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Synthesis, cytotoxic activity and DNA interaction of Pd(II) complexes bearing *N*′-methyl-3,5-dimethyl-1-thiocarbamoylpyrazole

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ABSTRACT

A new series of complexes of general formulae $[PdX_2(tmdmPz)]$ {X = Cl (**1**), Br (**2**), I (**3**), SCN (**4**); tmdmPz = *N*′-methyl-3,5-dimethyl-1-thiocarbamoylpyrazole} have been synthesized and characterized by elemental analysis, molar conductivities, IR, ^1H and $^{13}C\{^1H\}$ NMR spectroscopy. In these complexes, the tmdmPz coordinates to Pd(II) center as a neutral N,S-chelating ligand. The geometries of the complexes have been optimized with the DFT method. Cytotoxicity evaluation against LM3 (mammary adenocarcinoma) and LP07 (lung adenocarcinoma) cell lines indicated that complexes **1**–**4** were more active than cisplatin. The binding of the complexes with a purine base (guanosine) was investigated by ^1H NMR and mass spectrometry, showing that the coordination of guanosine occurs through N7. Electrophoretic DNA migration studies showed that all of them modify the DNA tertiary structure.

1. Introduction

The incorporation of transition metal ions for the design of new chemotherapeutic agents offers a potentially rich and challenging research area [1–8]. The enormous success of *cis*-diamminedichloroplatinum(II) (cisplatin) in clinical practice to treat several types of human malignancies has encouraged vigorous efforts in the development of new metal-based compounds for diagnostic and/or therapeutic purposes. The evident analogy between Pt(II) and Pd(II) coordination stereochemistry has prompted numerous studies on palladium(II) derivatives as new fascinating drug candidates for cancer treatment as well as grow inhibition of pathogenic microorganisms [9–14]. Advances in this sense have been focused on Pd(II) complexes bearing *N,S*-donor chelating ligands, which impels the *cis*-coordination to the more labile ligand (e.g. two chloro groups) [15]. Such approach has demonstrated to be very attractive to synthesize new active Pd(II) derivatives against tumor cell lines and parasites [16]. For instance, Quiroga et al. [17] have synthesized the compound *cis*-$[PdCl_2(N\frown S)]$ (N⌢S = phenylacetaldehyde-thiosemicarbazone) that showed good cytotoxicity towards several tumour cell lines including cisplatin-resistant lines, with promising therapeutic index. This compound displayed an enhanced capacity to form DNA interstrand cross-links in comparison with cisplatin. Within this context, special attention has also been paid to 1-thiocarbamoylpyrazolyl molecules based on their remarkable structural analogy with thiosemicarbazones (Fig. 1).

Besides that, the incorporation of the pyrazole nucleus in the molecular structures of these *N,S*-donor derivatives represents an interesting strategy to design new pharmaceutical candidates as compounds comprising the pyrazole moiety have displayed promising antitumor [18], antimicrobial [19], antiviral [20], anticonvulsant [21], anti-inflammatory [22], antipyretic activities [23].

Recently, Lv et al. [24] have reported that the compound 3-(3,4-dimethylphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide exhibited a potent epidermal growth factor receptor (EGFR) inhibitory activity with IC_{50} of 0.07 μM, comparable to the positive control erlotinib. Such *N,S*-based pyrazolyl compound also showed significant cytotoxicity against MCF-7 tumor cell lines with IC_{50} of 0.08 μM.

1-Thiocarbamoylpyrazolyl derivatives are well known chelating ligands able to coordinate the Pd(II) ion through the sulfur atom and the pyridine-like nitrogen (N2) at pyrazolyl moiety, affording a stable five-membered ring [25]. The chemistry of Pd(II) complexes of 1-thiocarbamoylpyrazolyl ligands has been receiving considerable attention largely because of their pharmacological

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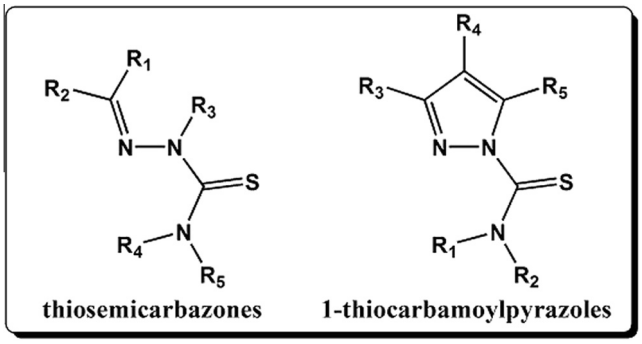


Fig. 1. Structures of thiosemicarbazones and 1-thiocarbamoylpyrazoles.

properties [26–29]. It seems plausible that the combination of 1-thiocarbamoylpyrazolyl ligands with ions like Pd(II), which are able to bind DNA, may produce synergistic inhibition of cancer cells growth. In fact, we have recently reported the synthesis of compounds of the type $[PdX_2(tdmPz)]$ {X = Cl, Br, I, SCN; tdmPz = 3,5-dimethyl-1-thiocarbamoylpyrazole} that showed interesting IC_{50} values towards some murine mammary adenocarcinoma cell lines in the range 20–30 μM, being similar to that observed for cisplatin [30]. These findings have motivated us to prepare new analogous Pd(II) derivatives and investigate their cytotoxic activities as well as their interactions with DNA, a potential pharmacological target.

