Synthesis and Characterization of New Palladium(II) Complexes with Ligands Derived from Furan-2-carbaldehyde and Benzaldehyde Thiosemicarbazone and their *in vitro* Cytotoxic Activities against Various Human Tumor Cell Lines

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With the ligands 4-phenyl-1-(furan-2-carbaldehyde)thiosemicarbazone, $HTSC^1$, (**1**), 4-phenyl-1-(5′-phenyl-furan-2-carbaldehyde)thiosemicarbazone, $HTSC^2$ (**2**), *o*-methoxy-benzaldehydethiosemicarbazone, $HTSC^3$ (**3**), and *o*-cyano-benzaldehydethiosemicarbazone, $HTSC^4$ (**4**), the corresponding palladium(II) complexes, $Pd(TSC^1)_2$ (**5**), $Pd(TSC^2)_2$ (**6**), $Pd(TSC^3)_2$ (**7**), and $Pd(TSC^4)_2$ (**8**) were synthesized and characterized by elemental analysis and spectroscopic techniques. The crystal structure of $Pd(TSC^3)_2$ (**7**) was determined by single-crystal X-ray diffraction. Complex **7** shows a square-planar geometry, where two deprotonated ligands are coordinated to the Pd^{II} center through the nitrogen and sulfur atoms in a *trans* arrangement. *In vitro* antitumor studies against different human tumor cell lines have revealed that the palladium(II) complexes **5**–**8** are more cytotoxic (IC_{50} values in the range of 0.21 – 3.79 μM) than their corresponding ligands (**1** – **4**) (> 60 μM). These results indicate that the antiproliferative activity is enhanced when thiosemicarbazone ligands are coordinated to the metal. Among the studied palladium(II) complexes, **8** exhibits high antitumor activity on K562 chronic myelogenous leukemia cells with a low value of the inhibitory concentration (IC_{50} = 0.21 μM).

Key words: Thiosemicarbazone, Palladium(II) Complexes, Crystal Structures, Antitumor/Cytotoxic Activity

Introduction

The interest to find new compounds derived from thiosemicarbazones and their transition metal complexes has increased because these compounds possess a wide variety of biological activities as antiviral, antimicrobial, anticancer, and antitumor agents [1 – 7].

Reports indicate that the *in vitro* antitumor activity against mammalian cells of chelating agents derived from (*N,N,S*) heterocyclic thiosemicarbazones is due to the inhibition of the ribonucleotide reductase enzyme, which is responsible for the synthesis of DNA precursors [8, 9]. The nature of the heteroatomic ring and the presence of the imine group (-N=CH-) are important factors in the inhibitory action against tumor cell growth [10]. Also, the cobalt, nickel, zinc, chromium, and manganese complexes with 1-(2-furanthiocarba)-3-thiosemicarbazide (*S,S*) and furan-2-carbaldehyde thiosemicarbazone (*N,S*) ligands present antitumor activity *in vitro* and *in*

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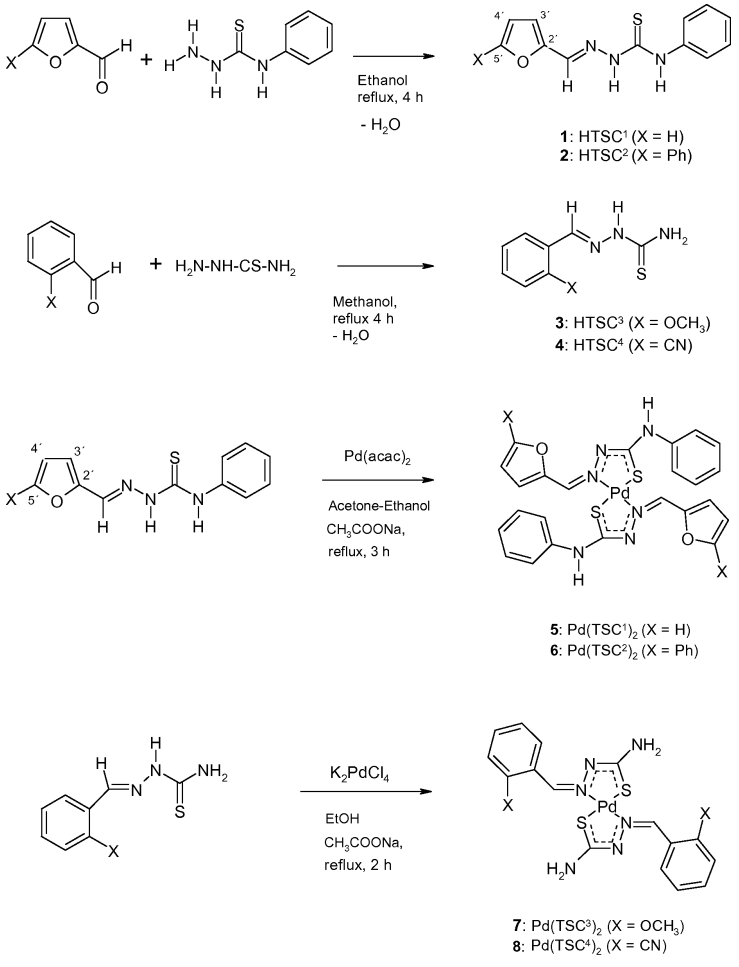

