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New palladium(II) complexes with pyrazole ligands

Part I. Synthesis, spectral and thermal studies, and antitumor evaluation

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Abstract The pyrazole ligand 3,5-dimethyl-4-iodopyrazole (HdmIPz) has been used to obtain a series of palladium(II) complexes (**1–4**) of the type $[PdX_2(HdmIPz)_2]$ {X = Cl^- (**1**); Br^- (**2**); I^- (**3**); SCN^- (**4**)}. All compounds have been isolated, purified, and characterized by means of elemental analysis, IR spectroscopy, ^1H and $^{13}C\{^1H\}$-NMR experiments, differential thermal analysis (DTA), and thermogravimetry (TG). The TG/DTA curves showed that the compounds released ligands in the temperature range 137–605 °C, yielding metallic palladium as final residue. The complexes and the ligand together with cisplatin have been tested in vitro by MTT assay for their cytotoxicity against two murine cancer cell lines: mammary adenocarcinoma (LM3) and lung adenocarcinoma (LP07).

Keywords Palladium(II) · Pyrazoles · IR and NMR spectroscopy · Thermal behavior

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Introduction

A rich coordination and supramolecular chemistry have been developed around pyrazolyl ligands since they can interact with metals in many different ways. They usually coordinate to metals through the 2-N (the pyridine nitrogen), in a neutral monodentate mode. When deprotonated, pyrazole becomes the pyrazolate ion, which can coordinate through the pyridine-type nitrogen, in an anionic monodentate mode, or through both nitrogen atoms as exo/endobidentate anionic modes. The nucleophilicity of the nitrogen and their accessibility may be fine tuned by appropriate ring substitution [1–5]. In addition, the hydrogen bonding donor capability of the pyrrolic nitrogen atom has been widely employed in the self-assembly of new metallosupramolecules sustained by coordination and/or hydrogen bonds [6–9].

In particular, mononuclear Pd(II) and Pt(II) pyrazolyl compounds have attracted considerable attention in recent years because of their promising antitumor activity. Budzisz et al. have reported that the *trans*-$[PdCl_2L_2]$ complex (L = 5-(2-hydroxyphenyl)-1,3-dimethyl-4-methoxycarbonyl-1H]-2-pyrazole) showed significant cytotoxicity against the HL-60 and NALM-6 leukemia cell lines [10]. Keter et al. have demonstrated that the $[PtCl_2L_2]$ (L = pyrazole) compound induces apoptosis in Jurkat, CaSki, and HeLa human cancer cells [11].

As part of our ongoing studies on structure [12–17], thermal behavior [18–23] and biological activity [24–27] of organometallic and coordination compounds, we report herein the synthesis, spectroscopic characterization and thermal investigation of the compounds $[PdCl_2(HdmIPz)_2]$ (**1**), $[PdBr_2(HdmIPz)_2]$ (**2**), $[PdI_2(HdmIPz)_2]$ (**3**), $[Pd(SCN)_2(HdmIPz)_2]$ (**4**) (HdmIPz=3,5-dimethyl-4-iodopyrazole). The complexes and the ligand together with cisplatin have been

tested in vitro by MTT assay for their cytotoxicity against two murine cancer cell lines: mammary adenocarcinoma (LM3) and lung adenocarcinoma (LP07).

Experimental

General comments

All the synthesis have been carried out at room temperature. All reagents were obtained from commercial suppliers. $[PdCl_2(MeCN)_2]$ was prepared according to the literature procedures [26].

Synthesis of the 3,5-dimethyl-4-iodopyrazole (HdmIPz)

The 3,5-dimethyl-4-iodopyrazole was synthesized and isolated in a similar manner to the work-up described for 4-iodopyrazole, using 3,5-dimethylpyrazole instead of pyrazole [28]. Yield: 80%. M.p. 137–140 °C. Anal. Calcd. for $C_5N_2H_7I$ (%): C, 27.05; N, 12.62; H, 3.18. Found: C, 26.99; N, 12.53; H, 3.24. ^{1}H NMR (dmso-d_6, 25 °C): 2.09 (6H, s, $-CH_3^{(3)/(5)}$), 12.60 ppm (1H, s, NH). $^{13}C\{^1H\}$ NMR (dmso-d_6, 25 °C): 11.26 ($CH_3^{(5)}$), 13.73 ($CH_3^{(3)}$), 61.95 (C–I), 140.78 ($C^{(5)}$), 148.97 ppm($C^{(3)}$).

