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Antimycobacterial and antitumor activities of Palladium(II) complexes containing isonicotinamide (isn): x-ray structure of trans-[Pd'('N IND.3') IND.2''(isn) IND.2']

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Original article

Antimycobacterial and antitumor activities of Palladium(II) complexes containing isonicotinamide (isn): X-ray structure of *trans*-[$Pd(N_3)_2(isn)_2$]

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ABSTRACT

Complexes of the type *trans*-[$PdX_2(isn)_2$] {X = Cl (**1**), N_3 (**2**), SCN (**3**), NCO (**4**); isn = isonicotinamide} were synthesized and evaluated for *in vitro* antimycobacterial and antitumor activities. The coordination mode of the isonicotinamide and the pseudohalide ligands was inferred by IR spectroscopy. Single crystal X-ray diffraction determination on **2** showed that coordination geometry around Pd(II) is nearly square planar, with the ligands in a *trans* configuration. All the compounds demonstrated better *in vitro* activity against *Mycobacterium tuberculosis* than isonicotinamide and pyrazinamide. Among the complexes, compound **2** was found to be the most active with MIC of 35.89 μM. Complexes **1**–**4** were also screened for their *in vitro* antitumor activity towards LM3 and LP07 murine cancer cell lines.

1. Introduction

Tuberculosis (TB) is a disease of antiquity and was responsible for about a quarter of all deaths in Europe in the middle of the 19th century [1]. Drugs such as isoniazid (INH) and pyrazinamide (pza) have historically been successful in the treatment of TB infections. However, since the 1980s the disease has been undergoing a resurgence driven by a variety of changes in social, economic and medical factors, and with increasing urbanization in developing countries and the increased mobility of human populations [2]. Current estimates of the World Health Organization (WHO) suggest that one third of the world's population is infected with TB bacteria [3]. TB is now the leading infectious cause of death worldwide and there are an estimated 9.2 million new cases of TB every year [3]. Concomitant with the resurgence of TB it has been observed the occurrence of multi-drug resistant TB, because the treatment involves administration of multi-drug regimen over a long period of time, which leads to patient noncompliance [4]. For all these reasons, there is an urgent need of new potent drugs for TB therapy.

Isonicotinamide (isn) is a pyridine derivative with an amide group in 4-position and possesses a structural analogy with the first-line anti-TB drugs INH and pza (Fig. 1).

Isonicotinamide and their derivatives constitute a class of biologically important compounds. For instance, some isn based-compounds are promising reactivators for the acetylcholinesterase inhibited by sarin [5] and paraoxon organophosphorus agent [6]. Another relevant property of isn is related to its ability to enhance Sirt1 deacetylase activity by competing with the endogenous Sirt1 inhibitor nicotinamide [7]. Taking into account that Sirt1 deacetylase was recently found to both suppress tumorigenesis and cancer growth [8], isonicotinamide represents also a good choice for the design of new antitumor drugs.

Among the compounds used in the cancer chemotherapy, cisplatin is one of the most effective and potent antitumor drugs [9]. Since the introduction of cisplatin in clinical treatments, intensive efforts have been channeled towards the search for cytotoxic compounds with more acceptable toxicity profiles but retentive or even expansive activity. For a long time, the design of new platinum antitumor drugs has mainly concentrated on direct cisplatin analogues. More recently, structures that violate the empirical structure–activity relationships of platinum compounds, such as multinuclear complexes, transplatin derivatives and complexes based on other transition metals [10], are developed to

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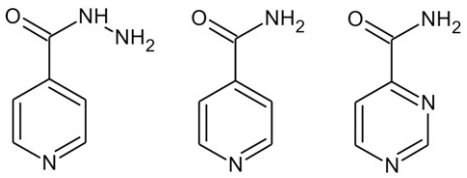

Fig. 1. Structural representation of INH (on the left), isn (center) and pza (on the right).

discover "non-classical" drugs that can act in a different way from cisplatin. Within this context, palladium(II) derivatives were quickly selected due to their structural analogy with square planar platinum(II) complexes.