Scheme 1. Synthesis of the ligands derived from furan-2-carbaldehyde and benzaldehyde thiosemicarbazone.

Scheme 2. Synthesis of the palladium(II) bis-chelate complexes of furan-2-carbaldehyde and benzaldehyde thiosemicarbazone.

vivo against several mice and human tumor cell lines [11, 12]. On the other hand, the square-planar palladium(II) and platinum(II) complexes M(L)Cl and ML_2 with (*N,N,S*) ligands derived from 2-formyl and 2-acetyl pyridine thiosemicarbazone show high antiproliferative activity *in vitro* and *in vivo* against different human cancer cell lines and P388 mouse leukemia cells, respectively [13, 14]. Moreover, the octahedral nickel(II) complexes with phenanthrenequinone and 1,2-naphthoquinone thiosemicarbazone (*O,N,S*) of ML_2 type exhibit high cytotoxicity *in vitro* against the T47D and MCF-7 human breast cancer cell lines [15, 16]. Recently, *in vitro* studies of the platinum(II) and palladium(II) bis-chelate complexes with benzaldehyde thiosemicarbazone derivatives against different human tumor cell lines showed that these metal complexes are more cytotoxic than their respective ligands [17]. Probably, the high cytotoxicity of these square-planar palladium(II) complexes may be related to the intercalation between pairs of DNA bases, or to the breaking of DNA strands [18, 19].

The present work describes the synthesis, characterization and antitumor activity of palladium(II) bis-chelate complexes of the type $Pd(TSC^{1-4})_2$ (**5** – **8**) with the ligands 4-phenyl-1-(furan-2-carbaldehyde)-thiosemicarbazone, $HTSC^1$ (**1**), 4-phenyl-1-(5′-phenyl-furan-2-carbaldehyde)thiosemicarbazone, $HTSC^2$ (**2**), *o*-methoxy-benzaldehydethiosemicarbazone, $HTSC^3$ (**3**), and *o*-cyano-benzaldehydethiosemicarbazone, $HTSC^4$ (**4**).

Results and Discussion

Synthesis and characterization

The ligands $HTSC^{1-2}$ (**1**, **2**) derived from furan-2-carbaldehyde thiosemicarbazones, and the ligands

$HTSC^{3-4}$ (**3**, **4**) derived from benzaldehyde thiosemicarbazone were prepared according to the literature [17, 20, 21] as shown in Scheme 1.

The ligands were obtained in good yields (62 – 90 %) and characterized by elemental analysis and IR, and ^1H and ^{13}C NMR spectroscopy. The palladium(II) complexes were obtained in satisfactory yields (48 – 66 %) as shown in Scheme 2 and characterized by IR and ^1H and ^{13}C NMR spectroscopy.

Analytical and spectroscopic data obtained for the thiosemicarbazone ligands and their palladium(II) complexes are in agreement with the proposed structures.

The complex $Pd(TSC^3)_2$ (**7**) was recrystallized from an acetone solution, and single crystals suitable for the structure determination by X-ray diffraction were obtained.

Infrared spectra

The broad ν(N-H) bands of the -NH group observed for the ligands at 3132 – 3292 cm^{-1} have disappeared in the spectra of the corresponding complexes, indicating the deprotonation of the NH-CS group. The strong ν(C=N) bands of the thiosemicarbazones at 1600 – 1620 cm^{-1} are shifted in the spectra of the complexes by about 15 – 25 cm^{-1} towards lower frequencies, indicating coordination of the azomethine nitrogen atoms [22 – 24]. All ligands show medium bands in the 854 – 950 cm^{-1} range, ascribed to ν(C=S) vibrations [25, 26]. These absorption bands are shifted by 27 – 45 cm^{-1} towards lower frequencies for the palladium(II) complexes, suggesting coordination of the thiocarbonyl sulfur atoms [17]. These results indicate that the thiosemicarbazone ligands are coordinated bidentately to the palladium(II) ion through the nitrogen and sulfur atoms.

