European Journal of Medicinal Chemistry 44 (2009) 4611–4615

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Antitumor and antimycobacterial activities of cyclopalladated complexes: X-ray structure of [Pd(C^2,*N*-dmba)(Br)(tu)] (dmba = *N*,*N*-dimethylbenzylamine, tu = thiourea)

Antonio C. Moro [a], Antonio E. Mauro [a,*], Adelino V.G. Netto [a], Sandra R. Ananias [a], Marcela B. Quilles [b], Iracilda Z. Carlos [b], Fernando R. Pavan [c], Clarice Q.F. Leite [c], Manfredo Hörner [d]

[a] *Departamento de Química Geral e Inorgânica, Instituto de Química, UNESP, CEP, 14801-970 Araraquara, SP, Brazil*
[b] *Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas, UNESP, CEP, 14801-902 Araraquara, SP, Brazil*
[c] *Departamento Ciências Biológicas, Faculdade de Ciências Farmacêuticas, UNESP, CEP, 14800-900 Araraquara, SP, Brazil*
[d] *Departamento de Química, Centro de Ciências Naturais e Exatas, UFSM, CEP, 97105-900 Santa Maria, RS, Brazil*

ARTICLE INFO

Article history:
Received 31 March 2009
Received in revised form
27 May 2009
Accepted 29 June 2009
Available online 4 July 2009

Keywords:
Cyclopalladated complex
Crystal structure
Cytotoxicity assays
Mycobacterium tuberculosis
Antitumor
Thiourea

ABSTRACT

The reactions between [Pd(C^2,*N*-dmba)(μ-X)]$_2$ (dmba = *N*,*N*-dimethylbenzylamine; X = Cl, Br) and thiourea (tu) in the 1:2 molar ratio at room temperature resulted in the mononuclear compounds [Pd(C^2,*N*-dmba)(Cl)(tu)] (**1**) and [Pd(C^2,*N*-dmba)(Br)(tu)] (**2**), which were characterized by elemental analyses and infrared (IR), ^{1}H- and ^{13}C{^{1}H} NMR spectroscopies. The crystal and molecular structures of **2** were determined by single-crystal X-ray diffraction. In vitro cytotoxicity assays of the compounds **1**, **2**, tu, dmba and cisplatin were carried out using two murine tumor cell lines, namely mammary adenocarcinoma (LM3) and lung adenocarcinoma (LP07). The compounds **1**, **2**, tu and dmba were also tested against *Mycobacterium tuberculosis* and their MIC values were determined.

1. Introduction

Cis-Diamminedichloroplatinum (II) is a clinically important antitumor drug [1,2] which reacts with nucleophilic sites in DNA forming monoadducts as well as intra- and interstrand cross-links, but its administration in humans shows many side-effects such as nausea, nephrotoxicity and neurotoxicity [3,4]. Due to these adverse effects, research moved on to second and third-generation platinum compounds like carboplatin [1] and oxaliplatin [4], respectively, as well as to other metal complexes with improved pharmacological properties [5,6]. Among the non-platinum metal complexes studied for cancer treatment, palladium(II) derivatives were readily chosen due to their structural analogy with those containing Pt(II) [4,7]. The design of Pd(II) derivatives with anticancer activity is an exciting challenge, since Pd(II) compounds show ligands-exchange kinetics 10^5 times greater than the Pt(II) analogous [7]. Due to this rapid ligands-exchange, palladium (II) derivatives do not maintain their structural integrity in biological fluids for long enough to reach the pharmacological target. To overcome this high lability, chelating ligands have been used to yield high thermodynamically stable and kinetically inert Pd(II) complexes [4]. Within this context, the tertiary amine *N*,*N*-dimethylbenzylamine (dmba) represents a good choice to prepare new ortho-cyclopalladated complexes with promising in vivo and in vitro cytotoxicity [3,4]. For instance, the compound [Pd$_2$(C^2,*N*–$S_{(-)}$dmpa)$_2$(μ-dppf)(Cl)$_2$] (dmpa = *N*,*N*-dimethyl-1-phenethylamine; dppf = 1,1′-bis(diphenylphosphine)ferrocene) has shown antimetastatic effects [8]. Moreover, transition metal complexes containing *S*-donor ligands have received considerable attention largely because of their bioinorganic relevance [9]. Some bipyridyl complexes of Pt(II) containing thiourea have been reported as a new class of efficient DNA intercalators, whose mode of action differs from that of cisplatin, since it is based on non-covalent interactions [10]. Another point of discussion is the ability of the thiourea to reduce the nephrotoxicity of the platinum drugs [11].