Synthesis of the complexes

The compound $[PdCl_2(HdmIPz)_2]$ (**1**) was prepared as follows: 0.39 mmol (86 mg) of 3,5-dimethyl-4-iodopyrazole, in 2 mL of CH_3OH, was added to an orange solution of $[PdCl_2(MeCN)_2]$ (50 mg; 0.19 mmol) dissolved in 10 mL of CH_3OH, affording a yellow suspension. The solid was isolated by filtration, washed with cold CH_3OH, and dried under vacuum. Yield 68%. Compounds **2–4** were readily obtained by metathetical reactions of the $[PdCl_2(HdmIPz)_2]$ with salts of the appropriate anions in methanol media. The resulting suspensions were filtered off and the solid were washed with CH_3OH and dried under vacuum. Yield 60–85%.

Instrumentation

Elemental analyses of carbon, nitrogen, and hydrogen were performed on a microanalyzer elemental analyzer CHN, model 2400 Perkin–Elmer. Infrared spectra were recorded in KBr pellets on a Nicolet model SX-FT-Impact 400 spectrophotometer in the 4000–400 cm^{-1} spectral range. ^{1}H- and $^{13}C\{^1H\}$-NMR spectra were recorded in dmso-d_6 solutions at room temperature on a Varian INOVA 500 spectrometer. Melting points were determined on an MAPFQ apparatus. Thermogravimetry (TG) and differential thermal analysis (DTA) were carried out using a TA *Instruments* model SDQ 600, under flow of dry synthetic air (50 mL min^{-1}), temperature up to 900 °C, and at heating rate of 20 °C min^{-1} in α-alumina sample holders. The reference substance was pure α-alumina in DTA measurements. X-ray powder diffraction patterns were measured on a Siemens D-5000 X-ray diffractometer using CuK_α radiation ($\lambda = 1.541$ Å) and setting of 34 kV and 20 mA. The peaks were identified using ICDD bases [29].

In vitro antitumor assays

Cytotoxic activity studies have been done according to the literature procedures [30].

Results and discussion

The analytical results confirmed the proposed formulae for the synthesized compounds. The results of the analyses and melting points are presented in Table 1.

Infrared spectra

The main IR data of the complexes are presented in Table 2. The neutral monodentate coordination of pyrazolyl ligands was evidenced by the presence of intense *v*NH bands over the spectral range 3230–3080 cm^{-1}. The shift of the *v*NN band to higher frequencies ($\Delta v \cong 60$–125 cm^{-1}) compared to that of the free ligands indicates a strengthening of the N–N bond as a result of the interaction between palladium(II) and pyridine-like nitrogen atom [31]. The ν_{as}SCN vibration for **4** appears as an intense and very sharp band at 2121 cm^{-1}, being consistent with terminal *S*-bonded thiocyanate [32]. This frequency value agrees well with those observed for analogous $[Pd(SCN)_2L_2]$ complexes (L = pyrazole,1-phenyl-3-methylpyrazole,3,5-dimethylpyrazole) which molecular structures were determined by single crystal X-ray diffraction [33, 34].

