-GMP, adenine and SGH), and pyridine and picoline derivatives does not lead to ring opening of the eight-member M–S ring core of the tetranuclear structure nor to ring cleavage of the chelate metal–TSCN ring [63]. This behavior may be attributed to the strong M–S bond [64] and the tetranuclear nature. However some authors have observed a M–S bond cleavage in the reaction of orthometallated TSCN tetranuclear complexes with a strong chelating phosphane [40]. The same authors have even observed M–N bond cleavage for terdentate ligands CNN (only in dinuclear and mononuclear complexes) but never M–C bond cleavage [65].

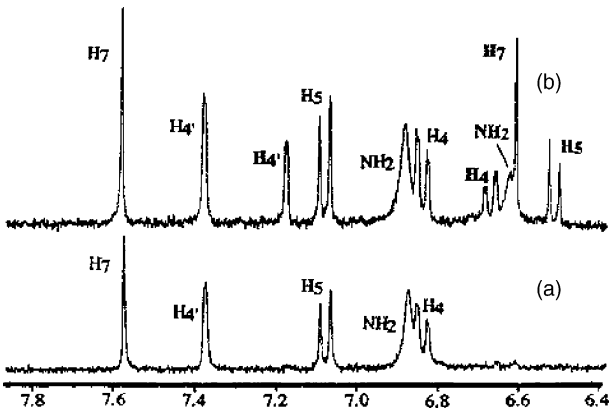


Fig. 3. Aromatic region of the ^{1}H NMR spectrum of complex **2** along time (a) $t = 0$ h; (b) $t = 24$ h. Copyright 1998, Am. Chem. Soc.

2.1.2. Pd and Pt complexes with p-isopropylbenzaldehyde n-protected thiosemicarbazones p-is.TSCN-NR

In order to identify new metal-thiosemicarbazone compounds endowed with specific cytotoxic properties we synthesized Pd(II) and Pt(II) compounds derived from *p*-isopropylbenzaldehyde N-protected thiosemicarbazone **2**, **3**, **4** and **5** (see Scheme 2). The presence of at least one NH group in these platinum compounds, which could potentially interact with cellular components, appears to be relevant to the structure–activity relationships [66]. The incorporation of additional groups in the terminal amine group of the thiosemicarbazone moiety has been used not only to increase the solubility but also to study the role of the NH group in the structure–activity relationships of Pt complexes.

Most of the thiosemicarbazone ligands can be used to isolate tetranuclear complexes. The reaction between $Pd(AcO)_2$ and the ligands **2**–**5**, in acetic acid, under the same conditions used with ligand **1**, resulted in the precipitation of the complexes $[PdL]_4$, **2a**, **3a**, **4a** and **5a**, respectively. Chromatographic purification provided the separation of cyclometallated cluster complexes (**2a**–**5a**) but a cyclometallated complex mixture is also detected for each ligand, but could not be separated. Although two isomeric orthometallated palladium complexes were separated using ligand **1**, the other expected palladium isomers "**b**", which are probably present in the mixture, represent a minimum yield from the reaction. Subsequently, the desired formation of the isomer "**b**" seems to be unfavorable, perhaps due to the bulky substituents in the amine moiety for the ligands **2**–**5**.

Orthoplatination was achieved only for ligands **2** and **3**, by reacting with $[Pt(\mu\text{-}Cl)(\eta^3\text{-}C_4H_7)]_2$, which leads to the formation of chloroplatinated compounds $[PtL]_4$, **2c** and **3c**, in very good yield, due to its η^3-η^1 isomerization [30]. However, reaction of ligand **4** or **5** with $[Pt(\mu\text{-}Cl)(\eta^3\text{-}C_4H_7)]_2$ afforded the dinuclear and not cyclometallated complexes, complexes $[Pt(p\text{-is.TSCN-R})(\mu\text{-}Cl)]_2$ in which the thiosemicarbazone acts as a NS-bidentate chelate ligand.[2] This type of dinuclear chloro-bridged complex has been reported as the first step of the cyclometallation process [35]. Although it is known that the dinuclear chloro-bridged complexes tends to yield the tetranuclear unit [35,36], this transformation has not been observed for complex **4c**[3] and only a low yield of complex **5c** was obtained. This fact reveals the difficulty of the cycloplatination process for this kind of ligand, probably due to the steric impediments of a bulky heterocyclic group. A general scheme for the synthesis of palladium and platinum complexes with *p*-is.TSCN-R ligands is shown in Scheme 2.

The tetranuclear complexes obtained, **2a**–**5a** and **2c**–**3c** and **5c** were characterized by the usual techniques. The spectroscopic data for these complexes are consistent with tetranuclear orthometallated units. The mass spectrum irrefutably indicate the tetranuclear unit. IR spectra indicate that each ligand coordinates to the metal (Pd or Pt) via azomethinic nitrogen atom and its thiolic sulfur atom. The NMR data of the complexes also clearly show the cyclometallated moiety of the complexes [33].

2.2. Phenylacetaldehyde thiosemicarbazone derivatives

By comparison with the other TSCN species studied, phenylacetaldehyde thiosemicarbazone [b.TSCN-NH_2], **6**, does not have *para* substituents in the phenyl group and also has a methylene –(CH_2) group between the phenyl and the TSCN moiety.

In general, the reactivity of ligand **6** differs from the other ligands **1**–**5**. For instance, [b.TSCN-NH_2] does not afford orthometallated complexes under the same reaction conditions already described for ligands from **1** to **5**. The incorporation of a CH_2 group tends to make the isolation of the tetranuclear complex more difficult and it makes this ligand problematic in orthometallation reactions. The tetranuclear complex was finally isolated from a monomer complex (as discussed in the following section) but no orthometallation is observed in this complex. These observations clearly demonstrate how changing the distance from the phenyl group to the TSCN moiety changes the final coordination of the metal atom. Discussion of the reactivity of [b.TSCN-NH_2] is presented in Sections 2.2 and 3.1. Synthesis pathways for [b.TSCN-NH_2] are shown in Scheme 3.

The tetranuclear complex derived from b.TSCN-NH_2, was finally isolated from the monomer **6i** which is described in Section 4.1. A chloroform solution of complex **6i** with stirring for three days at room temperature, afforded a precipitate of the complex **6j** in low yield [63]. The tetranuclear structure of complex **6j** is confirmed by its FAB mass spectrum. This conversion from monomer to polynuclear complex has been observed to occur with some thiosemicarbazones [14], and related ligands [67] and can be attributed to the strong Lewis-basic nature of the sulfur lone pair electrons and the spatial arrangement of such pairs [64]. No platinum tetranuclear complex has been isolated or found in studies of b.TSCN-NH_2.