Despite the extensive work devoted to the antitumor properties of palladium (II) compounds, studies on the antimycobacterial activity towards *Mycobacterium tuberculosis* involving Pd(II) species

* Corresponding author. Tel.: +55 016 33016625; fax: +55 016 33227932.
E-mail address: mauro@iq.unesp.br (A.E. Mauro).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.06.032

are scarce. In developing countries, tuberculosis (TB) is a leading cause of morbidity and mortality. Its coinfection with the human immunodeficiency virus (HIV) has been responsible for changes in the TB epidemiologic situation and also corroborates the urgency for the development of drugs able to act against multidrug-resistant strains of the *M. tuberculosis* [12,13]. In the same context, some thiourea derivatives exhibited good in vitro antimycobacterial activity against sensitive and resistant strains of *M. tuberculosis* [14]. In addition, the thiourea isoxyl (thiocarlide; 4,4-diisoamyloxydiphenylthiourea) is a clinically used drug effective against a range of multidrug-resistant strains of *M. tuberculosis* [15].

Our interest in palladium (II) compounds bearing thiourea type-ligands is focusing on preparing new species with promising biological activities [16,17]. We have developed a procedure for the synthesis of mononuclear cyclopalladated compounds of general formulae [Pd(C^2,*N*-dmba)(X)(tu)] (X = Cl, N_3, NCO) from cleavage reactions of [Pd(C^2,*N*-dmba)(μ-X)]$_2$ with thiourea [17].

In order to present some of our recent contributions in the coordination chemistry of palladium (II) complexes containing halides and pseudohalides as coligands [17–19], in this report we have evaluated the in vitro cytotoxic potential (IC_{50}) of the compounds [Pd(C^2,*N*-dmba)(Cl)(tu)] (**1**) and [Pd(C^2,*N*-dmba)(Br)(tu)] (**2**), (dmba = *N*,*N*-dimethylbenzylamine, tu = thiourea) against the LM3 and LP07 murine tumor cell lines, as well as their minimal inhibitory concentration (MIC) values against *M. tuberculosis*. In addition, this work also describes the synthesis, spectroscopic and structural characterization of [Pd(C^2,*N*-dmba)(Br)(tu)] (**2**). To the best of our knowledge, [Pd(C^2,*N*-dmba)(Br)(tu)] is the first cyclopalladated complex containing the thiourea ligand whose structure was determined by single-crystal X-ray diffraction.

2. Chemistry

The starting material [Pd(C^2,*N*-dmba)(μ-Cl)]$_2$ was prepared as previously described [20]. The compound [Pd(C^2,*N*-dmba)(μ-Br)]$_2$ was synthesized from [Pd(C^2,*N*-dmba)(μ-Cl)]$_2$ by an anion exchange reaction. Complex [Pd(C^2,*N*-dmba)(Cl)(tu)] (**1**) was obtained according to the method described in the literature [17]. The reaction between the cyclopalladated compound [Pd(C^2,*N*-dmba)(μ-Br)]$_2$ and tu in the 1:2 molar ratio readily occurred, leading to the new complex [Pd(C^2,*N*-dmba)(Br)(tu)] (**2**), Fig. 1.

3. Results and discussion

The elemental analyses for the synthesized compounds were in agreement with the proposed formulae. Spectroscopic results of compound **1** have already been reported and discussed elsewhere [17]. Complex [Pd(C^2,*N*-dmba)(Br)(tu)] (**2**) was characterized by infrared (IR), ^{1}H- and ^{13}C{^{1}H} NMR spectroscopies as well as by single-crystal X-ray diffraction method. The data obtained from these techniques are discussed as follows.

3.1. Infrared spectrum

The coordination of the tu in **2** was clearly evidenced by the appearance of additional IR bands [16,21,22] at 3408–3190 cm^{-1} (νN–H), and 1627–1622 cm^{-1} (δNH_2). The characteristic bands of the cyclometallated ring at ca. 3055 cm^{-1} ($\nu C–H_{ar}$), 2983–2839 cm^{-1} ($\nu C–H_{aliph.}$) and 1593 cm^{-1} (νC=C) in the IR spectrum of **2** were found unchanged when compared with those observed for the precursor, indicating that the coordination of the thiourea to the metallic center did not affect the integrity of the ortho-metallated ring [19].