Despite the fact that antitumor [11], antibacterial [12], anti-trypanosomal [13], antifungal [14], antiherpetic [15], anti-inflammatory [16], antiamoebic [17] and anti-tubercular properties [18] of palladium(II) compounds have been the subject of many reports, studies on the biological activity of isonicotinamide-based Pd(II) complexes remain unknown in literature.

For dichlorobis(isonicotinamide)palladium(II), $[PdCl_2(isn)_2]$, an additional site to be changed in this compound is occupied by chlorido ligands which can be substituted by other anionic ligands. The variation of the labile Cl ligand in metal complexes by pseudohalides has been extensively used [18,19]. Such replacement is expected to decrease the substitution rates for pseudohalides compared with chlorido ligands which may contribute to stabilization and biological activity in some of these Pd(II) species [20].

In the framework of our current research on the coordination and biological chemistry of palladium(II) compounds [21], we synthesized some palladium(II) compounds of the general formulae $[PdX_2(isn)_2]$ (X = Cl, N_3, SCN, NCO; isn = isonicotinamide) and evaluated their antimycobacterial and antitumor activities against TB bacillus and murine tumor cell lines (LP07 and LM3), respectively. The X-ray molecular structure of the compound *trans*-$[Pd(N_3)_2(isn)_2]$ was also described in this work.

2. Chemistry

2.1. Materials

The reagents isonicotinamide (Aldrich), pseudohalide salts (Riedel-de-Haën), $PdCl_2$ (Degussa) and solvents (Merck) were purchased commercially and used without further purification. Literature procedure was followed for the synthesis of $[PdCl_2(MeCN)_2]$ [22].

2.2. Physical measurements

Microanalysis (C, H, and N) was performed by the Central Analítica, Universidade de São Paulo, on a PerkinElmer 2400 CHN elemental analyzer. Melting points were determined on a Microquimica MQAPF-302 apparatus. The infrared spectra were recorded on an FT-IR Impact 400 model of the Nicolet Instrument Corporation spectrophotometer (KBr pellets, 4000–400 cm^{-1}). ^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.

2.3. Synthesis of the complexes

2.3.1. $[PdCl_2(isn)_2]$ (**1**)

To a reddish-brown solution of $[PdCl_2(MeCN)_2]$ (100 mg, 0.385 mmol) in $CHCl_3$ (20 mL) under magnetic stirring, was added a solution of isn (94.0 mg, 0.770 mmol) in CH_3OH (5 mL). After 1 h, the yellow precipitated formed was separated by filtration, washed with CH_3OH and dried in vacuo. Yield: 80%. Elemental anal. Calcd for $C_{12}H_{12}Cl_2N_4O_2Pd$: C, 34.19; H, 2.87; N, 13.29. Found: C, 34.05; H, 2.72; N, 13.03. IR data (KBr, cm^{-1}): νN–H 3412–3186(s); νC=O 1705(s), δNH_2 1614(m). ^1H NMR (DMSO-d_6, ppm): 8.89 [d, ^3J = 6.67 Hz, 4H, $H^{2,6}$]; 8.38 [s, 2H, NH]; 7.90 [s, 2H, NH]; 7.86 [d, ^3J = 6.67 Hz, 4H, $H^{3,5}$]. $^{13}C\{^1H\}$ NMR (DMSO-d_6, ppm): 164.93 [C=O]; 153.53 [$C^{2,6}$]; 141.00 [C^4]; 123.02 [$C^{3,5}$]. Melting point (dec.): >300 °C.