NMR spectra

The chemical shift (δ) data were extracted from the ^1H NMR and ^{13}C NMR spectra of the ligands and their metal complexes recorded in deuterated dimethylsulfoxide. In the ^1H NMR spectra of the ligands, the =N-NH protons were observed as singlets at δ = 11.42 – 11.97, while on complexation these signals disappeared, thus indicating the deprotonation of the =N-NH group. For all ligands, the signals of the HC=N protons appear as singlets at δ = 8.08 – 8.41 [17, 26]. In the spectra of the complexes, these signals are shifted by 0.07 – 0.21 ppm upfield for the $Pd(TSC^{1-2})_2$ compounds (**5**, **6**), while for $Pd(TSC^3)_2$ (**7**) and $Pd(TSC^4)_2$ (**8**) they are shifted by 0.64 ppm upfield and 0.62 ppm downfield, respectively. These results confirm the coordination of the imine nitrogen to the metal center [17, 24, 27, 28]. The NHPh aromatic proton signals of the $HTSC^1$ (**1**) and $HTSC^2$ (**2**) ligands appear at δ = 7.18 – 7.83, and the resonance lines found correspond to the calculated multiplicity. The resonance lines of the protons corresponding to the furan ring were observed at δ = 6.65 – 7.21, in agreement with the chemical shifts found for other ligands derived from furan-2-carbaldehyde thiosemicarbazone [20, 24, 26]. For the ligands $HTSC^3$ (**3**) and $HTSC^4$ (**4**) the aromatic proton signals are affected by the presence of the methoxy and cyano substituents at the *ortho* position of the phenyl moiety. These signals are shifted upfield for the protons in the *meta* (0.40 ppm) and *para* (0.04 ppm) positions for the TSC^3(**3**) ligand, while for the TSC^4 ligand (**4**) the aromatic proton signals are shifted downfield for the *meta* (0.39 ppm) and *para* (0.15 ppm) positions, relative to the unsubstituted phenyl moiety [17]. The resonance signals of the furan and phenyl protons in all ligands do not suffer relevant changes in the chemical shifts for the complexes. The NH_2 protons of the thioamide group in the ligands $HTSC^3$ (**3**) and $HTSC^4$ (**4**) show doublets in the range of δ = 7.93 – 8.14 and δ = 7.95 – 8.47, respectively, which are attributed to the restricted rotation of the -NH_2 group around the C–N bond, due to the delocalization of the lone pair on the NH_2 nitrogen [17].

In the ^{13}CNMR spectra, the carbon signals of the HC=N group appear at δ = 132.47 – 137.93. These results are similar to the chemical shifts found for the ligands furan-2-carboxaldehydethiosemicarbazone and *m*-cyanobenzaldehydethiosemicarbazone (δ = 132.56 and 139.66, respectively) [17, 20]. The C=S signals observed at δ = 175.64 – 178.64 are characteristic for this thiocarbonyl group in all the ligands. For the ligands $HTSC^1$ (**1**) and $HTSC^2$ (**2**) the resonance lines of the furan carbons appear at δ = 108.54 – 154.83, and these chemical shifts are in agreement with those found for other thiosemicarbazone ligands [29 – 31]. For the ligands $HTSC^3$ (**3**) and $HTSC^4$ (**4**) the aromatic carbons are observed at δ = 109.57 – 157.76.

The ^{13}C NMR spectrum of the complex $Pd(TSC^3)_2$ (**7**) shows a duplication of the signals for the methoxy (δ = 56.03, 55.59), aryl (δ = 158.47 – 111.02), imine (δ = 148.59, 143.99) and thiocarbonyl (δ = 176.81, 174.51) groups, while for the complex $Pd(TSC^4)_2$ (**8**) this duplication of the signals appears for the cyano

(δ = 119.69, 118.06) and aryl (δ = 153.13 – 126.95) groups. These results could indicate that the *cis*- and *trans*-isomers of Pd(TSC^3)$_2$ (**7**) and Pd(TSC^4)$_2$ (**8**) coexist in DMSO solution.

Structural data

The complex Pd(TSC^3)$_2$ (**7**) crystallizes in the monoclinic space group *C*2/*c* with 4 molecules in the unit cell, as a bis-chelate with C_i molecular symmetry (Fig. 1).