3.2. ^{1}H- and $^{13}C\{^1H\}$ NMR spectra

The ^{1}H- and ^{13}C{^{1}H}-NMR data (DMSO-d_6) also strongly support the cleavage of the dimer [Pd(C^2,*N*-dmba)(μ-Br)]$_2$ into the monomeric compound **2**. Concerning the ^{1}H NMR spectrum of **2**, the signals of the chelating C^2,*N*-dmba moiety [19,23] were observed at δ 7.31–6.78 (m, 4H, CH_{ar}), 3.94 (s, 2H, CH_2) and 2.70 (s, 6H, CH_3), whereas the appearance of a broad signal at δ 8.04 clearly indicated the coordination of the tu ligand (bs, 4H, –NH_2) [16]. The downfield shifting of the NH_2 signal when compared with that of the free ligand (δ 7.06) [24] could be associated to a substantial reduction in the electron density around the nitrogen atom, suggesting the Pd–S bond formation. The ^{13}C{^{1}H} NMR spectrum of **2** showed the typical signals [19,23] of the ortho-metallated dmba group at δ 148.5 (C_{ar}–Pd), 133.6–121.9 (C_{ar}), 72.28 (–N–CH_2–) and 51.24 [–N(CH_3)$_2$]. The quaternary carbon resonance of the tu ligand at δ 178.2 was found quasi 6 ppm upfield [24] when compared with the tu free resonance (δ 183.9). The upfield shift is attributed to a lowering of the C=S bond order upon coordination, which causes a shift of the electron density from the nitrogen to the carbon atom, increasing the double bond character of the C–N bond, as observed in other thiourea metal complexes [24,25]. These results are consistent with *S*-monodentate coordination of thiourea to palladium (II).

3.3. X-ray structure of [Pd(C^2,N-*dmba*)(Br)(tu)] (**2**)

A perspective drawing of the molecular structure of [Pd(C^2,*N*-dmba)(Br)(tu)], together with its atom labeling scheme is depicted in Fig. 2. A selection of bond lengths and angles is shown in Table 1.

The molecular structure of compound [Pd(C^2,*N*-dmba)(Br)(tu)] consists of a palladium atom to which a chelating *N*,*N*-dimethylbenzylamine-C^2,*N* moiety is bound through the N1 atom and the aromatic C11 atom, providing a five-membered cyclometallated ring. The coordination sphere around the palladium (II) center is completed by a bromo group *trans*-positioned to the carbopalladated

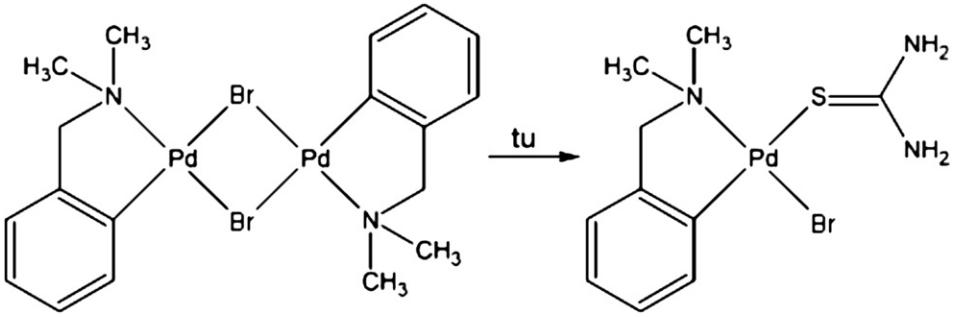

Fig. 1. A schematic representation of the cleavage reaction of the dimeric cyclopalladated compound [Pd(C^2,*N*-dmba)(μ-Br)]$_2$ by thiourea.

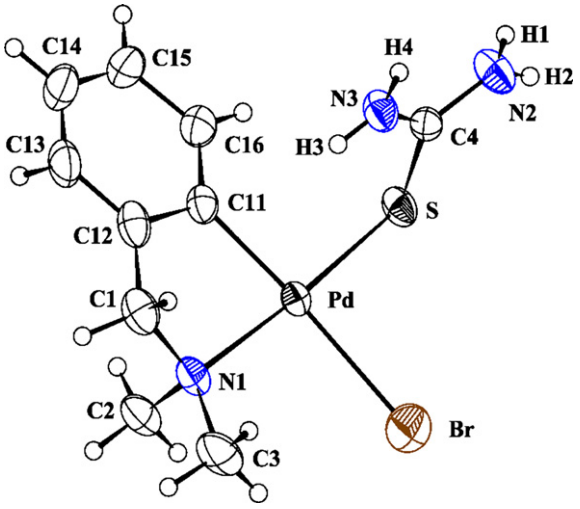

Fig. 2. An ORTEP representation of the molecular structure of the cyclopalladated compound **2**.

site and a sulphur atom from the thiourea ligand *trans* to the N1 atom, resulting in a slightly distorted square-planar geometry.