2.3.2. $[Pd(N_3)_2(isn)_2]$ (**2**)

To a solution of $[PdCl_2(MeCN)_2]$ (100 mg, 0.385 mmol) in $CHCl_3$ (20 mL), was added isn (94.0 mg, 0.770 mmol) in CH_3OH (5 mL). Subsequently, 2 mL of H_2O/CH_3OH (1:1) containing NaN_3 (50.1 mg, 0.770 mmol) was added in the mixture that was kept under stirring for 1 h. The dark-yellow suspension was filtered off and the solid was washed with deionized water and CH_3OH, and dried in vacuo. Yield: 85%. Elemental anal. Calcd for $C_{12}H_{12}N_{10}O_2Pd$: C, 33.16; H, 2.78; N, 32.22. Found: C, 33.17; H, 2.74; N, 31.99. IR data (KBr, cm^{-1}): νN–H 3406–3207(m); $\nu_{as}N_3$ 2008(s); νC=O 1670(s), δNH_2 1610(s). ^1H NMR (DMSO-d_6, ppm): 8.89 [d, ^3J = 6.67 Hz, 4H, $H^{2,6}$]; 8.43 [s, 2H, NH]; 7.97 [d, ^3J = 6.67 Hz, 4H, $H^{3,5}$]; 7.77 [s, 2H, NH]. $^{13}C\{^1H\}$ NMR (DMSO-d_6, ppm): 164.77 [C=O]; 152.03 [$C^{2,6}$]; 144.06 [C^4]; 123.91 [$C^{3,5}$]. Melting point (dec.): >220 °C.

2.3.3. $[Pd(SCN)_2(isn)_2]$ (**3**)

This complex was prepared similarly to **2** with the exception that NaSCN was used (62.4 mg, 0.770 mmol). The suspension was filtered off and the orange solid was washed with deionized water and CH_3OH, and dried in vacuo. Yield: 90%. Elemental anal. Calcd for $C_{14}H_{12}N_6O_2S_2Pd$: C, 36.02; H, 2.59; N, 18.00. Found: C, 35.89; H, 2.40; N, 17.91. IR data (KBr, cm^{-1}): νN–H 3369–3205(s); ν_{as}SCN 2118(s); νC=O 1674(s), $\delta(NH_2)$ 1626(s). Melting point (dec.): >300 °C.

2.3.4. $[Pd(NCO)_2(isn)_2]$ (**4**)

Complex **4** was synthesized similarly to **2**, employing KNCO (62.5 mg, 0.770 mmol) instead of NaN_3. After 1 h of magnetic stirring, a light-yellow solid was isolated from the reaction media by filtration, washed with deionized water and CH_3OH, and dried in vacuo. Yield: 90%. Elemental anal. Calcd for $C_{14}H_{12}N_6O_4Pd$: C, 38.68; H, 2.78; N, 19.33. Found: C, 38.26; H, 2.59; N, 19.68. IR data (KBr, cm^{-1}): νN–H 3381–3186(s); ν_{as}NCO 2258(s); νC=O 1707(s), $\delta(NH_2)$ 1612(m). ^1H NMR (DMSO-d_6, ppm): 8.88 [d, ^3J = 6.67 Hz, 4H, $H^{2,6}$]; 8.37 [s, 2H, NH]; 7.89 [s, 2H, NH]; 7.86 [d, ^3J = 6.67 Hz, 4H, $H^{3,5}$]. $^{13}C\{^1H\}$ NMR (DMSO-d_6, ppm): 164.95 [C=O]; 153.14 [$C^{2,6}$]; 144.23 [C^4]; 123.30 [$C^{3,5}$]. Melting point (dec.): >288 °C.

2.4. Crystal structure determination of complex **2**

Single crystals suitable for X-ray analysis were obtained by recrystallization from DMSO. The crystal was mounted on an Enraf-Nonius Kappa-CCD difractometer with graphite monochromated Mo Kα (λ = 0.71073 Å) radiation. The final unit cell parameters were based on all reflections. Data collections were made using the *COLLECT* program [23]; integration and scaling of the reflections were performed with the HKL Denzo-Scalepack system of programs [24]. Absorption corrections were carried out numerically using the Gaussian method [25]. The structure was solved by direct methods with *SHELXS-97* [26]. The model was refined by full-matrix least squares on F^2 by means of *SHELXL-97* [26]. All hydrogen atoms were stereochemically positioned and refined with the ridging model. Data collection and experimental details are summarized in Table 1.