The deprotonated ligand coordinates bidentately through the S and N atoms. The coordination of the Pd atom is square planar with a *trans* arrangement of the coordinating atoms. The coordination leads to a lengthening of the S1–C1 bond and to a shortening of the C1–N2 bond. Selected bond lengths and angles are listed in Table 1.

Strong hydrogen bonds between the hydrogen atoms of the amino groups and the N2 atoms of the chelate rings lead to a one-dimensional chain structure, related by centers of inversion (Fig. 2). The bond parameters for this hydrogen bonds are: N1–H2N 0.81 Å, N2···H2N 2.25(2) Å, N1···N2 3.050(2) Å.

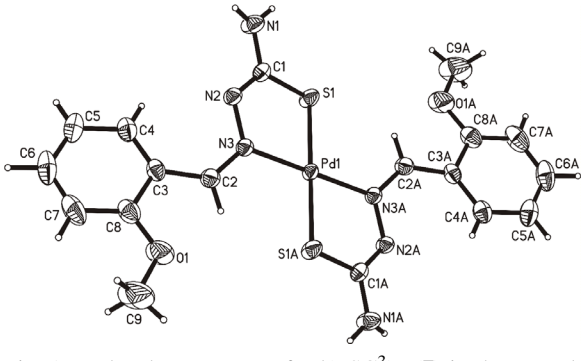

Fig. 1. Molecular structure of Pd(TSC^3)$_2$ (**7**) in the crystal. The displacement ellipsoids are drawn at the 50 % probability level, hydrogen atoms with arbitrary radii.

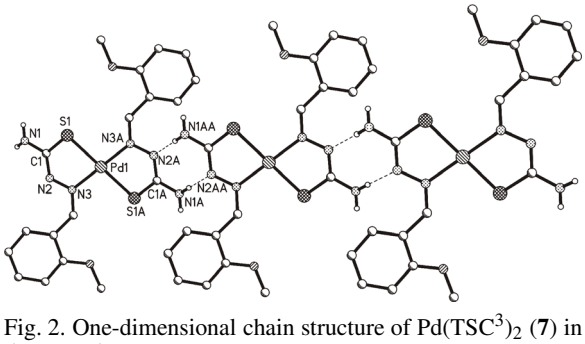

Fig. 2. One-dimensional chain structure of Pd(TSC^3)$_2$ (**7**) in the crystal.

Table 1. Selected bond lengths (Å) and angles (deg) for Pd(TSC^3)$_2$ (**7**).

Distances		Angles	
Pd1–S1	2.2922(5)	S1–Pd1–N3	82.96(4)
Pd1–N3	2.026(1)	Pd1–N3–N2	120.2(1)
N2–N3	1.391(2)	N3–N2–C1	113.5(1)
C1–N2	1.312(2)	N2–C1–S1	124.8(1)
C1–S1	1.745(2)	N2–C1–N1	118.3(2)
C1–N1	1.339(2)	N1–C1–S1	116.9(1)

Antitumor evaluation

The ligands derived from the thiosemicarbazone molecule have IC_{50} values in the range of 60 – 100 μM (micromolar concentration inhibiting 50 % cell growth) against the tested human tumor cell lines. The test results shown in Table 2 indicate that the palladium(II) complexes are more cytotoxic (IC_{50} = 0.21 – 3.79 μm) than their respective thiosemicarbazone ligands [11, 12, 24]. These results reveal that the metal chelates exhibit greater antiproliferative activity than their free ligands, due perhaps to the higher lipophilic character that favors a facile transport through the cellular membrane. Thus the complex may intercalate faster between the nitrogen bases of the DNA, producing conformational changes in the double helix and then causing cell death [17, 32, 33].

The Pd(TSC^2)$_2$ (**6**) and Pd(TSC^4)$_2$ (**8**) complexes show strong cytotoxic activity against all human tumor cell lines, with IC_{50} values of 0.28 – 0.67 and 0.21 – 0.99 μM, respectively. On the other hand, the complexes Pd(TSC^3)$_2$ (**7**) and Pd(TSC^4)$_2$ (**8**) are more cytotoxic at low micromolar concentrations (IC_{50} = 0.69 and 0.21 μM, respectively) against the (K562) human chronic myelogenous leukemia cell line, when compared to the palladium(II) bis-chelate complexes of benzaldehyde and *o*-nitro benzaldehyde thiosemicarbazone (IC_{50} = 6.15 and 3.04 μM, respectively). Probably, the presence of the *o*-methoxy and *o*-cyano substituents in the benzene ring may be an important factor in the activity. In addition, the Pd(TSC^1)$_2$ (**5**) and Pd(TSC^2)$_2$ (**6**) complexes (IC_{50} = 0.30 and 0.31 μM, respectively) tested *in vitro* against the (K562) human chronic myelogenous leukemia cell line appeared to be more cytotoxic with respect to other palladium(II) complexes of the acetone Schiff bases of *S*-methyl and *S*-benzyldithiocarbazate (IC_{50} = 5.84 and 5.00 μM, respectively) assayed in the human T-lymphoblastic leukemia cell line [34]. Pd(TSC^1)$_2$ (**5**) and Pd(TSC^2)$_2$ (**6**) (IC_{50} = 1.05 and 0.29 μM, respectively) tested *in vitro* against the (HT-29) colon adeno-