The donor atoms of the chelating *N*,*N*-dimethylbenzylamine-C^2,*N* ligand, by occupying mutually *cis* positions, impose an acute bite angle of 81.3(2)°. Other organopalladated compounds bearing the C^2,*N*-chelated dmba ligand exhibit comparable *N*–Pd–*C* angles, e.g. [Pd(C^2,*N*-dmba)(μ-N_3)]$_2$ [23] and [Pd(C^2,*N*-dmba)(NCO)(2,3-lutidine)] [26], whose bite angles are 82.2(3)° and 83.1(2)°, respectively.

The Pd–N1 bond length of 2.118(4) Å is longer than the sum of the covalent radii of Pd and N(sp^2) atoms (2.011 Å) [27] and is also slightly longer than the mean value of 2.07 Å observed for similar cyclopalladated compounds [23,26]. This lengthening indicates a weakening of the Pd–N1 bond due to the great *trans* influence of the sulfur atom from thiourea ligand.

The Pd–C11 distance (2.002(6) Å) is ca. 0.03 Å longer than those found in related complexes [23,26], but shorter than the predicted value of 2.081 Å (based on the sum of the covalent radii for C(sp^2) and Pd, 0.771 and 1.31 Å, respectively) [27]. The Pd–Br distance of 2.566(2) Å is significantly longer than the sum of the covalent radii (2.45 Å) [27] but lies in the range of 2.568(1)–2.546(1) Å obtained for similar complexes containing the Pd–Br bond *trans* to the carbometallated site [28,29].

The thiourea ligand acts in a *S*-monodentate mode, as also reported for other palladium (II) complexes containing structurally related thioureas [11,16]. The Pd–S distance of 2.314(1) Å agrees well with that found in the compound *cis*-[Pd(Cl)$_2$(tu)(PPh$_3$)] (2.319(1) Å) [16].

Since the results from the characterization techniques gave important structural information of the synthesized compounds, investigation on the antitumor and antimycobacterial activities of **1** and **2** were undertaken.

Table 1
Selected bond lengths (Å) and angles (°) for **2**.

Bond lengths (Å)		Bond angles (°)	
Pd–N(1)	2.118(4)	N(1)–Pd–(C11)	81.3(2)
Pd–C(11)	2.002(6)	C(11)–Pd–S	94.4(2)
Pd–S	2.314(1)	S–Pd–Br	89.9(1)
Pd–Br	2.566(1)	Br–Pd–N(1)	94.4(1)
		Pd–S–C(4)	106.6(2)

Table 2
Cytotoxicity data (IC_{50}) of the tu and dmba ligands and their palladium (II) complexes against murine LM3 and LP07 tumor cell lines.

Compound	IC_{50} (μM) ± SD	
	LM3	LP07
tu	>140	>140
dmba	>140	>140
1	72.4 ± 3.92	76.6 ± 1.99
2	29.6 ± 0.18	22.6 ± 1.93
Cisplatin	30.3 ± 3.72	4.34 ± 0.45

4. Biological activity

4.1. Cytotoxic activities against murine tumor cell lines

The cytotoxic activities of the tu and dmba ligands and their palladium (II) complexes were tested against murine mammary adenocarcinoma (LM3) and lung adenocarcinoma (LP07) tumor cell lines. The results are reported in Table 2 in terms of IC_{50} values (the concentration needed to inhibit 50% of the cellular proliferation). For comparison purposes, the cytotoxicity of cisplatin, a standard antitumor drug, was evaluated under the same conditions.

Recent literature has shown that cyclopalladated compounds might have promising potential anticancer applications [3,4,8], since these planar metal complexes can lead to possible alternative modes of cytotoxic action, such as intercalative DNA lesion, as opposed to the cisplatin-induced intrastrand guanine–guanine DNA lesion [30]. In addition, the square-planar coordination geometry is the more suitable for intercalative binding; furthermore, electronic and steric properties can be modulated by using appropriate ligands or substituents at the ligands [4,10]. Concerning the DNA intercalator compounds of the type [Pt(bipy)(tu)$_2$]Cl$_2$ (bipy = 2,2′-bipyridine), the presence of the thiourea group as *S*-donor ligand avoids unwanted covalent reactions with nucleobases [10].