Table 1
Crystallographic data and refinement details for *trans*-$[Pd(N_3)_2(isn)_2]$ (**2**).

Empirical formula	$C_{12}H_{12}N_{10}O_2Pd$
Formula weight	434.72
Temperature (K)	296(2)
Crystal system	Monoclinic
Space group	C2/c
a (Å)	8.3829(4)
b (Å)	9.8649(5)
c (Å)	18.9459(8)
β (°)	92.763(3)
Volume (Å^3)	1564.94(13)
Z	4
Density calculated (g/cm^3)	1.845
Absorption coefficient (mm^{-1})	1.218
F(000)	864
Crystal size (mm^3)	0.182 × 0.150 × 0.115
θ range for data collection (°)	3.19 to 26.00
Index ranges	$-10 \le h \le 10$ $-12 \le k \le 10$ $-22 \le l \le 23$
Reflections collected	5046
Independent reflections (R_{int})	1514 (0.0284)
Data/restraints/parameters	1514/0/116
Goodness-of-fit on F^2	1.071
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0369$, $wR_2 = 0.0924$
R indices (all data)	$R_1 = 0.0480$, $wR_2 = 0.1014$
Largest diff. peak and hole/e Å^{-3}	0.876 and −0.706
Extinction coefficient	0.0032(7)
Completeness to $\theta = 26.00°$	99.1%

CCDC 694418 contains the supplementary crystallographic data for the structure reported in this paper. Copies of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/products/csd/request/, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk.

3. Pharmacology

3.1. Cell culture and MTT assay

LM3 and LP07 cell lines were generously supplied by Prof. Elisa Bal De Kier Joffé from Cell Biology Department, Research Area, Instituto de Oncología 'Angel H. Roffo', Universidad de Buenos Aires, Buenos Aires, Argentina. LM3 and LP07 cells were maintained in MEM (Sigma) supplemented with 10% heat-inactivated FBS, 2 mmol L^{-1} 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 [27]. Passages were made by trypsinization of confluent monolayers (0.25% trypsin and 0.02% EDTA in Ca^{2+}–Mg^{2+} free PBS). Cell number was counted by the Trypan blue dye exclusion method.

Test solutions of the compounds (1000 μmol L^{-1}) were freshly prepared by dissolving the substance in 50 μL of DMSO completed with 4950 μL of culture medium. Afterwards, the tested compounds were diluted in culture medium to reach the final concentrations ranging from 500 to 50 μmol L^{-1}. The DMSO solvent in the concentrations used in test did not reveal any cytotoxic activity.

For the cytotoxicity evaluation (MTT assay), 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 compounds for 24 h to allow adaptation of cells prior to the addition of the test agents. Then, supernatants were removed and 200.0 μL of the compounds in concentrations ranging from 2 to 140 μmol L^{-1} or 200.0 μL of MEM-Complete as cell control of viability was added to each well. The effects of the compounds under the cells were determined 24 h after culture incubation. Then, supernatants were removed and 100.0 μL of solution of [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) was added in each well containing the samples [28]. MTT assay was performed and the plates were incubated for 3 h. Then, absorbances were measured and the cytotoxic midpoint value, the concentration of 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 two independent experiments through the equation of graphic line obtained (Microcal Origin 5.0™). Each compound in a given concentration was tested in triplicates in each experiment.

3.2. Antimycobacterial activity assay

Antimycobacterial activities of each tested compound 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 compound concentrations ranged from 0.15 to 250 μg mL^{-1}. The microplate Alamar Blue assay [29] (MABA) was used to measure the minimal inhibitory concentration (MIC) for the tested compounds (minimum concentration necessary to inhibit 90% growth of *Mycobacterium 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.