Table 1 Results of chemical analyses and melting points of the compounds **1–4**

Complex	m.p./°C	Found/calcd./%		
		C	H	N
1	252 (dec)	19.73/19.33	2.19/2.27	9.13/9.02
2	242 (dec)	17.05/16.91	2.05/1.99	7.99/7.89
3	214 (dec)	14.89/14.93	1.85/1.75	7.04/6.97
4	212 (dec)	21.42/21.62	2.27/2.21	12.24/12.61

Table 2 Selected infrared frequencies (cm^{-1}) for HmIPz and compounds **1–4**

	*v*NH	*v*CH	νCH_3	ν_{ring}	δCH_3	*v*NN	γ_{ring}
HdmIPz	3200–2400 *br*		[a]	1575 m	1463 m	1034 s	590 w
1	3180 s	–	2970 w	1560 m	1473 m	1091 m	658 m
2	3190 s	–	2970 w	1560 m	1473 m	1092 m	654 m
3	3230 s	–	2970 w	1554 m	1469 m	1084 m	656 m
4	3160 s	–	2960 w	1572 m	1477 m	1094 m	622 w

v stretching, δ in-plane bending, γ out-of-plane bending. *s* strong, *m* medium, *w* weak, *sh* shoulder

[a] Uncovered

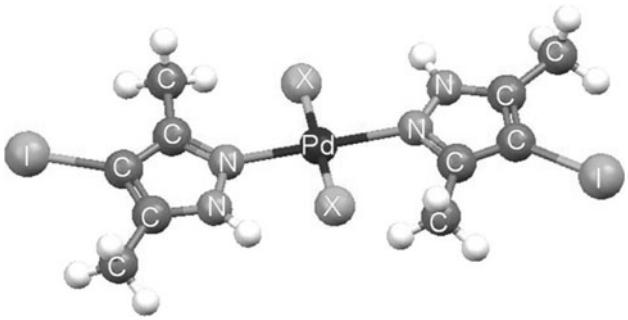


Fig. 1 Proposed structure of the compounds $[PdX_2(HdmIPz)_2]$ (X = Cl (**1**), Br (**2**), I (**3**), SCN (**4**))

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

The chemical shifts and assignment of NMR experiments are summarized in Table 3.

The ^1H-NMR spectra show that the pyrazole ligand is coordinated in a neutral monodentate fashion due to the non-equivalence of the methyl groups at positions 3 and 5 as well by the appearance of one single signal at ca. 13–14 ppm attributed to NH group. The $^{13}C\{^1H\}$-NMR data agree well with the neutral monodentate character of the pyrazole-type ligands. The additional ^{13}C signal at ~117 ppm, observed in the spectra of **4**, is assigned to the carbon atom from the *S*-thiocyanato group [35].

The analytical, IR, and NMR results obtained for compounds **1–4** suggest a square planar environment around the Pd atom whose coordination sites are occupied by two pyrazolyl ligands and two anionic X^- groups (X = Cl, Br, I, SCN). The *trans* configuration is attributed to these complexes on the basis of the known X-ray structures of their $[PdX_2(L)_2]$ analogs [33, 34, 36] (Fig. 1).

Thermogravimetric analysis

The TG curves for the complexes **1–4** are illustrated in Fig. 2. Table 4 lists the initial and final temperatures (°C),

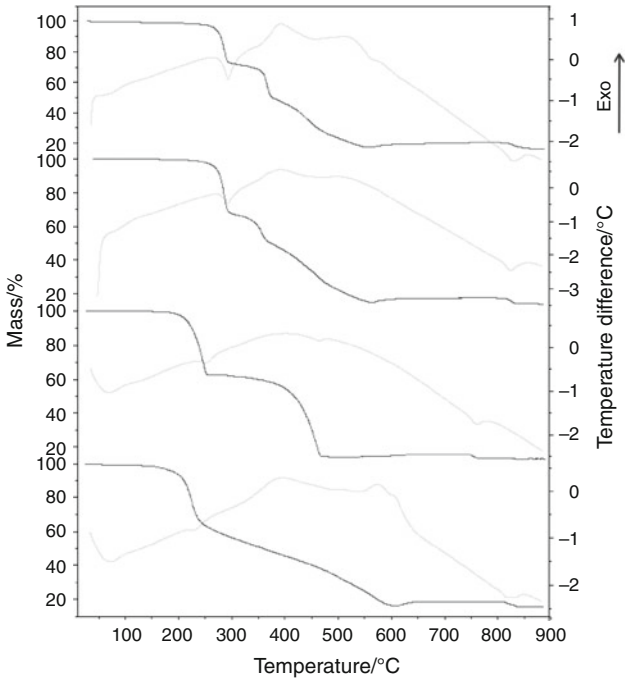
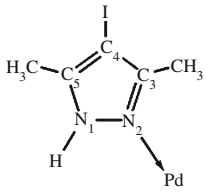