It was observed that the compounds **1** and **2** demonstrated a noticeable cytotoxicity against LM3 and LP07 cells when compared with the free ligands, implying that the biological activity is largely ascribed by the presence of the palladium (II) metal center. As depicted in Table 2, compound **2** was approximately 2.4 and 3.4 times more potent than **1** against LM3 and LP07 cell lines, respectively. These results suggest that the replacement of the chlorido by the bromido ligand increased the cytotoxic activity of the palladium (II) compound in both cell lines, as observed for other screening of antitumor agents [31]. In addition, we have verified that compound **2** was almost five fold less effective than cisplatin against LP07, but as cytotoxic as cisplatin for LM3.

4.2. Antimycobacterial activities

The minimum inhibitory concentration (MIC) values for thiourea and *N*,*N*-dimethylbenzylamine ligands and their Pd(II) complexes against *M. tuberculosis* are shown in Table 3. The comparison of the results allows a more precise interpretation of the data by correlating

Table 3
MIC values of the tu and dmba ligands and their palladium (II) complexes against *M. tuberculosis* H_{37}Rv.

Compound	MW	MIC (μM)	MIC (μg mL^{-1})
tu	76.07	>3286.4	>250
dmba	135.2	>1848.7	>250
1	352.1	88.74	31.2
2	396.6	57.99	23.0
Isoniazid	137.2	0.22	0.030

MW = molecular weight.

Table 4
Crystal data and structure refinement for **2**.

Empirical formula	$BrC_{10}H_{16}N_3PdS$
Formula weight ($g\,mol^{-1}$)	396.63
Temperature (K)	295(2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	$P2_1/n$
a (Å)	5.79120(10)
b (Å)	15.1668(3)
c (Å)	15.0500(3)
α (°)	90
β (°)	93.7790(10)
γ (°)	90
Volume ($Å^3$)	1319.03(4)
Z	4
D_{calc} ($Mg\,m^{-3}$)	1.997
Absorption coefficient (mm^{-1})	4.572
F(000)	776
Crystal size (mm)	$0.26 \times 0.04 \times 0.03$
θ Range for collected (°)	2.69–30.06
Index ranges	$-8 \le h \le 7$, $-21 \le k \le 21$, $-21 \le l \le 21$
Reflections collected	15092
Independent reflections [R_{int}]	3785 [0.0282]
Completeness to theta = 30.06° (%)	97.9
Max. and min. transmission	0.8750 and 0.3828
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3785/0/151
Goodness-of-fit on F^2	1.074
Final R indices [I > 2sigma(I)]	$R_1 = 0.0516$, $wR_2 = 0.1560$
R indices (all data)	$R_1 = 0.0627$, $wR_2 = 0.1655$
Largest difference peak and hole ($e\,Å^{-3}$)	1.842 and −2.853
CCDC No.	687610

them with the chemical structures of the studied compounds. Isoniazid, employed extensively for tuberculosis treatment nowadays [13], was used as standard antitubercular drug.

The tu and dmba ligands displayed poor inhibition ability against *M. tuberculosis*, but their coordination to palladium (II) caused a significant increase in their antitubercular activities. In addition, the MIC values demonstrated that **2** is more effective than **1** for this bacteria. Thus, the influence of the halide on the antitubercular activity was also verified. All Pd(II) complexes tested were less effective than isoniazid against the *M. tuberculosis* [13] (MIC value of 0.030 $\mu g\,mL^{-1}$). On the other hand, **1** and **2** possess higher inhibitory activity than pyrazinamide (MIC value of 50–100 $\mu g\,mL^{-1}$) [32], another compound employed for tuberculosis treatment.

5. Conclusion

The biological evaluation of two cyclopalladated compounds bearing thiourea has been described in this work. The results suggested that the complex [Pd(C^2,*N*-dmba)(Br)(tu)] displayed an interesting cytotoxic activity towards the mammary adenocarcinoma (LM3) and lung adenocarcinoma (LP07) murine tumor cell lines as well as against the *M. tuberculosis*, showing that the use of bromido instead of chlorido improved the biological activity. Further investigations are required to confirm this findings and to elucidate the mechanism of action of these Pd(II) compounds.