4. Results and discussion

The precursor $[PdCl_2(MeCN)_2]$ reacts with isonicotinamide (isn) to afford $[PdCl_2(isn)_2]$ (**1**). Compounds $[Pd(N_3)_2(isn)_2]$ (**2**), $[Pd(SCN)_2(isn)_2]$ (**3**) and $[Pd(NCO)_2(isn)_2]$ (**4**) are readily obtained by metathesis of the chlorido ligands in **1** by azide, thiocyanate and cyanate salts, respectively. A general scheme which represents the strategy employed for the synthesis of the complexes is illustrated in Fig. 2.

4.1. IR and NMR spectroscopy

Isonicotinamide molecule possesses three potential donor sites: (i) pyridine ring nitrogen; (ii) amino nitrogen; (iii) carbonyl oxygen. Among spectroscopic techniques employed to infer the bonding mode of the isonicotinamide [30,31], IR spectroscopy is the most widely used method. The vibrational modes associated

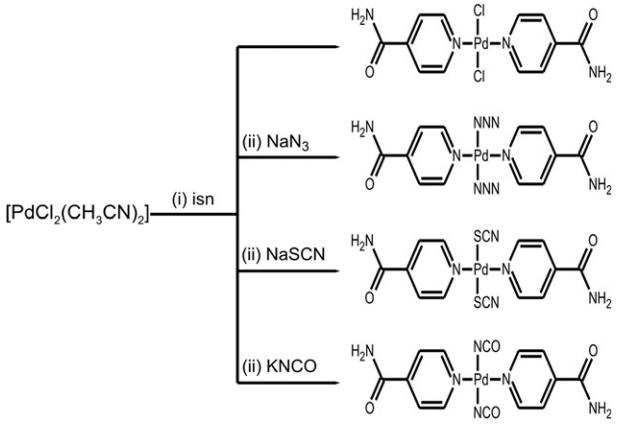

Fig. 2. General scheme for the synthesis of the complexes.

with amide group and pyridine ring give important information about the coordination fashion of the isn ligand. It is well established that, if coordination takes place through carboxyl oxygen, it is expected a shift to lower frequencies of the νC=O band when compared to that one of the free ligand (1666 cm^{-1}) [30–32]. On the other hand, when the amino nitrogen atom is involved in coordination, νNH_2 and δNH_2 absorptions shift to lower frequencies [30,31]. The coordination of the pyridine-type nitrogen shifts to higher frequencies some vibrational modes of the heterocyclic ring (e.g. ring breathing) due to coupling with M–N_{py} bond vibrations [33].

The IR spectra of **1**–**4** exhibited the characteristic bands of amide group at 3412–3186 cm^{-1} (νNH_2), *ca.* 1689 cm^{-1} (νC=O) and ~1615 cm^{-1} (δNH_2). These absorptions remain either unchanged or undergo a slight high frequency shift when compared to those of the free ligand [30,31], indicating that amide group does not take part in coordination. The pyridine ring breathing mode of coordinated isn in **1**–**4** at ~1062 cm^{-1} is shifted to higher frequencies when compared to that observed for free ligand (993 cm^{-1}) [30–32], which is attributed to the coordination of isonicotinamide through pyridine-type nitrogen atom. In addition, the existence of terminally coordinated pseudohalides is evidenced in the IR spectra of **2**, **3** and **4** by the presence of one single and strong band at 2008 cm^{-1} (**2**), 2118 cm^{-1} (**3**) and 2258 cm^{-1} (**4**), assigned to $\nu_{as}N_3$ [34], ν_{as}SCN [35] and ν_{as}NCO [36] modes, respectively.

NMR studies in DMSO-d_6 also support the attachment of isonicotinamide via pyridine-type nitrogen atom. The ^{1}H NMR spectrum of isonicotinamide (isn) consists of two dublets at 8.71 ppm (^3J = 6.0 Hz) and 7.76 ppm (^3J = 6.0 Hz), associated to the two adjacent protons present on either side of the pyridine ring, whereas the two broad signals at 8.23 and 7.70 ppm are attributed to the amide protons. In the ^{1}H NMR spectra of freshly prepared samples of **1**, **2** and **4**, there is a downfield shift of the ring proton signals (8.71–8.89 ppm and 7.76 to *ca.* 7.92 ppm) whereas the two amide hydrogens do not undergo significant shifts. On the other hand, compound **3** displays a very complex NMR spectrum with multiple broad signals over the spectral range 9.3–7.6 ppm, suggesting that **3** is fluxional at room temperature.