In the framework of our current research on the coordination and biological chemistry of Pd(II) based compounds [31–36], we describe herein the antitumor evaluation and some DNA interaction studies on compounds of the type $[PdX_2(tmdmPz)]$ {X = Cl (**1**), Br (**2**), I (**3**), SCN (**4**); tmdmPz = *N*′-methyl-3,5-dimethyl-1-thiocarbamoylpyrazole}.

2. Results and discussion

2.1. Synthesis of the complexes

The ligand *N*′-methyl-3,5-dimethyl-1-thiocarbamoylpyrazole (tmdmPz) was synthesized according to the method described by Barik et al. [37]. The preparation route of the new complexes is shown in Fig. 2. Compound **1** is prepared by the displacement of CH_3CN ligands from $[PdCl_2(CH_3CN)_2]$ by the tmdmPz. Compounds **2**–**4** were readily obtained by metathetical reactions of the $[PdCl_2(tmdmPz)]$ (**1**) with salts of the appropriate anions.

All the complexes are solids and decomposed above *ca.* 200 °C. They are insoluble in organic solvents such as acetone, chloroform and methanol but soluble in dmf and dmso. The molar conductivities of complexes **1**–**4** in dmf are between 22 and 38 Ω^{-1} cm^2 mol^{-1}, in agreement with their nonelectrolytic nature [38].

Table 1
Main characteristic IR vibrational bands of the free ligand and the complexes.

Compound	ν (cm^{-1})			
	N–H	CN_{ring}	$CN_{thioamide}$	C=S
tmdmPz	3332 *ms*	1579 *s*	1521 *s*	800 *m*
1	3385 *ms*	1594 *s*	1572 *ms*	770 *w*
2	3392 *ms*	1595 *s*	1574 *ms*	768 *w*
3	3391 *ms*	1593 *s*	1567 *ms*	763 *w*
4	3275 *ms*	1601 *s*	1571 *ms*	776 *w*

s = strong; ms = medium-strong; m = medium; w = weak.

2.2. IR spectroscopy

The main IR vibrational bands of the free ligand and its complexes are listed in Table 1. Selected diagnostic bands, especially those containing considerable ν(CS) or ν(CN) character, showed useful information for determining the coordination mode of tmdmPz in complexes **1**–**4**.

The spectrum of free tmdmPz exhibits a strong band at 1579 cm^{-1}, assigned to the C=N stretching of the pyrazole ring [39], which shifts 15–20 cm^{-1} to higher frequency in the IR spectra of **1**–**4**, being consistent with pyridine ring nitrogen atom coordination. The coordination via the thiocarbonyl sulfur atom is inferred by the shift to lower frequency of the νC=S band from 800 cm^{-1} (free ligand) to 770 (**1**), 768 (**2**), 763 (**3**) and 776 cm^{-1} (**4**). A shift to higher frequency of νCN absorption of the thioamide group (1521 cm^{-1} in the free ligand) rules out the idea of coordination through the nitrogen atom of this group. Moreover, the presence of the νNH band in the complexes confirms the neutral NS donor bidentate character of tmdmPz ligand in **1**–**4**. For complex **4**, the appearance of two ν_{as}SCN bands at 2124 and 2090 cm^{-1} supports the terminal *S*-bonded coordination mode and also the *cis* configuration [40–42].

2.3. NMR spectroscopy

The *N*,*S*-coordination (via pyridine ring nitrogen atom) is also supported by ^{1}H and ^{13}C NMR spectroscopy, which chemical shifts and assignment are summarized in Table 2.

In the NMR spectra of complexes **1**–**4**, the ^{1}H and ^{13}C nuclei of 3-CH_3 group are deshielded as a result of participation of the N2 atom in the coordination. This downfield shift is related to the σ-charge donation from the N(2)-donor to the Pd(II) centre, reducing the electron density at the position-3 near to the metal centre.

It is well known that the M–S bond formation in complexes bearing thioamide-type ligands can be detected on basis of NMR data [43]. Upon *S*-coordination, the C=S bond is weakened causing an upfield of the ^{13}C=S resonance [28]. Simultaneously, the C–N bond order is increased due to an enhancement in π electron

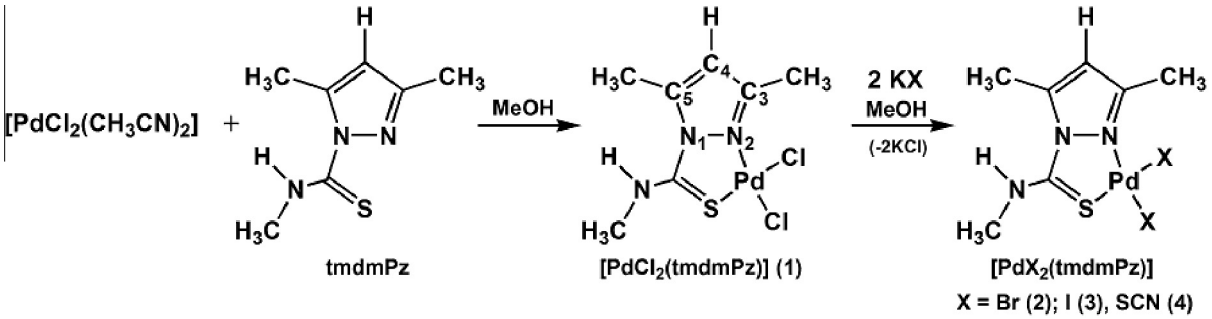


Fig. 2. Synthesis of compounds **1**–**4**.