Table 2. IC_{50} (μM) values of the palladium complexes $Pd(TSC^{1-4})_2$ (**5**–**8**) against the different human tumor cell lines[a].

Human tumor cell lines	$Pd(TSC^1)_2$ (**5**)	$Pd(TSC^2)_2$ (**6**)	$Pd(TSC^3)_2$ (**7**)	$Pd(TSC^4)_2$ (**8**)
Lung large cell carcinoma (H460)	1.23	0.32	3.79	0.99
Prostate carcinoma (DU145)	1.54	0.67	2.02	0.55
Breast adenocarcinoma (MCF-7)	1.15	0.28	1.58	0.42
Amelanotic melanoma (M-14)	1.65	0.46	2.74	0.88
Colon adenocarcinoma (HT-29)	1.05	0.29	1.08	0.34
Chronic myelogenous leukemia (K562)	0.30	0.31	0.69	0.21

[a] IC_{50} corresponds to the concentration required to inhibit 50 % of the cell growth when the cells are exposed to the compounds during 48 h. Each value is the average of two independent experiments.

carcinoma cell line were more cytotoxic with respect to the nickel(II) complex of 5-methyl-2-furaldehyde thiosemicarbazone (IC_{50} = 37 μM) assayed *in vitro* and *in vivo* against the (CaCo-2) mice tumor cell line [12]. With respect to other nickel(II) and cobalt(II) bis-chelate complexes of octahedral geometry (ML_2) of tridentate phenanthrenequinone thiosemicarbazone (*O,N,S*) ligands (IC_{50} = 1.92 and 2.04 μM, respectively) assayed in the T47D human breast cancer cell line [15], the complex $Pd(TSC^4)_2$ (**8**) assayed *in vitro* against the MCF-7 human breast adenocarcinoma cell line turned out to be about ten times more cytotoxic than the nickel(II) and cobalt(II) complexes.

In summary, we have synthesized the palladium(II) bis-chelate complexes with ligands derived from furan-2-carbaldehyde and benzaldehyde thiosemicarbazone. The molecular structure of $Pd(TSC^3)_2$ (**7**) shows a square-planar geometry with two deprotonated ligands coordinated to Pd(II) through the nitrogen and sulfur atoms in a *trans* arrangement. The palladium(II) complexes are more cytotoxic at low micromolar concentrations compared to the free ligands.

Experimental Section

Materials and measurements

Chemicals were reagent grade. Palladium(II) bis(acetylacetonate), potassium tetrachloropalladate, thiosemicarbazide, 4-phenylthiosemicarbazide, furan-2-carbaldehyde, 5-phenyl-furan-2-carbaldehyde, *o*−cyano-benzaldehyde, and *o*-methoxy-benzaldehyde were purchased from Aldrich. Melting points were determined on a Büchi melting point B-545 apparatus. Elemental analyses were determined on a Fisons-Carlo Erba Elemental Microanalyzer. Infrared spectra were recorded as KBr pellets (4000 – 400 cm^{-1}) on a Bruker FT-IR IFS 55 Equinox spectrophotometer. NMR spectra were recorded on a Bruker Avance DRX 300 spectrometer in [D_6]DMSO operating at 300 and 75.5 MHz (^{1}H, ^{13}C). The chemical shifts were measured in ppm relative to tetramethylsilane.

Synthesis of the ligands

General method

To a hot solution of 4-phenyl thiosemicarbazide (3.3 g, 20 mmol) or thiosemicarbazide (1.8 g, 20 mmol) in ethanol (70 mL) or methanol (100 mL) was added dropwise a solution of a furan-2-carbaldehyde derivative (20 mmol) in 50 mL of ethanol, or a solution of a benzaldehyde thiosemicarbazone derivative (20 mmol) in 60 mL of methanol during 30 min. The mixture was refluxed for 3 – 4 h and stirred for 24 h at r. t. The solid product was filtered, washed severals times with cold ethanol and dried *in vacuo*. The ligand $HTSC^2$ (**2**) was obtained as large yellow needles after slow evaporation of a dichloromethane-methanol mixture. For the ligand $HTSC^3$ (**3**), the final reaction mixture was kept in the refrigerator, and after several days small colorless needles were obtained. Crystals suitable for X-ray crystallography were obtained by recrystallization from hot acetone.