3. Dinuclear complexes

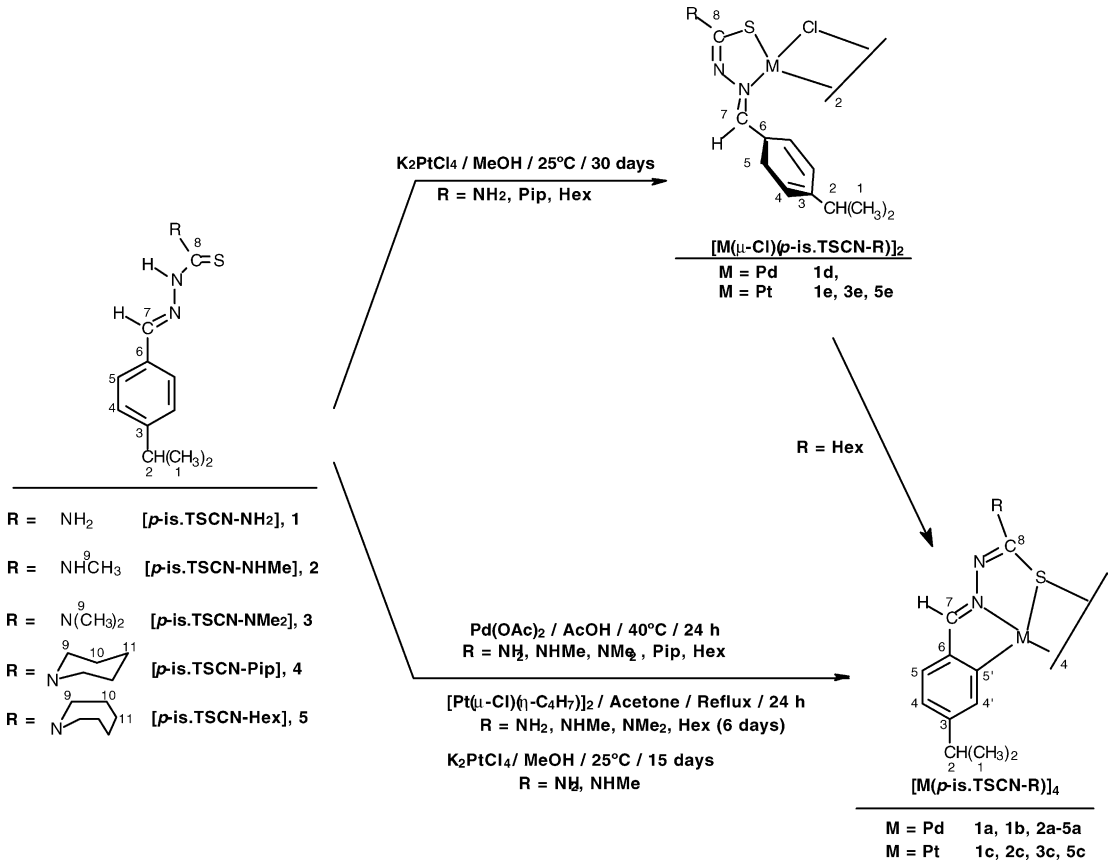
Chlorine bridging atoms hold the two metal atoms together in the palladium and platinum dinuclear complexes. No orthometallation is observed in any of the dinuclear complexes.

3.1. p-Isopropylbenzaldehyde thiosemicarbazone derivatives

Ligand **1** acts as bidentate ligand (S, N donor) in the dinuclear derivatives derived from *p*-is.TSCN-NH_2. However,

[2] Which has an electrophilic character and moreover can increase its co-ordinative unsaturation level.

[3] In the several attempts of reaction of ligand **4** with different platinum salts, there was no evidence for the presence of complex **4c**.



Scheme 3. Detailed synthesis pathway for Pd and Pt complexes with the ligands from **1** to **5**. Reproduced with permission from J. Inorg. Biochem. 1999, 75, 293–301. Copyright 1999 Elsevier.

ligand **1** is potentially a tridentate ligand. The basic reactions to afford cyclometallated complexes (of the carbon donor type) have been described in detail [68], the cyclometallation process involves first coordination of the metal to a donor atom present in the system (N coordination in particular) [69]. This may explain why cyclometallation takes place when $\{Pt(p\text{-is.TSCN-NH}_2)(\mu Cl)\}_2$ is stirred for 15 days in MeOH solution, and why the tetranuclear complex **1c** is isolated. The dinuclear bridge complexes represent the previous step (of intramolecular activation) to the cyclometallation of the thiosemicarbazone (see Scheme 2).

3.1.1. Pd and Pt complexes with p-isopropylbenzaldehyde thiosemicarbazone p-is.TSCN-NH$_2$

The dinuclear complexes $\{M(p\text{-is.TSCN-NH}_2)(\mu Cl)\}_2$, **1d** for M = Pd(II) and **1e** for M = Pt(II), were prepared by reaction of K_2PdCl_4 and K_2PtCl_4 respectively with the ligand *p*-is.TSCN-NH_2 **1**. Both complexes were characterized by the usual techniques. The analytical data agree with the stoichiometry $[MC_{11}H_{14}N_3SCl]$ and the IR spectra show coordination of the thiol (form II, Scheme 1) and imine group [32,55], but the ν(M–Cl) vibration are the most representative, being typical of chloro-bridged complexes [70,71]. The ^{1}H NMR spectra of both complexes confirm the coordination of the ligand in the thiol arrangement and the resonance of the AA′BB′ set of signals for the phenyl group are consistent with the absence of an orthometallated system.

3.1.2. Pd and Pt complexes with p-isopropylbenzaldehyde N-protected thiosemicarbazones p-is.TSCN-R$_2$

Any of the TSCNs studied can generate tetranuclear orthometallated complexes and the dinuclear chloro-bridged complexes are also formed as a intermediate step in affording the final product. Only two of these complexes were completely purified and characterized namely $\{Pt(p\text{-is.TSCN-NMe}_2)(\mu Cl)\}_2$, **3e** and $\{Pt(p\text{-is.TSCN-NHex})(\mu Cl)\}_2$, **5e**, and in both the TSCN acts as a bidentate ligand [34,63].

3.2. Phenylacetaldehyde thiosemicarbazone derivatives

Dinuclear and monomeric derivatives were isolated in reactions intended to produce tetranuclear orthometallated complexes with b.TSCN-NH_2, When the reagent is $Pd(AcO)_2$, the resulting compound is a dinuclear acetate bridged complex. However, reaction with either K_2PdCl_4 or K_2PtCl_4 produces monomer complexes. The dinuclear chloride bridge derivative could only be isolated by metathesis reaction of the dinuclear acetate bridged complex with NaCl. No orthometallation derivatives were observed for these attempts in any case.