Table 2
^{1}H and ^{13}C NMR data for the ligand and the complexes **1**–**4** at 298 K, in dmso-d_6, given as ppm, multiplicity, [integration].

Nuclei	Compound				
	tmdmPz	**1**	**2**	**3**	**4**
^{1}H NMR					
3-CH_3	2.18 s [3H]	2.48 s [3H]	2.48 s [3H]	2.56 s [3H]	2.47 s [3H]
H-4	6.16 s [1H]	6.29 s [1H]	6.29 s [1H]	6.34 s [1H]	6.28 s [1H]
5-CH_3	2.63 s [3H]	2.52 s [3H]	2.52 s [3H]	2.59 s [3H]	2.54 s [3H]
N–CH_3	3.04 s [3H]	3.15 s [3H]	3.18 s [3H]	3.20 s [3H]	3.17 s [3H]
NH	10.0 s [1H]	10.0 s [1H]	10.0 s [1H]	10.0 s [1H]	[a]
^{13}C NMR					
3-CH_3	13.19	15.37	15.92	14.83	14.62
5-CH_3	16.52	14.19	14.62	14.58	14.40
N–CH_3	31.86	38.59	38.58	36.03	38.46
C-3	143.75	144.61	144.64	144.48	143.52
C-4	111.37	110.86	110.67	110.67	110.45
C-5	148.60	144.61	144.64	144.48	143.52
C=S	176.61	155.17	[a]	[a]	[a]
SCN	–	–	–	–	118.03

[a] NOT observed; s = singlet.

density in the C–N bond, resulting in a downfield shift of the –NR_2 signals (R = H or organic substituents) [43].

In the ^{13}C{^{1}H} NMR spectra of complexes **1**–**4**, the N–CH_3 signal is found 5–7 ppm downfield compared to the free ligand, indicating that a partial double bond character in the C–N bond was produced upon *N,S*-coordination. The C=S resonance is detected only in the ^{13}C{^{1}H} NMR spectrum of compound **1**, in which it was observed an upfield shift of *ca.* 21 ppm of the C=S resonance, giving a clear evidence of Pd–S bond formation [30]. The additional ^{13}C signal at 118 ppm, observed in the ^{13}C{^{1}H} NMR spectrum of **4**, is assigned to the carbon atom from the *S*-thiocyanato group [44].

2.5. DFT studies

As no single crystal for X-ray diffraction studies could be obtained the structures of the compounds **1**–**4** have been optimized using the algorithm of Berny [45]. The optimized structure of a representative compound, [$PdCl_2$(tmdmPz)] (**1**), together with the electronic HOMO (highest occupied molecular orbital)-LUMO (lowest unoccupied molecular orbital) pictures is depicted in Fig. 3. The calculated geometries and HOMO–LUMO orbitals of complexes **2**, **3**, and **4** are given in the Supplementary material (Figs. S1–S3, respectively).

In all of these compounds, the palladium atom in **1**–**4** lies in a square planar coordination environment made by a chelating *N′*-methyl-3,5-dimethyl-1-thiocarbamoylpyrazole (through the pyridine-like N atom from pyrazole ring and the sulfur atom) and two halide ligands (**1**–**3**) or *S*-thiocyanato (**4**) groups. Except for compound **4**, the pyrazolyl ring and the thiocarboxamide group are essentially coplanar. A selection of calculated bond lengths and angles is shown in Table S1 (Supplementary material). The nitrogen and sulfur donor atoms of the chelating tmdmPz ligand determine a chelating bite angle of 82.5° (**1**), 82.0° (**2**), 82.0° (**3**), and 81.3° (**4**). The bond distances Pd–X were found to be slightly different, reflecting the different *trans* influences exerted by the sulfur and the pyridine-like N atoms. Depending on the anionic ligand (X), the $PdNSX_2$ coordination sphere in **1**–**4** is more or less distorted from the square-planar geometry. The highest distortion occurred in compound **4**, probably due to a combination of electronic effects and steric hindrance introduced by the thiocyanato groups.

It can be observed that, independently of the type of coordinated halide group, the frontier orbitals of **1**, **2**, and **3** exhibit a similar character. The HOMO levels show greater contributions of *p*-like orbitals at the halide atoms, with smaller contributions from *d*-orbitals at the Pd center. The LUMO orbitals are primarily localized on the pyrazolyl ring (π^*-type MOs), with further significant contributions from the *p*-like orbitals of the N and S atoms around the Pd atom and *d*-orbitals of the metal. DFT calculations for [$Pd(SCN)_2$(tmdmPz)] (**4**) (Fig. S3, Supplementary material) show that the HOMO is based on the *p* orbitals of the N and S atoms of the thiocyanate ligand with contributions from *d*-orbitals of the palladium atom. The LUMO are constructed mainly from π^* orbitals of pyrazolyl ring, thioamide moiety and thiocyanate ligands, with a significant contribution from *d* orbitals of the metal center.