6. Experimental protocols

6.1. Physical measurements

Elemental analyses of carbon, nitrogen and hydrogen were performed on an EA1110–CHNS–O microanalyzer from CE-Instruments. Melting points (M.p.) were determined on a Microquímica apparatus. Infrared spectra were recorded on a Nicolet Impact 400 spectrophotometer in the spectral range 4000–400 cm^{-1} using the KBr pellets technique. ^{1}H- and ^{13}C{^{1}H} NMR spectra were obtained from DMSO-d_6 (Merck) solutions and are referred to the high field $Si(CH_3)_4$ signal on a Bruker AC-200 spectrometer working at 200 MHz for ^{1}H and at 50 MHz for ^{13}C.

6.2. X-ray crystallography

Single-crystals suitable for X-ray studies were obtained from the mother liquor of the reaction between [Pd(C^2,*N*-dmba)(μ-Br)]$_2$ and tu in the 1:2 molar ratio. The data were collected on a Bruker APEX II CCD area-detector diffractometer with graphite-monochromatized Mo-*Kα* radiation ($\lambda = 0.71073$ Å). The crystal and experimental data are listed in Table 4. The structure was solved by direct methods using SIR2004 [33]. The non-hydrogen atoms were refined anisotropically by the full-matrix least-squares method using SHELXL97 [34].

6.3. Chemistry

6.3.1. Materials

The reagents thiourea (Merck), *N*,*N*-dimethylbenzylamine (Aldrich), $PdCl_2$ (Degussa), KBr (Merck) were employed without further purification. Methanol, chloroform and *n*-pentane of analytical purity were purchased from Merck.

6.3.2. Preparation of the complex **2**

*6.3.2.1. [Pd(C^2,N-dmba)(Br)(tu)] (**2**).* A 10 mL chloroform solution of [Pd(C^2,*N*-dmba)(μ-Br)]$_2$ (0.10 g; 0.16 mmol) was mixed with a 5 mL methanol solution of thiourea (0.024 g; 0.32 mmol) at room temperature. The mixture was stirred for 1 h and the yellow solid formed was filtered off, washed with methanol, chloroform, *n*-pentane and then dried under vacuum. Yield: 60%. M.p. > 146.4 °C (dec.). Anal. Calcd. for $BrC_{10}N_3H_{16}PdS$ (%): C, 30.28; H, 4.07; N, 10.60. Found: C, 30.98; H, 4.01; N, 10.63.

6.3.3. Solubility

The synthesized palladium (II) compounds are soluble in DMSO.

6.4. Biological evaluation

6.4.1. Antitumor activity

6.4.1.1. Cells. LM3 and LP07 cells were maintained in Eagle's Minimum Essential Medium, MEM (Sigma), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, and 80 $\mu g\,mL^{-1}$ gentamicin, defined as complete medium, in plastic flasks (Corning) at 37 °C in a humidified 5% CO_2 atmosphere [35]. Passages were made by trypsinization of confluent monolayers (0.25% trypsin and 0.02% EDTA in Ca^{2+}–Mg^{2+} free phosphate-buffered saline). The cell number was counted by the Trypan blue dye exclusion method.

6.4.1.2. Compounds. Test solutions of the compounds (1000 μM) were freshly prepared by dissolving the substances in 50 μL of DMSO and completing with 4950 μL of the culture medium. Afterwards, the tested compounds were diluted in a culture medium to reach the desired concentrations ranging from 10 to 140 μM. The DMSO solvent did not reveal any cytotoxic activity in the concentrations used in test. Cisplatin (Sigma) was employed as the standard antitumor drug.

6.4.1.3. MTT assay. For the cytotoxicity evaluation, 200.0 μL samples of LM3 and LP07 cells (5×10^4 cell mL^{-1}, adjusted in MEM) were added to each well of a 96-well tissue culture plate (Corning) and then preincubated in the absence of the compounds for 24 h to allow

adaptation of the cells prior to the addition of the test agents. Afterwards, the supernatants were removed and 200.0 μL solutions of the compounds in concentrations ranging from 10 to 140 μM or 200.0 μL of MEM-Complete as cell control of viability was added to each well. The effects of the compounds towards the cells were determined 24 h after the culture incubation. After that, the supernatants were removed and 100.0 μL solutions of [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) were added in each well containing the samples [36]. The MTT assay was performed and the plates were incubated for 3 h. Then, the absorbances were measured and the cytotoxic midpoint value, i.e. the concentration of the chemical agent needed to reduce the spectrophotometric absorbance to 50%, was determined by linear regression analysis with 95% of confidence limits. The IC_{50} was defined as the medium of three independent experiments through the equation of graphic line obtained (Microcal Origin 5.0™). Triplicates tests were performed for each concentration of each compound.