3.2.1. Pd complexes with phenylacetaldehyde thiosemicarbazone [b.TSCN-NH_2]

b.TSCN-NH_2 ligand with $Pd(AcO)_2$ and acetic acid as solvent leads to precipitation of a yellow acetate-bridged solid $\{Pd(b.TSCN\text{-}NH_2)(\mu AcO)\}_2$, **6h** insoluble in most of organic solvents, except DMSO [63]. IR data show the presence of two new bands typical of ν(C=O) stretching frequencies from the bridged acetate groups. The *trans* arrangement of the ligands in the complex is inferred from the fact that the acetate bridge methyl groups appear as only one singlet at 1.8 ppm (the *cis* arrangement of the ligands in the complex shows the methyl groups appearing as two singlets). The lack of the NH signal in the ^{1}H NMR spectra demonstrate that the TSCN is deprotonated in this complex and acts in its thiolic form (form II, Scheme 1). Coordination through the nitrogen is confirmed not only by the strong downfield change in the chemical shift of the CH_2 group, which links the phenyl group, but also from the change observed in the multiplicity of these protons. The CH_2 protons become non equivalent protons, contrary to the same protons in the free ligand. The acetate groups can force the two square planes of each palladium to have a relatively small dihedral angle, resulting in the molecule adopting a non-planar open-book structure [72]. This kind of structure has been of special interest in the cyclometallation process [27], and the "open book" disposition [73] of this structure may be the reason for the steric impediments around CH_2 in the structure which produces the different multiplicity.

Dinuclear complexes, which are linked by two bridging acetate groups, can easily afford the corresponding unfolded chloro-bridged complexes by metathesis with NaCl [27]. The acetate–chlorine exchange reaction of the $\{Pd(b.TSCN\text{-}NH_2)(\mu AcO)\}_2$ complex with NaCl leads to the corresponding chloro-bridged complex in which the analytical data are in agreement with the molecular formula: $\{Pd(b.TSCN\text{-}NH_2)(\mu Cl)\}_2$, **6d**. The IR spectrum of the complex is consistent with the chloro-bridged structure. The methylene group $-CH_2-$ in the ^{1}H NMR spectra appear as equivalent protons [63], which suggests that the structure of the complex should be unfolded where the methyl protons do not find steric impediments.

Attempts to obtain crystals of the acetate-bridged complex from DMSO solution causes bridge splitting, and affords mixtures difficult to deal with. This fact, together with the low insolubility of these complexes, becomes an obstacle to the suitability for reproducible cytotoxicity studies.

4. Mononuclear complexes

4.1. Phenylacetaldehyde thiosemicarbazone derivatives

As a part of our program directed to the synthesis of TSCN compounds b.TSCN-NH_2 is valuable to use as a cisplatin structural model ML_2X_2. The antitumor activity of this model depends on the nature of the inert group L, and the leaving group X (which generates the active specie, MLn^{2+}). The b.TSCN-NH_2 monomer complexes offer potential leaving groups that could afford active species.

The importance of the cisplatin model is that the DNA species provide several nucleophilic centers to the active MLn^{2+} to which it may bond forming different DNA adducts, resulting in specific geometric dispositions.

Monomer complex derivatives from b.TSCN-NH_2 were synthesized in two different ways: bridge cleavage reactions with the corresponding acetate-bridged complex as starting material and the direct synthesis between b.TSCN-NH_2 and the desired metal salt. The ligand b.TSCN-NH_2 is bidentate in both structures of general formulae $M(b.TSCN\text{-}NH_2)Cl_2$, where TSCN can act as protonated or deprotonated ligand (see Scheme 3).

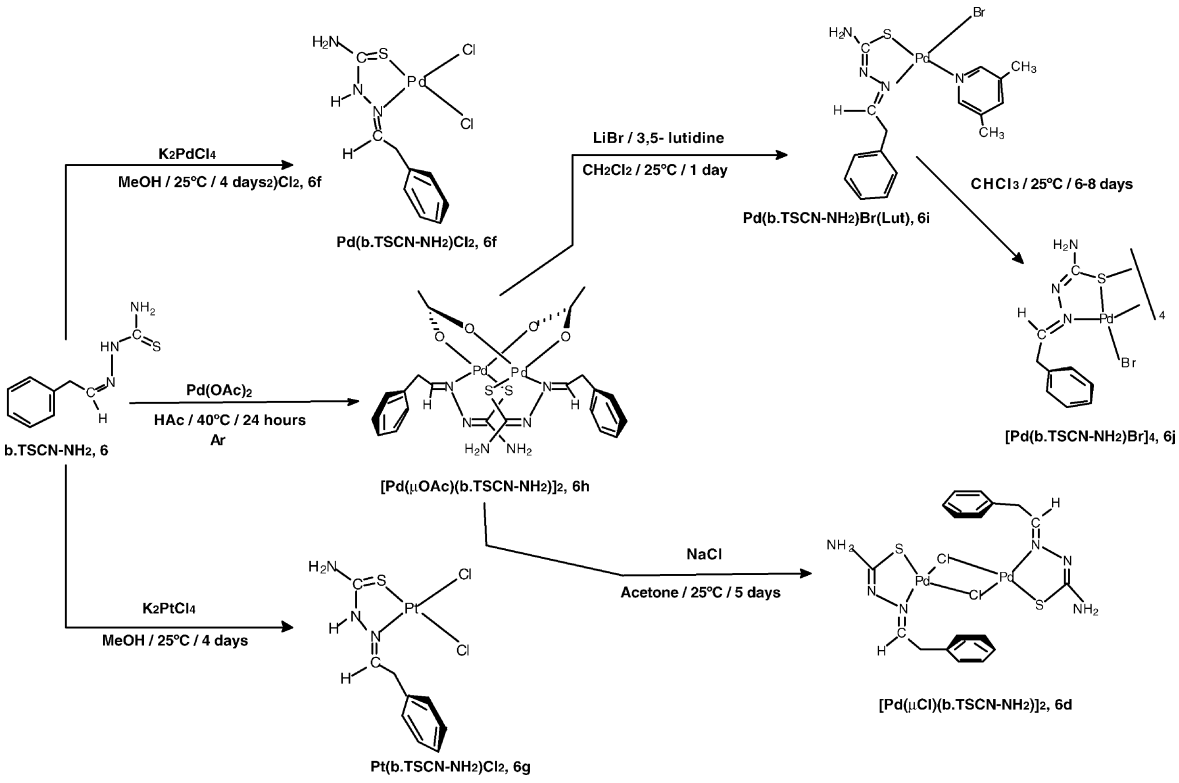
4.1.1. Pd complexes with phenylacetaldehyde thiosemicarbazone b.TSCN-NH_2

The reaction of the dinuclear acetate complex **6h** with bases such as 3, 5-lutidine afforded a monomer complex $\{Pd(b.TSCN\text{-}NH_2)Br(Lut)\}$ **6i**. We have been studying the reactivity of acetate dinuclear complexes to afford monomers [27,58], and in every case the addition of bases leads to cleavage of the acetate-bridge of the complex. We were especially interested in this monomer complex formation because the unfolded structure of the monomer will make the methylene protons equivalent again as in the starting material ligand **6**. This fact provides further support for the folded acetate dinuclear complex structure in **6h**. On the other hand, the thiolic form (form II, Scheme 3) of the TSCN which is deprotonated in this monomer, only allows one possible leaving group in the complex structure, $\{Pd(b.TSCN\text{-}NH_2)Br(Lut)\}$. So the complex **6i** will afford species such as $[M(TSCN)Base]^+$ having one less active position against the DNA compared with the cisplatin model and its active species MLn^{2+}.