Table 3
IC_{50} values (μM) values of **1**–**4**, tmdmPz and cisplatin against LM3 and LP07 cell lines.

Compound	IC_{50} (μM)	
	LM3	LP07
[$PdCl_2$(tmdmPz)] (**1**)	3.29 ± 0.20	2.56 ± 0.14
[$PdBr_2$(tmdmPz)] (**2**)	3.40 ± 0.44	1.84 ± 0.14
[PdI_2(tmdmPz)] (**3**)	2.53 ± 0.06	1.65 ± 0.44
[$Pd(SCN)_2$(tmdmPz)] (**4**)	3.17 ± 0.48	1.66 ± 0.10
Tmdmpz	>140	>140
Cisplatin	30.3 ± 3.72	4.3 ± 0.45

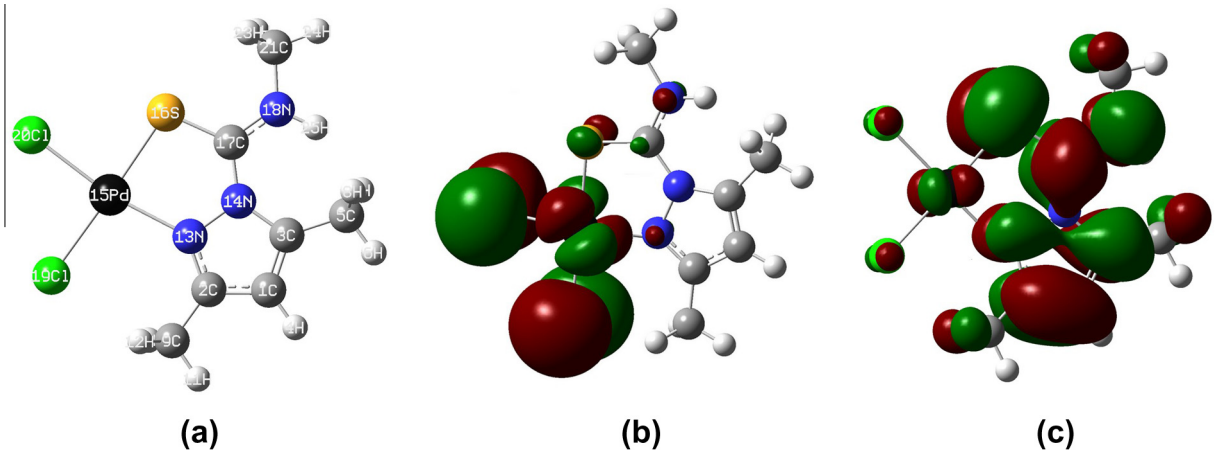


Fig. 3. Energy minimized structure of 1 (a), (b) HOMO and (c) LUMO. The red (dark) and green (light) isosurfaces correspond to positive and negative isosurface values, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.6. Anti proliferative activity

The cytotoxic activity of the complexes was evaluated in LM3 (murine mammary adenocarcinoma) and LP07 (murine lung adenocarcinoma) after 24 h using the MTT assay. Table 3 summarizes the obtained IC_{50} values.

According to the cytotoxicity data, the free ligand tmdmPz is considered inactive, since it showed no drug response at drug concentrations <140 μM in both cell lines. In our previous studies on the cytotoxicity of compounds $[PdX_2(tdmPz)]$ {X = Cl, Br, I, SCN; tdmPz = 3,5-dimethyl-1-thiocarbamoylpyrazole} [30], the iodo-complex was the most active towards LM3 tumor cells, with a IC_{50} value of 24 μM. The compounds **1**–**4** displayed remarkable cytotoxic levels over the 2.5–3.4 μM concentration range, being *ca.* 10-fold more potent than $[PdI_2(tdmPz)]$ and cisplatin against the LM3 cell line. With respect to the cytotoxic effects on LP07 cells, none of the $[PdX_2(tdmPz)]$ were more active than cisplatin [30]. In this work, the IC_{50} values found for compounds **1**–**4** (2.6–1.6 μM) demonstrated that these Pd(II) derivatives are approximately 2.5 times more cytotoxic than cisplatin. These findings suggested that the cytotoxic effects *in vitro* increased with the replacement of hydrogen at the N atom of the thioamide moiety by a methyl group, implying that a combination of lipophilicity and steric hindrance effects may be involved in the activity. However, such structure–activity relationship is only preliminary taking into account that they were based on only two murine cell lines. Thus, testing on further human tumor cell lines is necessary in order to confirm the proposed structure–activity relationship.

Taking into account that DNA is the main cellular target of some Pd(II) complexes with thiosemicarbazones [17], we performed some complementary assays with guanosine and pNFkB-luc plasmid DNA in order to check if the observed cytotoxicity may be related with the interaction of the Pd(II) complexes with DNA.