*4-Phenyl-1-(furan-2-carbaldehyde) thiosemicarbazone, $HTSC^1$ (**1**)*

Colorless solid. Yield 82 %, m. p. 160 – 162 °C. – Anal. for $C_{12}H_{11}N_3OS$ (245.30 g/mol): calcd. C 58.76, H 4.52, N 17.13, S 13.07; found C 58.81, H 4.64, N 17.26, S 12.91. – IR (KBr): ν = 3296 (NHPh), 3132 (NH), 1620 (C=N), 925 (C=S) cm^{-1}. – ^{1}H NMR ([D_6]DMSO): δ = 6.65 (t, H^4, J = 3.3 Hz), 7.08 (d, H^3, J = 3.3 Hz), 7.85 (d, H^5, J = 3.3 Hz), 7.18 (t, $2H_{meta}$, NHPh, J = 8.7 Hz), 7.35 (t, $1H_{para}$, NHPh, J = 7.5 Hz), 7.57 (d, $2H_{ortho}$, NHPh, J = 9.0 Hz), 8.08 (s, 1H, HC=N), 9.86 (s, 1H, NHPh), 11.97 (s, 1H, =N-NH). – ^{13}C NMR ([D_6]DMSO): δ = 112.49, 113.26, 145.26, 149.39 (C^4,C^3,C^5,C^2; furan ring), 125.49, 128.26, 138.94 (NHPh), 132.92 (HC=N), 175.64 (C=S).

*4-Phenyl-1-(5'-phenyl-furan-2-carbaldehyde) thiosemicarbazone, $HTSC^2$ (**2**)*

Yellow needles. Yield 62 %, m. p. 178 – 180 °C. – Anal. for $C_{18}H_{15}N_3OS$ (321.40 g/mol): calcd. C 67.2, H 4.70, N 13.07, S 9.98; found C 67.00, H 4.94, N 13.33, S 9.81. – IR (KBr): ν = 3290 (NHPh), 3159 (NH), 1618 (C=N), 950 (C=S) cm^{-1}. – ^{1}H NMR ([D_6]DMSO): δ = 7.16 (dd, H^4, J = 3.7 Hz), 7.21 (d, H^3, J = 3.7 Hz), 7.24 (m, $4H_{meta}$, NHPh, J =

7.8 Hz), 7.60 (d, 2H$_{para}$, NHPh, J = 7.8 Hz), 7.83 (d, 4H$_{ortho}$, NHPh, J = 7.8 Hz), 8.10 (s, 1H, HC=N), 9.95 (s, 1H, NHPh), 11.92 (s, 1H, =N-NH). – ^{13}C NMR ([D$_6$]DMSO): δ = 108.54, 115.87, 148.99, 154.83 (C^4,C^3,C^5,C^2; furan ring), 125.40, 128.30, 129.52, 139.04 (NHPh), 124.07, 125.41, 128.34, 129.52 (phenyl-furan ring), 132.47 (HC=N); 175.65 (C=S).

*o-Methoxy-benzaldehyde thiosemicarbazone, HTSC^3 (**3**)*

Colorless crystals. Yield 82 %, m. p. 220 – 222 °C. – Anal. for $C_9H_{11}N_3OS$ (209.27 g/mol): calcd. C 51.65, H 5.30, N 20.08, S 15.32; found C 51.75, H 5.50, N 20.10, S 15.04. – IR (KBr): ν = 3410 (NH$_2$), 3292 (NH), 1600 (C=N), 874 (C=S) cm^{-1}. – ^{1}H NMR ([D$_6$]DMSO): δ = 3.81 (s, 1H, OCH$_3$), 8.08 (d, 1H$_{ortho}$, Ph, J = 7.8 Hz), 7.04 (d, 1H$_{meta}$, Ph, J = 7.8 Hz), 6.95 (t, 1H$_{meta}$, Ph, J = 7.8 Hz), 7.36 (t, 1H$_{para}$, Ph, J = 7.8 Hz), 8.41 (s, 1H, HC=N), 8.14, 7.93 (d, 2H, NH$_2$), 11.42 (s, 1H, =N-NH). – ^{13}C NMR ([D$_6$]DMSO): δ = 55.68 (OCH$_3$), 157.76, 131.33, 126.56, 122.18, 120.56, 111.64 (Ph), 137.93 (HC=N), 177.86 (C=S).