Fig. 2 TG and DTA curves of the complexes $[PdX_2(HdmIPz)_2]$ (X = Cl (**1**), Br (**2**), I (**3**), SCN (**4**))

Table 3 ^1H and $^{13}C\{^1H\}$-NMR data (ppm) for compounds **1–4** at 298 K, in dmso-d_6

Numbering scheme		^1H-NMR data*			$^{13}C\{^1H\}$-NMR data					
		NH	R_3	R_5	C_3	C_4	C_5	R_3	R_5	SCN
	**9**	13.87	2.61	2.25	150.91	64.24	145.67	15.55	11.77	–
	10	13.87	2.61	2.25	150.94	64.21	145.67	15.55	11.77	–
	11	13.78	2.53	2.27	151.21	63.87	145.99	16.65	11.71	–
	12	14.18	2.61	2.27	151.08	65.53	147.95	15.31	11.85	117.23

* All signals were found as singlets

[a] The $^{13}C\{^1H\}$-spectra of **5–8** could not be recorded

Table 4 Thermal analysis data for compounds $[PdX_2(HdmIPz)_2]$ (X = Cl (**1**), Br (**2**), I (**3**), SCN (**4**))

Complex	Step	ΔT/°C	Δm/%		DTA Peaks/°C		Assignment
			Obt.	Calc.	Endo	Exo	
1	1	251–292	−26.07	−26.13	288	–	–I, $–Cl^-$
	2	292–382	−24.84	−26.13	–	–	–I, $–Cl^-$
	3	382–556	−32.63	−30.62	–	390	–Organic compounds
	4	556–812	2.88	2.57	–	–	$0.5O_2$
	5	812–868	−3.01	−2.57	830	–	$-0.5O_2$
	Residue		16.46	17.14			
2	1	225–290	−29.74	−29.12	290	–	–I, $–Br^-$
	2	290–562	−55.22	−55.90	389	–	–Organic compounds, $–Br^-$
	3	562–811	2.39	2.25	–	–	$0.5O_2$
	4	811–865	−3.09	−2.25	827	–	$-0.5O_2$
	Residue		14.33	14.99			
3	1	173–251	−40.41	−43.38	–	–	$–I^-$, –HdmIPz
	2	251–471	−45.21	−43.38			$–I^-$, –HdmIPz
	2	471–744	1.14	1.99	–	–	$0.5O_2$
	3	744–820	−2.66	−1.99	764	–	$-0.5O_2$
	Residue		12.91	13.24			
4	1	137–605	−83.98	−84.02	–	392, 577	$–2SCN^-$, –2HdmIPz
	2	605–806	2.36	2.40	–	–	$0.5O_2$
	3	806–865	−2.82	−2.40	828	–	$-0.5O_2$
	Residue		15.45	15.98			

partial mass losses (%), and DTA peaks of the thermal studies on compounds **1**–**4** together with the assignments of each decomposition stage based on mass calculation. Therefore, the groups indicated at the right column of the Table 2 do not correspond necessarily to the gaseous final products of decomposition. The thermal decomposition of the studied complexes is a multi-stage process. After the pyrolysis of the ligands, all TG curves exhibited a slight mass increase up to ca. 800 °C due to the oxidation of the remaining Pd^0 to PdO. The X-ray powder diffractograms of the final products, obtained after the decomposition of PdO, showed the characteristic peaks of Pd (ASTM 05-0681).