6.4.2. Antimycobacterial assay

Antimycobacterial activities of each tested compound and of the standard drug isoniazid (Difco laboratories, Detroit, MI, USA) were determined in triplicate in sterile 96-well flat bottomed microplates (Falcon 3072; Becton Dickinson, Lincoln Park, NJ, USA) and Middlebrook 7H9 Broth (Difco) supplemented with oleic acid–albumin–dextrose–catalase (OADC) enrichment (BBL/Becton Dickinson, Sparks, MD, USA). The tested compounds concentrations ranged from 0.15 to 250 μg mL^{-1} and the isoniazid from 0.015 to 1.0 μg mL^{-1}. The microplate Alamar Blue assay [37] (MABA) was used to measure the minimal inhibitory concentration (MIC) for the tested compounds (minimum concentration necessary to inhibit 90% growth of *M. tuberculosis* $H_{37}Rv$ ATCC 27294). Fluorescence measurements were taken on a SPECTRAfluor Plus microfluorimeter (Tecan®) in bottom reading mode, with excitation at 530 nm and emission at 590 nm. As a standard control, the MIC value of isoniazid was determined on each microplate. The acceptable MIC of isoniazid ranged from 0.015 to 0.05 μg mL^{-1} [37].

Acknowledgements

We thank Prof. Dr. Lucas Luis Colombo from Institute of Oncology Ángel H. Roffo for providing the LM3 and LP07 murine tumor cell lines. We also thank Marisa C. P. Placeres for her technical assistance. This research was supported by CNPq, Capes and FAPESP.