Monomer complexes where b.TSCN-NH_2 acts in its thionic form (form I, Scheme 3) can also be prepared by simple treatment of b.TSCN-NH_2 with a metal salt using MeOH as a solvent. Equivalent amounts of b.TSCN-NH_2 and K_2PdCl_4 led to the formation of general formulae $Pd(b.HTSCN\text{-}NH_2)Cl_2$, **6f**. The not deprotonated form (form I, Scheme 3) of the TSCN allows comparison with the cisplatin model, as will be discussed below [74].

4.1.2. Pt complexes with phenylacetaldehyde thiosemicarbazone b.TSCN-NH_2

Equivalent amounts of b.TSCN-NH_2 and K_2PtCl_4 led to the formation of a complex with the general formulae $Pt(b.TSCN\text{-}NH_2)Cl_2$, **6g**. This complex is the platinum analogue of complex **6f** discussed in the paragraph above. In principle, compounds **6f**, **6g** and cisplatin represent a good model for comparison (see structures Scheme 4) to establish a good correlation of structure and activity. Two ν(Pt-Cl) stretching vibrations are observed as with the dinuclear complexes, but a mononuclear structure is proposed because the



Scheme 4. Scheme of [b.TSCN-NH_2] reactivity with Pd and Pt salts.

stretching frequencies are quite different [70] and the ligand is clearly not deprotonated as shown by the NMR spectra.

5. Cytotoxic activity of the complexes in cisplatin sensitive and resistant tumor cell lines

Our interest in searching for systems with antitumor activity and also in finding structure–activity relationships, led us to evaluate each of the complexes discussed in this review and analyze the differences in antitumor activity. The present study is based upon the following facts.

1. Thiosemicarbazones exhibit biological activity against parasites [1], several types of tumors [4,75] and hypoxic selectivity [76], such properties being more developed when a metal is present. *p*-is.TSCN-NH_2 represents an attractive starting point due to its previously established biological activity.
2. Orthometallated complexes display high antitumor activity. Different optical isomers of these compounds show different levels of antitumor activity [30,31].
3. Different substituents on the ligand modify the reactivity of the resulting complexes. For instance, it is believed that the presence of at least one NH group in the platinum compounds may be a key to the interaction of the compounds with the cellular components [77].
4. A number of studies have shown that thiols, especially glutathione (GSH), represent a significant cause of resistance to cisplatin [78]. Attempting to decrease inactivation by intracellular thiol concentration, we thought that introducing S-donor ligands in the complex structure might reduce such inactivation and afford complexes with a much lower nephrotoxicity effect [79].

5.1. Tetranuclear complexes

IC_{50} values (compound concentration that produces 50% of cell death) were calculated for the tetranuclear complexes derived from the ligands **1**–**5** against several tumor lines both sensitive to *cis*-DDP (Jurkat, Hela, 3T3, Pam 212), and resistant to *cis*-DDP (Pam-ras) (Table 1).[4] The lower solubility of the tetranuclear complex **6a** rendered the biological assays for this complex not viable.

The results reveal that our predictions regarding the potential antitumor activity were remarkably accurate, especially for derivatives derived from *p*-is.TSCN-NH_2 which appear to be the most promising of the complexes discussed here as a potential drug [33,36,80].

5.1.1. Cytotoxicity values of the TSCN-NH_2 derivatives

In general, complex **1a** is more active than its isomer **1b**. Moreover, the platinum analogue **1c** shows an intermediate

[4] Jurkat are acute T-cell leukemia cell lines, Hela are cervix epithelial carcinoma cell lines, NIH 3T3 are transformed murine fibroblasts, Pam (murine keratinocytes) and Pam-Ras (murine keratinocites transformed with the H-ras oncogene and resistant to cisplatin).

Table 1
IC_{50} values obtained against several tumor and normal cell lines for the tetranuclear complexes derived from *p*-is.TSCN-NR: **1**–**5**

IC_{50} (μM) ± S.D.						
	Cell lines					
	HL-60	JURKAT	HeLa	3T3	Pam-ras	Pam 212
p-is.TSCN-NH_2, **1**	87 ± 0.6	9 ± 0.2	78 ± 3	97 ± 5	65 ± 2	88 ± 6
[Pd(*p*-is.TSCN-NH_2)]$_4$, **1a**	28 ± 1	7 ± 0.3	4 ± 0.1	3 ± 0.2	5 ± 0.4	8 ± 0.3
[Pd(*p*-is.TSCN-NH_2)]$_4$, **1b**	9 ± 0.2	6 ± 0.3	45 ± 3	32 ± 0.6	37 ± 3	150 ± 9
[Pt(*p*-is.TSCN-NH_2)]$_4$, **1c**	20 ± 0.2	7 ± 0.5	20 ± 0.3	21 ± 0.3	9 ± 0.6	30 ± 3
p-is.TSCN-NHMe, **2**	19 ± 0.9	10 ± 0.8	84 ± 4	87 ± 3	64 ± 3	106 ± 4
[Pd(*p*-is.TSCN-NHMe)]$_4$, **2a**	22 ± 2	13 ± 1	86 ± 3	91 ± 5	62 ± 2	109 ± 6
[Pt(*p*-is.TSCN-NHMe)]$_4$, **2c**	17 ± 1	10 ± 0.8	82 ± 4	87 ± 3	58 ± 2	101 ± 7
p-is.TSCN-NMe_2, **3**	27 ± 3	20 ± 0.9	88 ± 4	102 ± 5	89 ± 7	123 ± 9
[Pd(*p*-is.TSCN-NMe_2)]$_4$, **3a**	29 ± 1	25 ± 3	92 ± 3	107 ± 4	96 ± 7	134 ± 7
[Pt(*p*-is.TSCN-NMe_2)]$_4$, **3c**	30 ± 1	27 ± 2	96 ± 6	89 ± 3	73 ± 3	113 ± 6
p-is.TSCN-Pip, **4**	15 ± 1	12 ± 0.5	80 ± 4	95 ± 3	72 ± 5	94 ± 5
[Pd(*p*-is.TSCN-Pip)]$_4$, **4a**	10 ± 0.7	9 ± 1	73 ± 3	94 ± 1	68 ± 2	90 ± 3
p-is.TSCN-Hex, **5**	24 ± 1	15 ± 2	92 ± 4	102 ± 5	81 ± 3	99 ± 6
[Pd(*p*-is.TSCN-Hex)]$_4$, **5a**	27 ± 2	18 ± 0.7	87 ± 3	104 ± 2	76 ± 2	104 ± 5
CDDP	7 ± 0.1	41 ± 0.7	7 ± 0.5	35 ± 0.7	160 ± 0.7	164 ± 0.8
Etoposide					136 ± 10	180 ± 12
Adriamicyn					156 ± 11	150 ± 5
Taxol					10 ± 0.4	8 ± 0.4