Besides the signals of the pyridine ring, the $^{13}C\{^1H\}$ NMR spectra of **1**–**4** showed the carbonyl resonance of amide moiety at *ca.* 165 ppm which is very close to that observed for the free ligand (166.39 ppm), indicating that carbonyl group does not take part in coordination.

4.2. X-ray structure of trans-[Pd(N$_3$)$_2$(isn)$_2$] (**2**)

The MERCURY representation of *trans*-[$Pd(N_3)_2(isn)_2$] (**2**) and a view of the 2D network structure formed by hydrogen bonding are shown in Fig. 3, which was generated by the Mercury 1.4.2 software [37]. Selected bond distances and angles are given in Table 2.

The palladium atom is located on the crystallographic inversion center giving rise to a slightly distorted square planar environment with four nitrogen atoms (two from the azido ligands and two from the isonicotinamide ligands) in *trans* configuration. The angle values are 92.25(15)° for N_4–Pd–N_1 and 87.75(15)° for N_4–Pd–N_1^*. The Pd–N_{isn} bond length of 2.016(3) Å is comparable to that found in *trans*-[$PdCl_2$(nicotinamide)$_2$] (2.017 Å) and *cis*-[Pd(bu$_2$bipy)(isn)$_2$]$^{+2}$ (average 2.03 Å) [38], whereas the Pd–N_{azide} bond distance (2.104 Å) is longer than those found in *trans*-[Pd(N$_3$)$_2$(1-phenyl-3-methylpyrazole)$_2$] (2.031 Å) [39] and *trans*-[Pd(N$_3$)$_2$(quinoline)$_2$] (2.038 Å) [40]. Another structural parameter of interest is the quasi-linearity and the asymmetry observed for the N–N distances in the azido group coordinated to palladium atom (N_1–N_2–N_3 = 174.59(6)°, N_1–N_2 = 1.130(7) Å and N_2–N_3 = 1.180(7) Å). It is worth mentioning that the existence of strong intermolecular N–H···O hydrogen bonds are responsible for the self-assembly of the monomers into a two-dimensional supramolecular array, Fig. 3(b).