References

[1] C. Pampillón, J. Claffey, K. Strohfeldt, M. Tacke, Eur. J. Med. Chem. 43 (2008) 122–128.
[2] L. Leyva, C. Sirlin, L. Rubio, C. Franco, R. Le Lagadec, J. Spencer, P. Bischoff, C. Gaiddon, J.P. Loeffler, M. Pfeffer, Eur. J. Inorg. Chem. (2007) 3055–3066.
[3] M.C. Rocha, A.M. Santana, S.R. Ananias, E.T. Almeida, A.E. Mauro, M.C.P. Placeres, I.Z. Carlos, J. Braz. Chem. Soc. 18 (2007) 1473–1480.
[4] A.S. Abu-Surrah, M. Kettunen, Curr. Med. Chem. 13 (2006) 1337–1357.
[5] C.S. Allardyce, P.J. Dyson, Platinum Met. Rev. 45 (2001) 62–69.
[6] C. Sirlin, J. Chengebroyen, R. Konrath, G. Ebeling, I. Raad, J. Dupont, M. Paschaki, F. Kotzyba-Hibert, C. Harf-Monteil, M. Pfeffer, Eur. J. Org. Chem. (2004) 1724–1731.
[7] J.S. Casas, E.E. Castellano, J. Ellena, M.S. García-Tasende, M.L. Pérez-Parallé, A. Sánchez, A. Sánchez-González, J. Sordo, A. Touceda, J. Inorg. Biochem. 102 (2008) 33–45.
[8] C. Bincoletto, I.L.S. Tersariol, C.R. Oliveira, S. Dreher, D.M. Fausto, M.A. Soufen, F.D. Nascimento, A.C.F. Caires, Bioorg. Med. Chem. 13 (2005) 3047–3055.
[9] S. Halder, S.M. Peng, G.H. Lee, T. Chatterjee, A. Mukherjee, S. Dutta, U. Sanyal, S. Bhattacharya, New J. Chem. 32 (2008) 105–114.
[10] G. Marverti, M. Cusumano, A. Ligabue, M.L. Di Pietro, P.A. Vainiglia, A. Ferrari, M. Bergomi, M.S. Moruzzi, C. Frassineti, J. Inorg. Biochem. 102 (2008) 699–712.
[11] L. Fuks, N. Sadlej-Sosnowska, K. Samochocka, W. Starosta, J. Mol. Struct. 740 (2005) 229–235.
[12] M. Silva, A.S. Lara, C.Q.F. Leite, E.I. Ferreira, Arch. Pharm. Pharm. Med. Chem. 334 (2001) 189–193.
[13] F.B. do Nascimento, G.V. Poelhsitz, F.R. Pavan, D.N. Sato, C.Q.F. Leite, H.S.S. Araújo, J. Ellena, E.E. Castellano, V.M. Deflon, A.A. Batista, J. Inorg. Biochem. 102 (2008) 1783–1789.
[14] P.P. Dixit, V.J. Patil, P.S. Nair, S. Jain, N. Sinha, S.K. Arora, Eur. J. Med. Chem. 41 (2006) 423–428.
[15] B. Phetsuksiri, M. Jackson, H. Scherman, M. McNeil, G.S. Besra, A.R. Baulard, R.A. Slayden, A.E. DeBarber, C.E. Barry, M.S. Baird, D.C. Crick, P.J. Brennan, J. Biol. Chem. 278 (2003) 53123–53130.
[16] A.C. Moro, F.W. Watanabe, S.R. Ananias, A.E. Mauro, A.V.G. Netto, A.P.R. Lima, J.G. Ferreira, R.H.A. Santos, Inorg. Chem. Commun. 9 (2006) 493–496.
[17] A.C. Moro, A.E. Mauro, S.R. Ananias, Ecletica Quim. 29 (2004) 57–61.
[18] K. Tomita, A.C. Caires, V.A. De Lucca Neto, A.E. Mauro, Acta Crystallogr. C50 (1994) 1872–1873;
A.V.G. Netto, R.C.G. Frem, A.E. Mauro, Mol. Cryst. Liq. Cryst. 374 (2002) 255–260;
A.E. Mauro, A.C.F. Caires, R.H.A. Santos, M.T.P. Gambardella, J. Coord. Chem. 48 (1999) 521–528.
[19] V.A. De Lucca Neto, A.E. Mauro, A.C.F. Caires, S.R. Ananias, E.T. de Almeida, Polyhedron 18 (1998) 413–417.
[20] A.C. Cope, E.C. Friedrich, J. Am. Chem. Soc. 90 (1968) 909–913.
[21] K. Nakamoto, Infrared Spectra of Inorganic and Coordination Compounds, fourth ed. Wiley, New York, 1986.
[22] M.W. Cooke, C.A. Murphy, T.S. Cameron, E.J. Beck, G. Vamvounis, M.A.S. Aquino, Polyhedron 21 (2002) 1235–1244.
[23] E.T. de Almeida, A.E. Mauro, A.M. Santana, S.R. Ananias, A.V.G. Netto, J.G. Ferreira, R.H.A. Santos, Inorg. Chem. Commun. 10 (2007) 1394–1398.
[24] S. Ahmad, A.A. Isab, W. Ashraf, Inorg. Chem. Commun. 5 (2002) 816–819.
[25] A.A. Isab, M. Fettouhi, S. Ahmad, L. Ouahab, Polyhedron 22 (2003) 1349–1354.
[26] S.R. Ananias, A.E. Mauro, K. Zutin, C.M.C. Picchi, R.H.A. Santos, Trans. Metal Chem. 29 (2004) 284–290.
[27] L. Pauling, The Nature of Chemical Bond, Cornell University Press, New York, 1960.
[28] J. Vicente, I. Saura-Llamas, J.A. García-López, B. Calmuschi-Cula, Organometallics 26 (2007) 2768–2776.
[29] J. Vicente, I. Saura-Llamas, M.G. Palin, P.G. Jones, M.C.R. de Arellano, Organometallics 16 (1997) 826–833.
[30] J. Dupont, C.S. Consorti, J. Spencer, Chem. Rev. 105 (2005) 2527–2571.
[31] L. Tusek-Bozic, M. Juribasic, P. Traldi, V. Scarcia, A. Furlani, Polyhedron 27 (2008) 1317–1328.
[32] A. Somoskovi, M.M. Wade, Z. Sun, Y. Zhang, J. Antimicrob. Chemother. 53 (2004) 192–196.
[33] M.C. Burla, R. Caliandro, M. Camalli, B. Carrozzini, G.L. Cascarano, L. De Caro, C. Giacovazzo, G. Polidori, R. Spagna, J. Appl. Crystallogr. 38 (2005) 381–388.
[34] G.M. Sheldrick, SHELXL97. Program for the Refinement of Crystal Structures, University of Göttingen, Göttingen, Germany, 1997.
[35] S. Galli, M.I. Labato, E.B. de Kier Joffé, M.C. Carreras, J.J. Poderoso, Cancer Res. 63 (2003) 6370–6377.
[36] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[37] L.A. Collins, S.G. Franzblau, Antimicrob. Agents Chemother. 41 (1997) 1004–1009.