2.7. Interaction with guanosine

It is generally accepted that the antitumor activity of various platinum containing drugs is related to the platination of DNA, most commonly via binding to guanine [46]. The interaction of the Pd(II) complexes with nucleotides has also been studied extensively [47] and it has been crystallographically proven the chelation take place through the guanine N7, similarly to cisplatin [48]. In this work, we use guanosine as a model system to study by ^{1}H NMR the reactivity of complexes **1**–**4**. However, due to the low solubility of **1**–**4** in water, the reaction between the Pd(II) complexes and guanosine was performed in dimethylformamide (dmf). In these conditions, complexes **1** and **2** reacted slowly with guanosine (completion after 48 h), affording a complex with molar ratio 1:2 palladium(II)–guanosine as detected by mass spectrometry (molecular peak at m/z = 840 associated with the corresponding isotopic signatures, Fig. 4). Their ^{1}H NMR spectra are in agreement with the coordination of the nucleotide to metal through guanine-N7. A new set of H8 signals is observed at δ 8.471 and δ 8.690, which represents a 0.5 ppm downfield shift compared to free guanosine and is in agreement with different chemical environments Scheme 1.

On the other hand, compounds $[PdI_2(tmdmPz)]$ (**3**) and $[Pd(SCN)_2(tmdmPz)]$ (**4**) did not interact with guanosine under the same conditions, possibly due to the smaller lability of iodo and thiocyanate than chloro and bromo ligands. In order to verify if the Pd–X bond is related to their lack of reactivity towards guanosine, we have attempted to replace the anionic X groups in **3** and **4** by labile ligands aiming at producing the more reactive and soluble complexes capable to readily interact with guanosine. In this context, excellent results are achieved by using the $AgNO_3$/dmf method [47,49,50] to produce *in situ* reactive and soluble solvento/nitrato Pd(II) and Pt(II) complexes. According to Lippert et al. [51], one of the reasons why this method provides good results

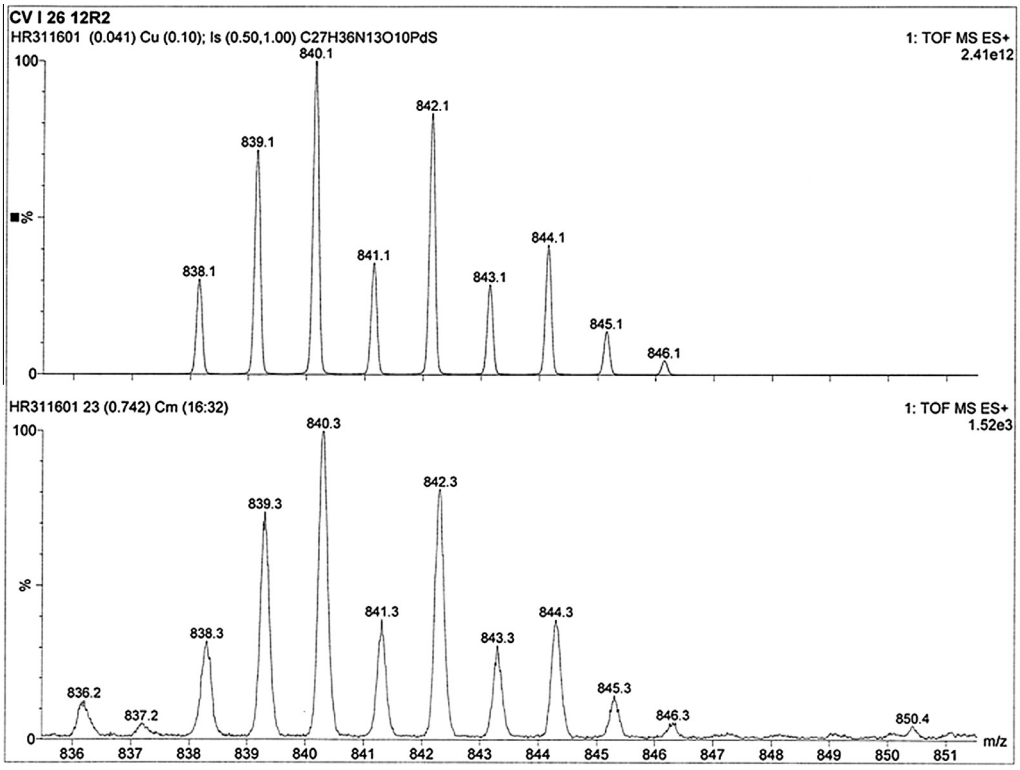
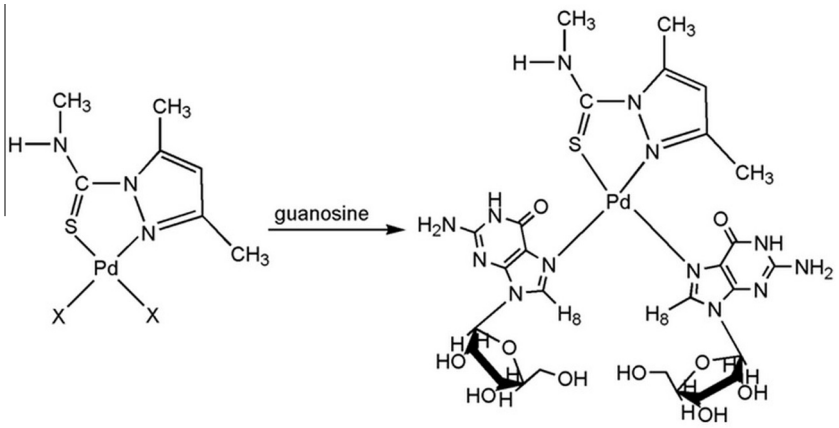


Fig. 4. Expansion of the peak m/z = 840 from the mass spectrum (top: simulated, bottom: measured) of the 1:2 palladium(II)–guanosine complex formed by the reaction between guanosine with **1** and **2** (or **3** and **4** pretreated with $AgNO_3$).