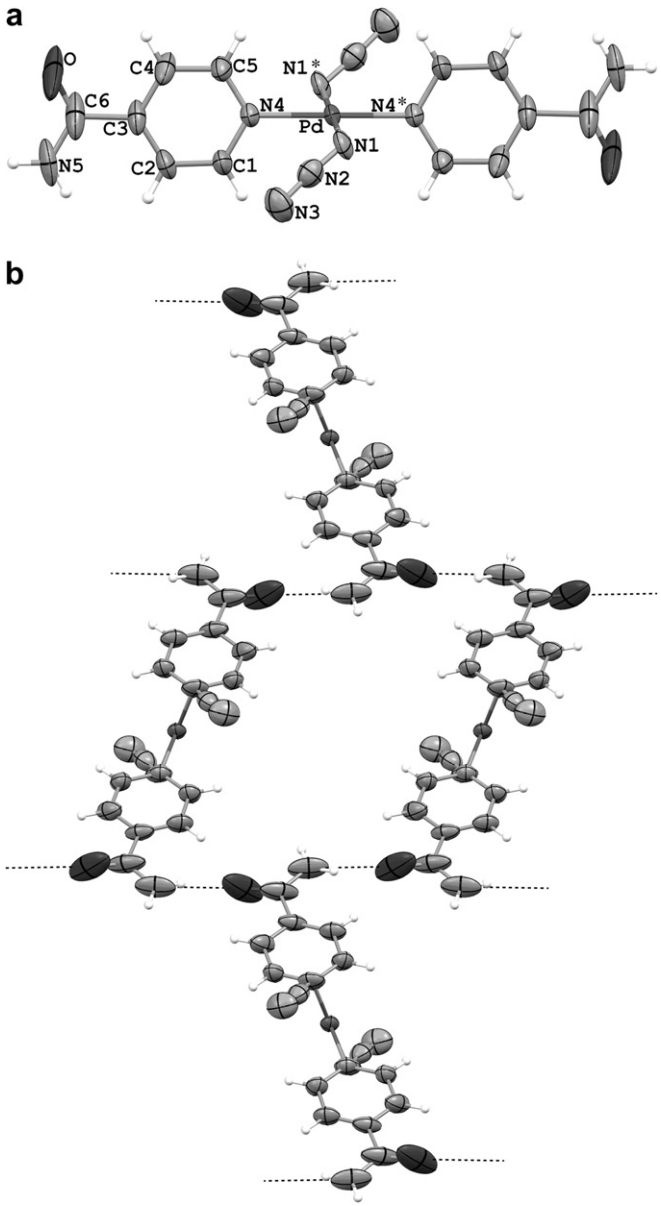

Fig. 3. (a) Mercury representation of *trans*-[$Pd(N_3)_2(isn)_2$] (**2**) showing the labeling of the atoms; (b) View of 2D network structure formed by hydrogen bonding in **2** along *a* axis.

Table 2
Selected bond lengths (Å) and angles (°) for the complex *trans*-[$Pd(N_3)_2(isn)_2$] (**2**).

Pd–N4	2.016(3)		N4–Pd–N4[a]	180.0(3)
Pd–N1	2.104(4)		N4–Pd–N1	92.25(15)
O–C6	1.21(1)		N4–Pd–N1[a]	87.75(15)
N5–C6	1.32(1)		N1–Pd–N1[a]	180.0(3)
N1–N2	1.130(7)		N1–N2–N3	174.59(6)
N2–N3	1.180(7)		Pd–N1–N2	115.3(4)
D–H···A	D···A	H···A	D–H···A	
N–H···O	2.885	2.038	167.98	
C–H···N	3.560	2.685	157.15	

[a] Symmetry transformations used to generate equivalent atoms: 1−x+3/2, −y+1/2, −z.

4.3. Antimycobacterial activity

Isonicotinamide, $[PdCl_2(MeCN)_2]$, complexes **1**–**4**, and a selection of pseudohalide salts (NaN_3, KNCO and NaNCS) were each evaluated for their antiproliferative activities against *M. tuberculosis*. The minimum inhibitory concentration (MIC) values are depicted in Table 3.

As shown in Table 3, isonicotinamide and $[PdCl_2(MeCN)_2]$ were essentially inactive against the pathogen. The coordination of the metal produced compounds which were more active than uncomplexed isonicotinamide and pseudohalide salts, with MIC values ranging from 35.6 to 297 μmol L^{-1}. It was apparent that the potency in the series was affected by the anionic ligand coordinated to Pd(II). The replacement of two chlorido by two thiocyanato (**1** → **3**) or two cyanato ligands (**1** → **4**) did not result in any increase in the anti-tubercular activity. On the other hand, the azido-complex *trans*-$[Pd(N_3)_2(isn)_2]$ (**2**) was *ca.* 8 fold more active than compounds **1**, **3** and **4**. In order to understand possible reasons for the different activity of **2**, the pseudohalides salts NaN_3, KNCO and NaNCS were tested in the same experimental condition. The results showed that NaN_3 inhibits the growth of *M. tuberculosis* at 39 μg mL^{-1} and is approximately threefold more toxic than NaNCS and KNCO salts. Such finding may indicate that coordination of azide ligand exerts an extra effect on the anti-tubercular activity of the isonicotinamide-based Pd(II) complex. Nevertheless, further studies on this class of compounds are required in order to rationalize the obtained MIC values in terms of structure–activity relationship as well as to understand the mechanism of action. It is important to point out that complex **2** is also more effective against *M. tuberculosis* than other palladium compounds of the type [Pd(C^2,*N*-dmba)(X)tu] (dmba = *N*,*N*-dimethylbenzylamine; X = Cl, Br; tu = thiourea) whose MIC values vary from 58 to 89 μmol L^{-1} [18].