Table 2
Complexes referred in this review and reference therein

Compound	References	Compound	References
[Pd(*p*-is.TSCN-NH_2)]$_4$ **1a**	[33,35]	[Pd(*p*-is.TSCN-NHex)]$_4$ **5a**	[32]
[Pd(*p*-is.TSCN-NH_2)]$_4$ **1b**	[31,34]	[Pt(*p*-is.TSCN-NHex)]$_4$ **5c**	[32]
[Pt(*p*-is.TSCN-NH_2)]$_4$ **1c**	[31,34]	[Pt(*p*-is.TSCN-NMe_2)(μ-Cl)]$_2$ **3d**	[32]
[Pd(*p*-is.TSCN-NH_2)(μ-Cl)]$_2$ **1d**	[33,34]	[Pt(*p*-is.TSCN-NPip)(μ-Cl)]$_2$ **4d**	[32]
[Pt(*p*-is.TSCN-NH_2)(μ-Cl)]$_2$ **1e**	[33,34]	[Pd(b.TSCN-NH_2)(μCl)]$_2$ **6d**	[32]
[Pd(*p*-is.TSCN-NHMe)]$_4$ **2a**	[34]	Pd(b-HTSCN-NH_2)Cl_2 **6f**	[70]
[Pt(*p*-is.TSCN-NHMe)]$_4$ **2c**	[32]	Pt(b-HTSCN-NH_2)Cl_2 **6g**	[70]
[Pd(*p*-is.TSCN-NMe_2)]$_4$ **3a**	[32]	[Pd(b.TSCN-NH_2)(μAcO)]$_2$ **6h**	[63]
[Pt(*p*-is.TSCN-NMe_2)]$_4$ **3c**	[32]	Pd(b-TSCN-NH_2)Br(Lut) **6i**	IP
[Pd(*p*-is.TSCN-NPip)]$_4$ **4a**	[32]	[Pd(b-TSCN-NH_2)Br]$_4$ **6j**	IP

cytotoxicity relative to that of isomers **1a** and **1b**, and also has the best therapeutic index (T.I.: 4.05).[5] The high cytotoxic values for the TSCN complexes were even higher than the clinically used drugs etoposide and adriamycin in the cisplatin-resistant cell line. Etoposide is one of the drugs currently used for the treatment of brain tumors [81].

Thus, from the anticancer screening data (see Table 2), complexes **1a**, **1b** and **1c** are potential antitumor agents particularly when one considers that they are active in tumor cells in which current clinical drugs such as *cis*-DDP and etoposide have poor or moderate cytotoxic activity. This fact indicates that these thiosemicarbazone complexes should have a biochemical mechanism of action different from that of *cis*-DDP.

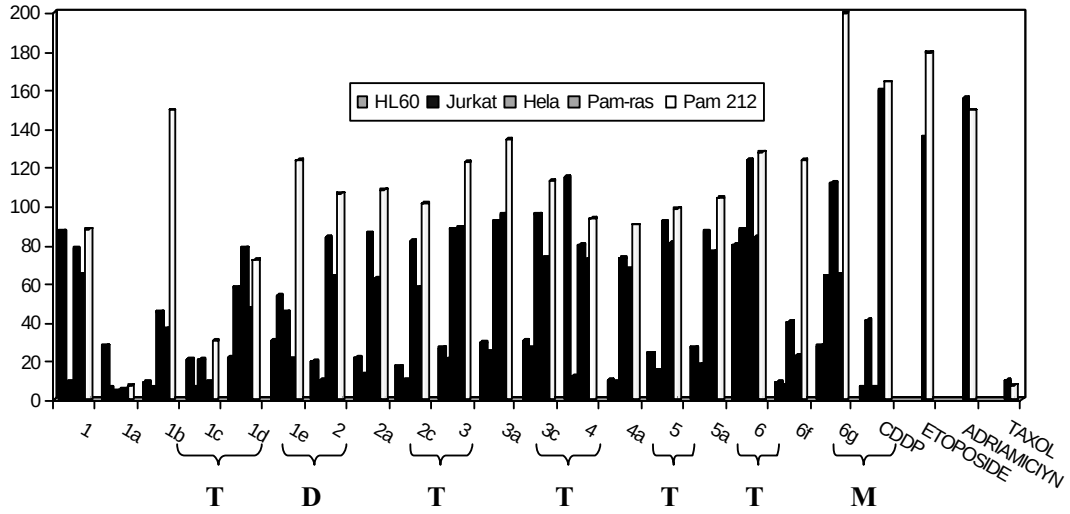
[5] T.I.: The Therapeutic Index is the comparison between the cytotoxicity of the compounds towards normal Pam cells versus transformed Pam-Ras cells.

5.1.2. Cytotoxicity values of the TSCN-R derivatives

Treatments of cisplatin resistance cell lines with the tetranuclear palladium and platinum complexes derived from ligands **2** to **5** have generally resulted in a weaker response than ligand **1** derivatives. However, despite the lower cytotoxic values in these lines, the tetranuclear complexes are more active than cisplatin and other clinically used drugs (see Scheme 5). Cytotoxicity values from platinum derivatives show a similar tendency to the **1c** complex, all of them being higher than for the palladium derivatives [34].

5.2. Dinuclear complexes

Dinuclear complex capacity to inhibit tumor cell growth has also been tested. Because of the higher toxicity values for tetranuclear derivatives of *p*-is.TSCN-NH_2, we elected to check the dinuclear complexes derived from ligand **1**. Scheme 5 includes the IC_{50} values of dinuclear complexes **1d** and **1e**. The dinuclear complexes show cytotoxic values higher than cisplatin in the Pam-ras cell lines. The



Scheme 5. Comparative chart of the cytotoxicity values from TSCN's discussed in this review. T: tetranuclear complexes, D: dinuclear complexes, M: mononuclear complexes.

antitumoral response of these dinuclear complexes is not as high as for the tetranuclear derivatives, but is still higher than the clinically used drugs taxol and adriamicine. Moreover, the platinum derivative shows a extraordinarily high therapeutic index value (IT:5.9).