The first (251–292 °C) and the second (292–382 °C) decomposition steps observed in TG curve of **1** are attributed, by mass calculation, to a loss of the iodo atoms from the HdmIPz ligands and two chloride. In the DTA curve, these processes are associated with an endothermic peak at 288 °C. The third step takes place between 382 and 556 °C and is accompanied by 32.63% of mass loss. It is assigned to the pyrolysis of the remaining organic fragment (Calcd. 30.62%), being associated with an exothermic peak at 390 °C. A slight mass increase of 2.88%, between 556 and 812 °C, is ascribed to the partial oxidation of Pd^0 to PdO which further degrades to Pd^0 in the last mass loss (3.01%) at 812–868 °C, accompanied by an endotherm at 830 °C.

The TG curve indicates that **2** is thermally stable up to 225 °C. Afterward, the release of the ligands takes place in two steps. In the first, observed in the 225–290 °C range, one iodo atom from the HdmIPz ligand and one bromine atom (29.74%) are released (Calcd. 29.12%). The loss of the remaining organic compounds and the bromide ligand occurs in the second step (55.22%), between 290 and 562 °C (Calcd. 55.90%). The DTA curve presents endothermic peaks at 290 and 389 °C corresponding to the elimination of the ligands. A progressive mass gain of 2.39% up to 811 °C is ascribed to the formation of additional PdO which further decomposed into Pd (Calcd.14.99%, Found 14.33%) at 811–865 °C.

Compound **3** started to decompose at 173 °C. A further heating to 251 °C resulted in an intermediate decomposition product attributed, by mass calculation, to the formation of the compound $[Pd_2I_3(HdmIPz)_3]$ (Calcd. 56.62%, Found 59.47%). The weight loss of 45.21% between 251 and 471 °C agrees well with the loss of one HdmIPz ligand and an iodide (Calcd. 43.38%), affording only Pd^0 as final product. The increase of mass (1.14%) observed at 471–744 °C is due to the oxidation of Pd^0 to PdO by uptake of O_2 (Calcd. 1.99%). The decomposition of PdO to Pd^0 is noticed in the last mass loss (2.66%) between 744 and 820 °C (Calcd. 1.99%), being associated with an endothermic peak at 764 °C.

The thermal decomposition of complex **4** started at lower temperature (137 °C) than the other compounds. Afterward, a mass change of 83.98% is caused by the complete elimination of the thiocyanate groups and HdmIPz ligands (Calcd. 84.02%). This process is accompanied by two exotherm peaks at 392 and 577 °C. In the range 605–806 °C the partial oxidation of Pd to PdO is observed. The last step corresponds to the decomposition of the remaining PdO to Pd at 806–865 °C. The final mass percent residue of 15.45% agrees well with the calculated amount of Pd (15.98%).

In vitro antitumor assays

The cytotoxic activities of HdmIPz and its palladium(II) complexes were tested against murine mammary adenocarcinoma (LM3) and lung adenocarcinoma (LP07) cell lines. Cells were exposed to a range of drug concentrations (140–20 μM) for 24 h and cell viability was analyzed by MTT assay. The cytotoxicity of cisplatin, a standard antitumor drug, was also evaluated under the same conditions. The results reveal that no significant cytotoxic activity was observed even at the highest concentration tested (140 μM). Cisplatin was able to induce 50% of cell death (IC_{50}) at 30.3 μM (LM3) and 4.34 μM (LP07).

Conclusions

The thermal decomposition of $[PdCl_2(HdmIPz)_2]$ (**1**), $[PdBr_2(HdmIPz)_2]$ (**2**), $[PdI_2(HdmIPz)_2]$ (**3**), $[Pd(SCN)_2(HdmIPz)_2]$ (**4**) has been described in this study. We have also reported the synthesis, spectroscopic characterization, and in vitro antitumor evaluation of the complexes. The thermal stability of the complexes (on the basis of the initial decomposition temperatures) can be ordered in the sequence **1** > **2** > **3** > **4**. The pyrazole ligand was coordinated in the neutral monodentate mode. This series of palladium(II) complexes does not display cytotoxicity on the tested tumor murine cell lines.

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