Scheme 1. Reaction of complexes **1**–**4** with guanosine 1:2 palladium–guanosine.

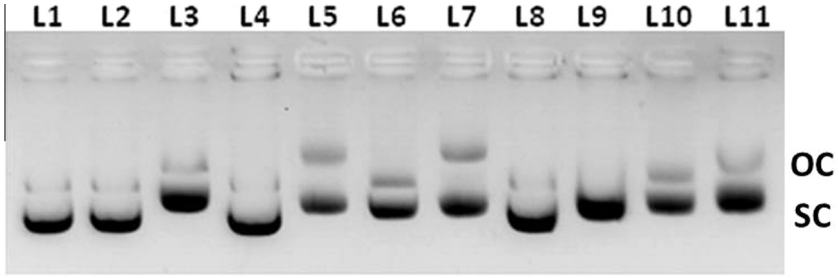


Fig. 5. Plasmid incubation with **1**–**4** and cisplatin after 24 h. Line 1: plasmid in water. Line 2: plasmid in water/dmf (5%). Line 3: cisplatin (10 μM). Line 4: 1 (10 μM). Line 5: 1 (100 μM). Line 6: 2 (10 μM). Line 7: 2 (100 μM). Line 8: 3 (10 μM). Line 9: 3 (100 μM). Line 10: 4 (10 μM). Line 11: 4 (100 μM). OC represents open circular pDNA and SC indicates supercoiled pDNA.

deals with the oxygen-bound ligands in the intermediate species, $[ML_n(O_d)]^{+x}$ (M = Pd(II), Pt(II); O_d = *O*1-dmf, NO_3^-), are much better leaving groups than the chloride ligand, leading to faster substitution kinetic. Thus, the solubility of compounds **3** and **4** in dmf has prompted us to carry out the halide abstraction using the $AgNO_3$/dmf method. After the halide/pseudohalide abstraction of complexes **3** and **4**, the solvento/nitrato derivative readliy reacted with guanosine yielding the $[Pd(tmdmPz)(guanosine)_2]^{+2}$ complex (Fig. 4). This fact suggests a role of the metal–halogen bond strength in the reactivity of the complexes towards guanosine.

2.8. Interaction with supercoiled plasmid

The effect of **1**–**4** on the structure of a supercoiled DNA was evaluated by their ability to modify the mobility of the circular pNFkB-luc plasmid in a gel electrophoresis assay. In these experiments, a DNA plasmid is incubated with different concentrations of the complex and then subjected to gel electrophoresis. It is well established that upon a one strand cleavage, the supercoiled structure relaxes, producing the nicked circular form which exhibits a slower migration [52]. Fig. 5 illustrates the electrophoretic mobility of pNFkB-luc plasmid DNA incubated with the synthesized compounds (**1**–**4**) at 10 or 100 μM concentration. To provide a basis for comparison, incubation of DNA with cisplatin was also performed at 10 μM under the same conditions. The reactions were conducted in aqueous solutions containing 5% dmf to help solubilize the complexes.

Control experiments demonstrate that relaxation of untreated plasmid does not occur in water (line 1) or water/dmf 5% (line 2). DNA is generally agreed to be the biological target of cisplatin with the major adduct being a 1,2-intrastrand crosslink between the N7 atoms of two adjacent purine residues [53]. As expected, cisplatin greatly modified the electrophoretic mobility of pNFkB-luc plasmid DNA at a concentration of 10 μM (line 3) which is in the activity range of cisplatin towards LM3 and LP07 cells (30.3–4.3 μM). At 100 μM, all the Pd(II) compounds produced an effect on the electrophoretic mobility of pNFkB-luc plasmid DNA. However, only the complexes $[PdBr_2(tmdmPz)]$ (**2**) and $[Pd(SCN)_2(tmdmPz)]$ (**4**) seemed to modify the tertiary structure of DNA at 10 μM.

These findings suggest that their cytotoxicity did not appear to be correlated to the DNA damaging activity of these compounds. The gel electrophoresis experiment (Fig. 5) indicated that compounds **2** and **4** induced more DNA damage at lower concentration than **1** and **3**, and would be expected to be the most active compounds if DNA damage was a major contributor to cytotoxicity. The comparable cytotoxic levels (2.6–1.6 μM) of **1**–**4** towards LM3 and LP07 lines suggest that their cytotoxicity mechanisms may involve not only DNA binding but also interaction with additional pharmacological targets.