5.3. Mononuclear complexes

The ability of mononuclear complexes described in Section 3 to inhibit tumor cell growth was tested under the same conditions as the tetranuclear and dinuclear complexes [74]. Complexes **6f** and **6g** are also more active than *cis*-DDP in the Pam-ras cell line. Both compounds show a good cytotoxicity therapeutic index, being notably higher for the platinum derivative. These results indicate that monomer complexes may also be considered as potential antitumor agents because they show not only a good therapeutic index, but also high IC_{50} values in tumor cells in which the antitumor drug *cis*-DDP has poor activity.

6. "In vitro" DNA binding studies of the complexes

The cytotoxic assays discussed in Section 4 clearly indicate that palladium and platinum thiosemicarbazone derivatives should have a biochemical mechanism of action different from that of cisplatin. It is very well known that DNA is the main pharmacological target of metal-based drugs, considering that we have analyzed the interaction of the most representative complexes of every group with DNA.

6.1. Tetranuclear and dinuclear complexes

6.1.1. Studies of the interaction with the secondary structure of DNA

Circular dichroism is a convenient method for monitoring the secondary structure of nucleic acids in solution, and studying the conformation changes of the DNA by comparison with the CD spectra already measured for the known DNA conformation [82]. We analyzed the effect of binding for tetranuclear and dinuclear complexes by comparison of the control DNA CD spectra with the CD spectra of compound:DNA complexes [33]. The evaluation of the spectra and the wavelength at which the maximum and minimum values of elipticity [θ] occur in the DNA incubated with the tetranuclear complexes **1a**, **1b** and **1c**, indicate that cyclopalladated compounds produce different conformational changes on DNA secondary structure from those induced by the cycloplatinated compound **1c**. A "tailing effect" appears at 315 nm in palladium:DNA complexes and also for platinum:DNA complexes [33]. This "tailing effect" is absent in cisplatin:DNA complexes and is attributed to the formation of DNA aggregates which may due to DNA interhelical cross-links formed by the metal [83]. To find out whether the "tailing effect" observed in the thiosemicarbazone complexes with DNA is due to interhelical cross-link formation, we analyzed the interaction of the higher cytoxicity complexes (which are the ligand **1** derivatives) with linear and supercoiled plasmid DNA. These experiments were carried out to detect the presence of DNA aggregates after DNA melting (thermal denaturation).

Fig. 4 shows the pattern of the linear double strand pBR322 DNA bands and the pBR322 DNA bands of the tetranuclear complexes: pBR322 DNA compounds (TC:DNA compounds) after melting (see caption of Fig. 4). As expected, melted native pBR322 DNA migrates as a smear which corresponds to a single strand DNA form (lane 2). The melted TC:DNA compounds also migrate as a smear of single DNA strand (middle of the lanes 3–11). Surprisingly, a fraction of high molecular weight DNA bands retained in the agarose wells were observed at all the periods of incubations of pBR322 with the tetranuclear complexes (top of the lanes 3–11). This clearly indicates that those

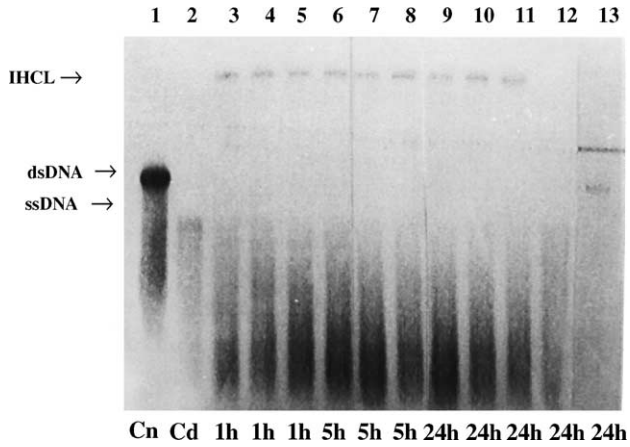


Fig. 4. Pattern of single and double-stranded DNA after melting of compound **1a**: pBR322 DNA (lanes 3, 6 and 9), compound **1b**: pBR322 DNA (lanes 4, 7 and 10), and compound **1c**: pBR322 complexes (lanes 5, 8 and 11) formed after incubation of linear pBR322 plasmid DNA with compounds **1a**, **1b** and **1c** at $r_i = 0.01$. Cn, native DNA; Cd, denaturated DNA; IHCL, interhelical cross-linked; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; **1**: *p*-is.TSCN-NH_2 (lane 12); DDP: cisplatin (lane 13). Reproduced with permission from J. Med. Chem. 41 (1998) 1399–1408. Copyright 1998 Am. Chem. Soc.

bands of unmelted DNA were forming aggregates due to the formation of interhelical cross-links between the compounds and several double-stranded DNA molecules. Those extensive interhelical CLs of the DNA fragments result in a DNA mixture of high molecular weight unable to penetrate through the agarose gel matrix [83]. The binding effect of dinuclear complexes on the DNA secondary structure shows very similar consequences to the tetranuclear complexes. Fig. 5 shows how compounds constituted by dinuclear complex:DNA compounds (DC:DNA compounds) after melting are retained in the agarose gel because a fraction of high

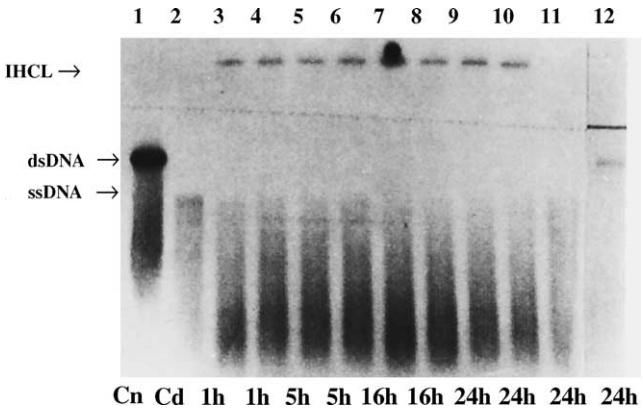


Fig. 5. Pattern of single- and double-stranded DNA after melting of thiosemicabazone complexes: pBR322 DNA compounds, formed after incubation of linear pBR322 plasmid DNA with complex **1d** (lanes 4, 6, 8 and 10) and complex **1e** (lanes 3, 5, 7 and 9) at $r_i = 0.1$. Cn, native DNA; Cd, denaturated DNA; IHCL: interhelical cross-linked; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; **1**: *p*-is.TSCN-NH_2 (lane 12); DDP: cisplatin (lane 13). Reproduced with permission from J. Inorg. Biochem. 75 (1998) 293–301–1408. Copyright 1999 Elsevier.