*o-Cyano-benzaldehyde thiosemicarbazone, HTSC^4 (**4**)*

Colorless solid. Yield 90 %, m. p. 210 – 212 °C. – Anal. for $C_9H_8N_4S$ (204,25 g/mol): calcd. C 52.92, H 3.95, N 27.43, S 15.70; found: C 52.83, H 4.05, N 27.19, S 14.85. – IR (KBr): ν = 3377 (NH$_2$), 3238 (NH), 2222 (C≡N), 1615 (C=N), 854 (C=S) cm^{-1}. – ^{1}H NMR ([D$_6$]DMSO): δ = 8.23 (d, 1H$_{ortho}$, Ph, J = 8.0 Hz), 7.86 (d, 1H$_{meta}$, Ph, J = 8.0 Hz), 7.71 (t, 1H$_{meta}$, Ph, J = 8.0 Hz), 7.55 (t, 1H$_{para}$, Ph, J = 8.0 Hz); 8.34 (s, 1H, HC=N), 8.47, 7.95 (d, 2H, NH$_2$), 11.79 (s, 1H, =N-NH). – ^{13}C NMR ([D$_6$]DMSO): δ = 117.78 (C≡N), 136.71, 133.66, 133.29, 129.95, 127.55, 109.57 (Ph), 137.72 (HC=N); 178.64 (C=S).

Synthesis of the palladium(II) complexes

General method

A solution of Pd(acac)$_2$ (0.153 g, 0.5 mmol) in acetone-ethanol (2 : 1, 90 mL), or a solution of K_2PdCl_4 (0.163 g, 0.5 mmol) in water-ethanol (2 : 1, 30 mL) was added dropwise to a stirred hot solution of the 2-furaldehyde thiosemicarbazone derivate (1.0 mmol) in 80 mL of acetone-ethanol or of the benzaldehyde thiosemicarbazone derivate in ethanol (60 mL). Sodium acetate (0.082 g, 1 mmol) in 5 mL of water was then added. The solution was refluxed for 2 – 3 h and stirred for 24 h at r. t. The precipitate was collected by filtration, washed three times with cold ethanol (50 mL) and dried under vacuum. For the complex Pd(TSC^3)$_2$ (**7**), crystals suitable for X-ray crystallography were obtained by slow evaporation from an acetone solution at r. t. after two weeks.

*Bis(4-phenyl-1-(furan-2-carbaldehyde) thiosemicarbazonato)palladium(II), Pd(TSC^1)$_2$ (**5**)*

Square-shaped red crystals. Yield 53 %, m. p. 235 – 236 °C. – IR (KBr): ν = 3415 (NH$_2$), 1604 (C=N), 880 (C=S) cm^{-1}. – ^{1}H NMR ([D$_6$]DMSO): δ = 6.70 (dd, 2H^3), 6.74 (dd, 2H^4), 7.95 (d, 2 H^5), 7.09 (t, 4H$_{meta}$, NHPh), 7.35 (t, 2H$_{para}$, NHPh), 7.52 (dd, 4H$_{ortho}$, NHPh), 8.01 (s, 2H, HC=N), 9.86 (s, 2H, NHPh).

*Bis(4-phenyl-1-(5′-phenyl furan-2-carbaldehyde) thiosemicarbazonato)palladium(II), Pd(TSC^2)$_2$ (**6**)*

Red solid. Yield 66 %, m. p. 284 – 285 °C. – IR (KBr): ν = 3421 (NH$_2$), 1601 (C=N), 910 (C=S) cm^{-1}. – ^{1}H NMR ([D$_6$]DMSO): δ = 6.87 (t, 2H^4), 6.98 (d, 2 H^3); 7.13, 7.06 (m, 10 H, 5-phenyl-furaldehyde ring), 7.19 (t, 4H$_{meta}$, NHPh), 7.38 (t, 2H$_{para}$, NHPh), 7.85 (d, 4H$_{ortho}$, NHPh), 7.89 (s, 2H, HC=N), 9.54 (s, 2H, NHPh).