3. Conclusion

In summary, we have successfully synthesized a series of neutral palladium complexes of the type $[PdX_2(tmdmPz)]$, where tmdmPz = *N*′-methyl-3,5-dimethyl-1-thiocarbamoylpyrazole; X = Cl (**1**), Br (**2**), I (**3**), SCN (**4**). Our results indicated that these chelated Pd(II) complexes were considerably more active against LM3 and LP07 cell lines than cisplatin and their analogous $[PdX_2(tdmPz)]$ complexes [30]. The fact that compounds 1–4 displayed similar cytotoxicity index suggest that changing halide/pseudohalide (X) has no significant effect on the biological activity of the complexes **1**–**4** *in vitro*. It seems probable that either aquation of the labile Pd(II) complexes (or displacement of the halide/pseudohalide by

other ligands) may take place and therefore the complexes are transformed to the same active intermediates or cleavage of the Pd–X bond is not essential for cytotoxicity.

On the other hand, binding studies of **1**–**4** towards guanosine has demonstrated that there is a relationship between the lability of group X and reactivity. Compounds with X groups of intermediate lability (X = Cl, Br) reacted with guanosine whereas those containing softer X groups (such as I and SCN) demonstrated to be inert under the same conditions. From the inspection of gel electrophoresis results, a different pattern has been observed. Compounds **2** (X = Br) and **4** (X = SCN) elicited a greater effect on plasmid DNA mobility at 10 μM, which is in agreement with their low IC_{50} values. On the other hand, the concentration of **1** (X = Cl) and **3** (X = I) required to produce an observable effect on DNA electrophoresis (100 μM) would not be pharmacologically relevant, being much higher than the range of their IC_{50} values. Therefore, the cytotoxicity of **1**–**4** is probably associated with their ability to interact with DNA and other additional pharmacological targets.

4. Experimental

4.1. Materials and instrumentation

All chemicals used were of reagent grade and used without further purification. The starting complex $[PdCl_2(MeCN)_2]$ [54] and the ligand *N*′-methyl-3,5-dimethyl-1-thiocarbamoylpyrazole [33] were prepared as previously described.

^1H and ^{13}C {^1H} NMR spectra were registered at 298 K, in dmso-d_6 solution, on a Varian model Inova 500 spectrometer operating at 500 and 126 MHz, respectively. Data are reported as chemical shifts (δ) in ppm. Residual solvent signals were used as internal references (^1H, ^{13}C). Electrospray (positive mode) high-resolution mass spectra were recorded on a Q-TOF micro spectrometer (Waters), using internal (H_3PO_4) and external lock masses (leucine-enkephalin $[M+H]^+$: m/z = 556.2766). IR spectra were recorded on a Spectrum 200 from Perkin Elmer, in a range between 4000–400 cm^{-1} using KBr pellets. Conductivities were measured with a Digimed-DM-31 conductometer using $1x10^{-3}$ mol L^{-1} dmf solutions. Elemental analyses were performed by the Central Analítica at IQ-University of São Paulo, Brazil.

4.2. Synthesis of the complexes

*$[PdCl_2(tmdmPz)]$ (**1**):* 0.19 mmol (33 mg) of *N*′-methyl-3,5-dimethyl-1-thiocarbamoylpyrazole in 1 mL of MeOH, was added to an orange solution of $[PdCl_2(MeCN)_2]$ (50 mg; 0.19 mmol) dissolved in 10 mL of MeOH, affording a yellow suspension. After stirring for 1 h, the solid was isolated by filtration, washed with MeOH, and dried under vacuum. Yield: 79%. *Anal.* Calc. for $C_7H_{11}Cl_2N_3PdS$: C, 24.26; H, 3.20; N, 12.12. Found: C, 24.74; H, 3.26; N, 11.95%. IR (KBr, cm^{-1}): 3385 *ms*, 3112 *w*, 2929 *w*, 1594 *s*, 1572 *ms*, 1469 *m*, 1388 *ms*, 1118 *w*, 997 *m*, 770 *w*.

*$[PdBr_2(tmdmPz)]$ (**2**):* 0.19 mmol (33 mg) of *N*′-methyl-3,5-dimethyl-1-thiocarbamoylpyrazole in 1 mL of MeOH, was added to an orange solution of $[PdCl_2(MeCN)_2]$ (50 mg; 0.19 mmol) dissolved in 10 mL of MeOH, affording a yellow suspension. After stirring for 1 h, 2 mL of H_2O containing KBr (46 mg; 0.39 mmol) was added in the mixture that was kept under stirring for 2 h. The light-yellow suspension was filtered off and the solid was isolated by filtration, washed with distillated water and MeOH, and dried under vacuum. Yield: 75%. *Anal.* Calc. for $C_7H_{11}Br_2N_3PdS$: C, 19.31; H, 2.55; N, 9.65. Found: C, 19.35; H, 2.43; N, 9.57%. IR (KBr, cm^{-1}): 3392 *ms*, 3097 *w*, 2928 *w*, 1595 *s*, 1574 *ms*, 1469 *m*, 1388 *ms*, 1117 *w*, 994 *m*, 768 *w*.

*$[PdI_2(tmdmPz)]$ (**3**):* the complex was prepared similarly to **2** with the exception that KI was used (64 mg; 0.39 mmol). The suspension was filtered off, the brown solid was washed with distillated water and MeOH and dried under vacuum. Yield: 85%. *Anal.* Calc. for $C_7H_{11}I_2N_3PdS\ 2H_2O$: C, 14.87; H, 2.67; N, 7.43. Found: C, 14.93; H, 2.35; N, 7.32%. IR (KBr, cm^{-1}): 3391 *ms*, 3097 *w*, 2925 *w*, 1593 *s*, 1567 *ms*, 1465 *m*, 1385 *ms*, 1118 *w*, 991 *m*, 763 *w*.