It is worth to emphasize that compounds **1**–**4** possess higher inhibitory activity than pyrazinamide (MIC value of 406–812 μmol L^{-1}), used for tuberculosis treatment [41]. On the other hand, none of the synthesized complexes was more active than isoniazid (MIC 0.05 μmol L^{-1}), one of the first-line anti-tubercular drugs [29].

4.4. Antitumor activity

IC_{50} values (the concentration that inhibited in 50% the cellular proliferation) on breast tumor cells (LM3) and lung tumor cells (LP07) are presented in Table 4. For comparison purposes, the cytotoxicity of cisplatin, a standard antitumor drug, was also evaluated under the same conditions.

Despite the fact that the free isonicotinamide is inactive against the tested culture cell lines, its coordination on palladium(II) center resulted in a significant decrease of the IC_{50} values. However, none of the synthesized complexes were more active than cisplatin.

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

Compound	MW	MIC (μmol L^{-1})	MIC (μg mL^{-1})
isonicotinamide	122.12	>2047	>250
NaN_3	65.01	599.9	39
NaSCN	81.08	>1542	>125
KNCO	81.11	>1541	>125
$[PdCl_2(CH_3CN)_2]$	252.43	495.2	125
trans-$[PdCl_2(isn)_2]$ (**1**)	421.58	296.5	125
trans-$[Pd(N_3)_2(isn)_2]$ (**2**)	434.72	35.89	15.6
trans-$[Pd(SCN)_2(isn)_2]$ (**3**)	466.83	267.8	125
trans-$[Pd(NCO)_2(isn)_2]$ (**4**)	434.70	287.5	125
Pyrazinamide[a]	123.11	406.1–812.2	50–100

MW = molecular weight.
[a] Standard drug [41].

Table 4
Cytotoxicity data (IC_{50}) of the isn and their palladium(II) complexes against murine LM3 and LP07 tumor cell lines.

Compound	IC_{50} (μmol/L)	
	LM3	LP07
trans-$[PdCl_2(isn)_2]$ (**1**)	338.58 ± 8.60	325.06 ± 5.89
trans-$[Pd(N_3)_2(isn)_2]$ (**2**)	316.71 ± 10.52	252.81 ± 6.33
trans-$[Pd(SCN)_2(isn)_2]$ (**3**)	325.73 ± 9.47	279.51 ± 8.88
trans-$[Pd(NCO)_2(isn)_2]$ (**4**)	269.48 ± 10.74	317.93 ± 8.14
Isonicotinamide	>500.00	>500.00
Cisplatin[a]	30.26 ± 3.72	4.34 ± 0.45

[a] Standard drug.

5. Conclusions

The synthesis, structural and spectroscopic characterization as well as the biological activity of palladium(II) compounds containing isonicotinamide were described in this work. The monodentate coordination mode of the isonicotinamide ligand via pyridine-like nitrogen atom was clearly evidenced for all prepared complexes. The complex *trans*-$[Pd(N_3)_2(isn)_2]$ (**2**) was the most active palladium(II) compound against *M. tuberculosis* and demonstrated to be more potent than pyrazinamide, a standard drug used for tuberculosis treatment. Evaluation of the antitumor activity indicated that all Pd(II) compounds were less active than cisplatin towards breast tumor cells (LM3) and lung tumor cells (LP07).

Further studies on the antitumor and antimycobacterial activities of palladium(II) and other metal-based derivatives are also underway in our laboratories in order to gain more information about the mechanism of action of these palladium(II) complexes bearing isonicotinamide.

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