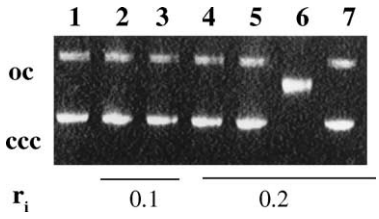


Fig. 6. Changes in the electrophoretic mobility of the ccc forms and oc forms of supercoiled pBR322 plasmid DNA after 24 h of incubation with compounds **1c** (lanes 2 and 4) and **1d** (lanes 3 and 5). oc: open circular form; ccc: covalently closed circular form; unmodified control DNA (lane 1). and *p*-is.TSCN-NH_2 (lane 7) and cisplatin (lane 6) at $r_i = 0.2$.

molecular weight has been formed (see caption of Fig. 5). Those aggregates might also be caused by interhelical CLs between several double-stranded linear DNA molecules. In both figures (Figs. 4 and 5), neither cisplatin nor ligand **1** produces any detectable DNA band at the top of the gel as an indication that they are unable to form DNA interhelical CLs. On the other hand, a DNA band corresponding to double strand unmelted is observed in both figures (lane 12 Fig. 4, and line 12 Fig. 5) for cisplatin. Cisplatin induces the formation of some interstrand CLs in pBR322 DNA [84].

6.1.2. Studies of the interaction with the tertiary structure of DNA

The effect of the binding of metallic complexes on DNA tertiary structure was determined by their ability to alter the electrophoretic mobility of the covalently closed circular (ccc) and the open circular form (oc) of the pBR322 plasmid DNA [30,36]. For instance, Fig. 6 shows the electrophoretic mobility pattern of the native supercoiled pBR322 plasmid, the pBR322 DNA incubated with the tetranuclear complex **1c** and with dinuclear complex **1e**. The tetranuclear complex and dinuclear complex incubation do not vary the mobility of the ccc and oc forms of pBR322 DNA. However, as expected, incubation of pBR322 DNA with cisplatin leads to delay in the mobility of the ccc forms and to an increase in mobility of the oc form. Cisplatin induces unwinding of the DNA superhelix resulting in a loss of negative supercoils and, therefore, a delay in electrophoretic mobility. Moreover, cisplatin also induces a DNA "shortening" effect leading to an increase in electrophoretic mobility [85].

Altogether, the data obtained from the analysis of the interaction of the tetranuclear and dinuclear complexes with DNA indicate that these tetranuclear complexes share a unique feature, namely, the formation of DNA interhelical CLs. Due to the fact that these compounds are active in *cis*-DDP-resistant cell lines where, on the other hand, *cis*-DDP is unable to form DNA interhelical CLs, it is possible that part of the biochemical mechanism of action of the tetranuclear cyclometallated complexes and the dinuclear complexes may be due to DNA interhelical CLs formation. In fact, it has been previously reported that bis-Pt complexes, which also form DNA interhelical CLs, are active in tumor cells resistant to *cis*-DDP [86].

Since the cytotoxic activity of the tetranuclear complexes is higher than the dinuclear complexes, and *cis*-DDP does not form interhelical CLs, we think that these polynuclear compounds preserve the oligomeric structure, and produce a different type of DNA adduct (interhelical CLs) than cisplatin. Taking into account that Pd or Pt atoms adopt a square-planar structure in the complexes, we postulate a nucleophilic attack from some nitrogen atoms of the bases (i.e., N7/N3 of guanine residues of different DNA molecules) at two or more metal centers of the cyclometallated tetramer without breaking up the cluster. The preservation of the clustered structure would be in agreement with the results obtained in the present paper indicating that tetranuclear compounds are more active than dinuclear and both form DNA aggregates by means of DNA interhelical CLs.

6.2. Mononuclear complexes

6.2.1. Studies of the interaction with the secondary structure of the DNA

The monomer complex interactions with linear plasmid DNA have also been analyzed.

Fig. 7a shows the pattern of DNA bands of mononuclear complexes: pBR322 DNA compounds (MC:DNA compounds) after melting. Just as expected, it was observed that control melted pBR322 DNA migrates as a DNA band which corresponds to the single-stranded DNA form and that the control unmelted DNA migrates as a DNA band of higher molecular weight which corresponds to double-stranded linear pBR322 DNA (see caption of Fig. 7). However, two DNA bands are observed in the melted MC:DNA compounds formed. The high mobility band corresponds to a melted single-stranded DNA and the other one of lower mobility corresponds to unmelted double-stranded DNA. The band of unmelted double-stranded DNA is indicative of formation of DNA interstrand CLs [86]. Cisplatin induces also the formation of DNA interstrand CLs but the double-stranded unmelted DNA represents only about 5% of the total DNA while in the same time, mononuclear complex (**6f** and **6g**) unmelted double-stranded DNA represents 40 and 90% of the total DNA, respectively.

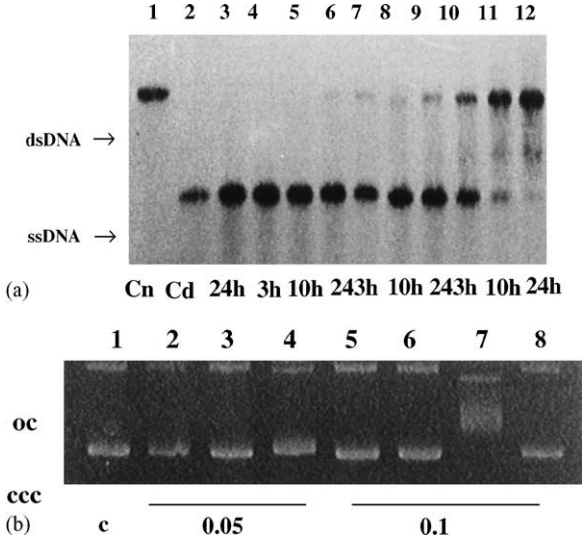


Fig. 7. (a) Pattern of single- and double-stranded DNA after melting of thiosemicabazone complexes: pBR322 DNA compounds, formed after incubation of linear pBR322 plasmid DNA with ligand **6** (lane3), cisplatin (lanes 4–6), complex **6f** (lanes 7–9), and complex **6g** (lanes 10–12) at $r_i = 0.05$. Cn, native DNA; Cd, denaturated DNA; IHCL: interhelical cross-linked; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA Reproduced with permission from J. Inorg. Biochem. 70 (1998) 2117–2123. Copyright 1998 Elsevier. (b) Changes in the electrophoretic mobility of the ccc forms and oc forms of pBR322 plasmid DNA after 24 h of incubation with complexes **6f**, **6g** and *cis*-DDP at $r_i = 0.05$ (lanes 2, 3 and 4, respectively) and 0.1 (lanes 5–7, respectively). Compounds **6** at $r_i = 0.1$ is in line 8. oc: open circular form; ccc: covalently closed circular form; c: unmodified control DNA. Reproduced with permission from J. Inorg. Biochem. 70 (1998) 2117–2123. Copyright 1998 Elsevier.