*$[Pd(SCN)_2(tmdmPz)]$ (**4**):* the complex was prepared similarly to **2** with the exception that KSCN was used (37 mg; 0.39 mmol). The suspension was filtered off, the orange solid was washed with distillated water and MeOH and dried under vacuum. Yield: 87%. *Anal.* Calc. for $C_9H_{11}N_5PdS_3$: C, 27.59; H, 2.83; N, 17.87. Found: C, 27.78; H, 3.10; N,17.35%. IR (KBr, cm^{-1}): 3275 *ms*, 3097 *w*, 2925 *w*, 2124 *ms*, 2090 *ms*, 1601 *s*, 1571 *ms*, 1477 *m*, 1381 *ms*, 1120 *w*, 999 *m*, 776 *w*.

4.3. Computational strategy

In this work, the employed quantum chemical approach to determine the molecular structures was Becke three-parameter hybrid theory [55] using the Lee–Yang–Par (LYP) correlation functional [56] and the basis sets used for calculations were: [4s] for H (^2S), [5s4p] for C (^3P) and N (^4S), [11s7p] for Cl (^2P), [12s7p] for S (^3P), [15s11p6d] for Br (^2P), [16s9p5d] for I (^2P) and [12s8p5d] for Pd (^1S) [57–59]. In order to better describe the properties of **1**–**4** in the implementation of the calculations, it was necessary to include polarization functions for all atoms of the compounds. The strategy to choice of the polarization functions was previously described [57–59]. The polarization functions are: α_p = 0.33353749 for H (^2S), α_d = 0.72760279 and α_d = 0.35416230 for C (^3P) and N (^4S), respectively, α_d=0.47236655 for Cl atom (^2P), α_d = 0.34993775 for S atom (^3P), α_f = 0.42912802 for Br (^2P), α_f = 0.51068618 for I (^2P), and α_f = 0.14057699 for Pd (^1S) atoms [57–59]. The calculations in this work were performed using the GAUSSIAN 09 routine [60]. In all cases, vibrational frequencies were calculated and compared with experimental data to ensure that the optimized geometries represented local minima.

4.4. Cell culture and in vitro cytotoxicity assays

LM3 and LP07 cells were maintained in MEM (Sigma) supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, and 80 μg mL^{-1} gentamicin, defined as complete medium, in plastic flasks (Corning) at 37 °C in a humidified 5% CO_2 atmosphere. Passages were made by trypsinization of confluent monolayers (0.25% trypsin and 0.02% EDTA in Ca^{2+} e Mg^{2+} free PBS). Cell number was counted by the Trypan blue dye exclusion method.

Dose–response curves were obtained for both LM3 and LP07 cells by incubating 5×10^4 cells mL^{-1} for 24 h in the absence and presence of various concentrations of tested compounds. Because of low aqueous solubility, the test compounds were dissolved in dmso first and then serially diluted in complete culture medium such that the effective dmso content did not exceed 0.5% (the solvent has no cytotoxic activity at this concentration). Cell viability was checked by MTT assay according to the literature procedures [61]. The sensitivity to the drug was evaluated by the drug concentration needed to inhibit cell growth by 50%. The IC_{50} obtained is a medium of two independent experiments.

4.5. Reactions with guanosine

*Complexes **1** and **2**:* Guanosine (0.022 mmol, 2.0 equiv) dissolved in 0.5 mL of warm dmf was added to a solution of the complex (0.011 mmol, 1.0 equiv) in 1 mL of dmf. The reaction was kept under agitation for 48 h. The solvent was removed under a high vacuum (pallet pump) at room temperature and the resultant solid

was taken-up in deuterated methanol. Insoluble materials were eliminated by centrifugation before being analyzed by ^{1}H NMR spectroscopy and mass spectrometry.

*Complexes **3** and **4**:* To the complex (0.011 mmol, 1.0 equiv) in dmf (1 mL), $AgNO_3$ (0.022 mmol, 2.0 equiv) was added. After stirring for 2 h in the dark, the white solid formed (AgCl) was filtered off through a Millipore filter. Guanosine (0.022 mmol, 2.0 equiv) in 0.5 mL of warm dmf was added to the filtrate. The reaction was kept under agitation for 48 h. The solvent was removed under a high vacuum (pallet pump) at room temperature and the resultant solid was taken-up in deuterated methanol. Insoluble materials were eliminated by centrifugation before being analyzed by ^{1}H NMR spectroscopy and mass spectrometry.

Interaction with supercoiled plasmid: Solutions (2000 and 200 μM) of the **1**–**4** were prepared in dmf: water, 1:1 v/v. 1.0 μL of each solution was added to 1.0 μL of pNFkB-luc plasmid in presence of 18.0 μL of $KClO_4$ (10^{-2} M), then incubated for 24 h. Samples at the two concentrations (10.0 and 100 μM) were analyzed by agarose gel electrophoresis (1% agarose gel in 0.5× tris–acetic acid EDTA buffer, pH = 7.9, 100 V). The gel was revealed with BET under UV-light.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.poly.2013.08.040.

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