*Bis(o-methoxy-benzaldehyde thiosemicarbazonato)-palladium(II), Pd(TSC^3)$_2$ (**7**)*

Needle-shaped orange crystals. Yield 55 %, m. p. > 251 °C (decomp.). – IR (KBr): ν = 3375 (NH$_2$), 1585 (C=N), 847 (C=S) cm^{-1}. – ^{1}H NMR ([D$_6$]DMSO): δ = 3.84 (s, 1H, OCH$_3$), 8.71 (d, 2H$_{ortho}$, Ph), 7.09 (d, 2H$_{meta}$, Ph), 6.95 (t, 2H$_{meta}$, Ph), 7.42 (t, 2H$_{para}$, Ph), 7.77 (s, 2H, HC=N), 7.13 (s, 4H, NH$_2$). – ^{13}C NMR ([D$_6$]DMSO): δ = 56.03, 55.59 (OCH$_3$); 158.43, 158.36, 133.05, 132.51, 132.46, 120.49, 120.07, 119.99, 111.14, 111.02 (Ph), 148.59, 143.99 (HC=N), 176.81, 174.51 (C=S).

*Bis(o-cyano benzaldehyde thiosemicarbazonato)-palladium(II), Pd(TSC^4)$_2$ (**8**)*

Orange solid. Yield 48 %, m. p. > 240 °C (decomp.). – IR (KBr): ν = 3419 (NH$_2$), 2216 (C≡N), 1590 (C=N), 815 (C=S) cm^{-1}. – ^{1}H NMR ([D$_6$]DMSO): δ = 8.63, 7.60, 7.23 (m, Ph), 8.96 (s, 2H, HC=N), 8.19 (s, 4H, NH$_2$). – ^{13}C NMR ([D$_6$]DMSO): δ = 119.69, 118.06 (C≡N), 153.13, 152.23, 135.99, 134.35, 133.82, 128.25, 126.95, 124.78, 104.96 (Ph), 143.78 (HC=N), 163.92 (C=S).

Crystal structure determination

The intensities were measured on a Stoe IPDS1 diffractometer and were corrected for Lorentz and polarization effects, and for absorption using the program DIFABS [35]. The relevant crystallographic data are listed in the Table 3. The structure was solved by Direct Methods, and refined with full-matrix least-squares procedures using SHELX-97 [36].

CCDC 777497 contains the supplementary crystallographic data for this paper. These data can be obtained free

Table 3. Crystal data and parameters pertinent to data collection and structure refinement for $Pd(TSC^3)_2$ (**7**).

Empirical formula	$C_{18}H_{20}N_6O_2S_2Pd$
Formula weight	522.92
Temperature, K	213(2)
Crystal size, mm^3	$0.3 \times 0.1 \times 0.1$
Crystal system	monoclinic
Space group	*C*2/*c*
a, Å	16.044(1)
b, Å	10.0538(8)
c, Å	13.7987(7)
β, deg	110.923(9)
Volume, $Å^3$	2079.0(3)
Z	4
Density, g cm^{-3}	1.67
Absorption coeff., mm^{-1}	1.1
θ range for data collect., deg	2.5 – 26.0
Index range *hkl*	±19, ±12, ±16
Reflections collected	8055
Independent refl. / R_{int}	1964 / 0.0291
Max. / min. transmission	0.7297 / 0.8962
Data / ref. parameters	1964 / 173
Final *R* indices R_1 / wR_2 [$I \geq 2\,\sigma(I)$]	0.0175 / 0.0419
R indices R_1 / wR_2 (all data)	0.0291 / 0.0427
Goodness-of-fit on F^2	0.850
Lgst. diff. peak / hole, e $Å^{-3}$	0.26 / −0.38

of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

Biological activity

Cell culture

The H460 (human lung large cell carcinoma), M-14 (human amelanotic melanoma), DU145 (human prostate carcinoma), MCF-7 (human breast adenocarcinoma), HT-29 (human colon adenocarcinoma), and K562 (human chronic myelogenous leukemia) cell lines were obtained from the research laboratory of the Faculty of Sciences and Philosophy, Universidad Peruana Cayetano Heredia. Cells were cultured in DMEM medium supplemented with 10 % fetal calf serum and 50 μg mL^{-1} gentamycin at 37 °C in 5 % CO_2 / 95 % air.

Assessment of cytotoxicity

Growth inhibition was evaluated by preparing serial dilutions in DMSO of the ligands or palladium(II) complexes (100 – 0.10 μM) and incubating the cells in 96-well plates in the presence or absence of these fractions for 48 h at 37 °C. The percent of inhibition of cell growth relative to the control essay was evaluated colorimetrically using the sulforhodamine B dye, according to a published procedure, with comparison to the control [37]. The IC_{50} value was defined as the concentration of a test sample resulting in a 50 % reduction of absorbance as compared with untreated controls that received a serial dilution of the solvent in which the test samples were dissolved, and was determined by linear regression analysis.

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