6.2.2. Studies of the interaction with the tertiary structure of the DNA

The effect of binding of mononuclear complexes on DNA tertiary structure was determined by the capacity of the compounds to alter the electrophoretic mobility of the covalently closed circular (ccc) and open circular (oc) forms of supercoiled pBR322 plasmid DNA. Fig. 7b shows that compounds **6f** and **6g** do not alter the electrophoretic mobility of the ccc and oc forms of pBR322 DNA. However, incubation of pBR322 DNA with cisplatin leads to a delay in the mobility of the ccc forms and to an increase in mobility of the oc form. Since mononuclear complexes do not alter the electrophoretic mobility of supercoiled DNA, it is most likely that the adducts formed by these compounds on DNA may stabilize, rather than unwind, supercoiled DNA. Thus, the absence of an unwinding effect in supercoiled DNA supports previous evidence indicating that monomer complexes form DNA interstrand CLs that are located at twists of the superhelix. This type of phenomenon has also been observed in the interaction of certain *cis*-Pt analogs with supercoiled plasmids [87].

Altogether, the data obtained from the analysis of the interaction of mononuclear complexes with plasmid DNA indicate that both compounds have an enhanced capacity to form DNA interstrand CLs in comparison to *cis*-DDP. The fact that compound **6g** exhibits higher cytotoxicity and forms more DNA interstrand CLs than compound **6f** may be correlated with the reactivity of palladium which is about 10^5 times higher than that of platinum [84]. Taking into account that compounds **6f** and **6g** have a metallic center in a *cis* configuration as *cis*-DDP but differ from *cis*-DDP in the inert group L, it is likely that steric reasons imposed by the TSCN group may be involved in the enhanced ability of compounds **6f** and **6g** to form DNA interstrand CLs. Thus, that is probably the reason for the higher rate of formation of DNA interstrand CLs by the monomer compounds in comparison with *cis*-DDP, and it may also explain the activity of these compounds in *cis*-DDP-resistant cells.

Table 3
IC_{50} values (μM ± S.D.) obtained against several tumor and normal cell lines for the binuclear and mononuclear complexes derived from **1** and **6** ligands

	Cell lines				
	HL-60	JURKAT	HeLa	Pam-ras	Pam 212
p-is.TSCN-NH_2, 1	87 ± 0.6	9 ± 0.2	78 ± 3	65 ± 2	88 ± 6
[Pd(*p*-is.TSCN-NH_2)Cl]$_2$, **1d**	22 ± 0.5	58 ± 0.1	58 ± 0.1	47 ± 2	72 ± 3
[Pt(*p*-is.TSCN-NH_2)Cl]$_2$, **1d**	30 ± 0.4	54 ± 0.4	45 ± 2	21 ± 0.5	124 ± 0.5
b-TSCN-NH_2, **6**	80 ± 0.7	88 ± 1	124 ± 3	84 ± 1	128 ± 5
Pd(b.HTSCN-NH_2)Cl_2, **6f**	9 ± 0.2	8 ± 0.3	40 ± 2	23 ± 0.1	124 ± 3
Pt(b.HTSCN-NH_2)Cl_2, **6g**	28 ± 28	64 ± 3	112 ± 2	65 ± 1	200 ± 9
CDDP	7 ± 0.2	41 ± 0.2	7 ± 0.5	157 ± 6	164 ± 6
Etoposide				136 ± 10	180 ± 12
Adriamicyn				156 ± 11	150 ± 5
Taxol				10 ± 0.4	8 ± 0.4

7. Apoptosis induction by the thiosemicarbazones derivatives

The cytotoxic assays show that complexes **1b**, **1c** and **1e** display specific cytotoxic activity against Pam-ras cell lines. While the clinical drugs cisplatin, etoposide or adrimicine (which are actually used in tumor treatment [88] associated with H-ras oncogene [89] alterations) are not as specific as any of the thiosemicarbazone complexes mentioned in this review, the thisemicarbazone complexes show much better therapeutic indexes (particularly **1b**, **1c** and **1e** complexes). How these two favorable outcomes are related with the apoptosis induction in Pam-ras cell lines [90,91] (with a low drug concentration) has also been studied [80]. Apoptosis assay results show how a strong DNA ladder indicative of apoptosis is obtained from the DNA of Pam-ras cells treated with the dinuclear and tetranuclear platinum compounds. On the other hand, palladium derivatives show a smear of DNA (instead of a DNA ladder) and the apoptosis quantification values is less than the 10%.

8. Recapitulation of the most relevant data

1. The reactivity of palladium and platinum salt with thiosemicarbazones afforded polynuclear complexes $(ML)_4$. Metal–carbon bonds are found in the structure where the metal is either palladium or platinum, and the ligand is the thiosemicarbazone in its thiolic form. The core of the structure is an eight-member ring of alternating metal and S atoms in a boat conformation.
2. The tetranuclear cyclometallated complexes are the first reported polynuclear complexes with thiosemicarbazone ligands. Different IC_{50} values are found for these tetranuclear complexes, depending on the structure and on the metal. Different isomers of the tetranuclear structures show different IC_{50} values. Forming a metal (palladium and platinum) complex enhances the biological activity of the thiosemicarbazone, and in general platinum derivatives are more cytotoxic that palladium derivatives.
3. The data obtained from the analysis of the interaction of the tetranuclear and dinuclear complexes with DNA indicate that these complexes share a unique feature, namely, the formation of DNA interhelical cross-links. Due to the fact that these compounds are active in *cis*-DDP-resistant cell lines and that, on the other hand, *cis*-DDP is unable to form DNA interhelical cross-links, the biochemical mechanism of action of the tetranuclear and dinuclear compounds may be related with DNA interhelical cross-link formation.
4. Since the cytotoxic activity of the tetranuclear complexes is higher than for dinuclear complexes and *cis*-DDP does not form interhelical cross-links, the maintenance of the oligomeric structure seems to be essential for production of this type of DNA adduct. Nucleophilic attack from some nitrogen atoms of the bases (i.e., N7/N3 of guanine residues of different DNA molecules) could occurs at two or more metal centers of the cyclometallated tetranuclear complexes, without breaking up the cluster.
5. The palladium and platinum mononuclear complexes derived from TSCN can also be considered potential antitumor drugs. Compared with the TSCN polynuclear complexes, a monomer structure produces a different interaction with DNA:interstrand cross-links. The palladium mononuclear complex **6f** in particular has an enhanced capacity to form interstrand cross-links, perhaps due to the higher reactivity of palladium compared to platinum.
6. Comparing the features of the ligands studied, the activity of the complexes may be related to hydrogen bonding interactions with DNA. Evaluating the way that the thiosemicarbazone compounds **1**–**6** differ (Table 3), we see that the higher activity is for ligand **1**, probably because ligand **1** has a NH_2 group free and available for hydrogen bonding with DNA base pairs.

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