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Original article

New palladium(II) complexes bearing pyrazole-based Schiff base ligands: Synthesis, characterization and cytotoxicity

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ABSTRACT

Reactions of 5-hydrazino-1,3-dimethyl-4-nitro-1*H*-pyrazole (**1**) with substituted benzaldehydes (**2**–**5**) in methanol gave the new substituted benzaldehyde (1,3-dimethyl-4-nitro-1*H*-pyrazol-5-yl)hydrazone Schiff base ligands (**6**–**9**) benzaldehyde (1,3-dimethyl-4-nitro-1*H*-pyrazol-5-yl)hydrazone (**H-BDH, 6**), 2,3-dimethoxybenzaldehyde (1,3-dimethyl-4-nitro-1*H*-pyrazol-5-yl)hydrazone (**MeO-BDH, 7**), 4-chlorobenzaldehyde (1,3-dimethyl-4-nitro-1*H*-pyrazol-5-yl)hydrazone (**Cl-BDH, 8**), and 4-hydroxybenzaldehyde (1,3-dimethyl-4-nitro-1*H*-pyrazol-5-yl)hydrazone (**OH-BDH, 9**) in moderate to excellent yields. Reactions of these pyrazole-based Schiff bases with $[PdCl_2(NCPh)_2]$ in acetone at room temperature gave the *trans*-palladium(II) complexes *trans*-$[PdCl_2(L)_2]$ (**10**–**13**) (L = **6**–**9**). The isolated compounds were characterized by their physical properties, elemental analysis, IR-, MS (EI)- and NMR-spectroscopy. The cytotoxic effect of these complexes against the fast growing head and neck squamous carcinoma cells SQ20B and SCC-25 has been studied. The influence was dose dependent and varies by cell type. The complexes **11**, **12**, and **13** had higher clonogenic cytotoxic effect than cisplatin when tested on SQ20B cell line.

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1. Introduction

The significant similarity between the coordination chemistry of palladium(II) and platinum(II) compounds has advocated studies of Pd(II) complexes as antitumor drugs. A key factor that might explain why platinum is most useful comes from the ligand-exchange kinetics. The hydrolysis in palladium complexes is too rapid: 10^5 times faster than for their corresponding platinum analogues [1]. They dissociate readily in solution leading to very reactive species that are unable to reach their pharmacological targets.

Compared to cisplatin, the corresponding cispalladium, *cis*-$[PdCl_2(NH_3)_2]$ does not show antitumoral activity. It is well known that it undergoes an inactive *trans*-conformation and that the two compounds hydrolyze very fast assuming that they interact *in vivo* with a lot of molecules particularly proteins preventing them to reach the DNA, their pharmalogical target [2].

The considerably higher activity of palladium complexes implies that if an antitumor palladium drug is to be developed, it must somehow be stabilized by a chelate or a strongly coordinated, bulky monodentate nitrogen ligand and a suitable leaving group [3,4]. Due to the steric effect that results from the bulk on the donor atoms, these ligands could minimize any possible *cis*-*trans* isomerism and insure the direct separation of the desired *trans*-Pd isomers [5]. In general, research results indicated that most of the *trans*-palladium complexes showed a better activity than the *cis*-platinum isomers and superior activity than that of the *cis*-palladium isomers. More importantly, they showed activities equal to (or superior than) those of cisplatin, carboplatin, and oxaliplatin (the anticancer drugs in clinical use) *in vitro* [3].

Studies of platinum and palladium compounds with biologically active carriers have yielded promising results in the field of anticancer chemistry and there is potential for varying the biological activity of these complexes by changing the structure of the carrier [6]. Significant advances have emerged from this methodology of design [1,3].

Recently, we reported about the synthesis and molecular structure of an enantiometrically pure, *trans*-palladium(II) complex, *trans*-$[PdCl_2\{(R)$-(+)-bornylamine$\}_2]$ that bears the bulky amine ligand *R*-(+)-bornylamine (*endo*-(1R)-1,7,7-trimethylbicyclo[2-2-1]-heptan-2-amine) [5]. The complex showed similar antitumor activity against HeLa cells when compared with the activity of the standard references, cisplatin, carboplatin and oxaliplatin.

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Complexes bearing naturally occurring compounds have also been utilized. The palladium complex which contains the bulky nitrogen ligand harmine (7-methoxy-1-methyl-9H-pyrido[3,4-b]indole), *trans*-[$PdCl_2$(harmine)(DMSO)] exhibits a greater cytotoxic activity against P388, L1210 and K562 cell lines than cisplatin [7].

Pyrazole arylhydrazones were designed as mixed-hybrid isosteres of two known inhibitors of prostaglandin synthase and 5-lipoxygenase enzymes, BW-755c and CBS-1108 [8]. Some derivatives of these compounds inhibit the *in vitro* platelet aggregation of citrated platelet-rich rabbit plasma. The compound, *p*-methoxyformylbenzene-5-(1-phenyl-3-methyl-4-nitropyrazolyl)hydrazone, which contains a methoxy group at the *p*-position of the aryl part, was found to be the most active with 62% inhibition of aggregation [9]. The antiedematogenic properties and structure-activity relationship of several 5-N-phenylpyrazole arylhydrazone derivatives have been evaluated, the compound 5-(1-phenyl-3-methyl-4-nitropyrazolyl)-based aryl hydrazone showed the best profile as antiedematogenic agent [10].

Several studies of pyrazole-based complexes of palladium(II) and platinum(II) have been reported [11]. However, as far as we know, the coordination chemistry of pyrazole arylhydrazones with palladium has not been investigated. As an extension of our studies on both the coordination chemistry of heteroatom containing ligands [12,13] and on the biological activity [5,14,15] of their metal complexes, we describe here the synthesis and characterization of new N-methylpyrazol-5-arylhydarazonyl-based Schiff base ligands and their corresponding square-palnar palladium(II) complexes. To confirm the identity of the compounds prepared in the present study, a variety of techniques including elemental analysis, MS (EI), IR, ^{1}H- and ^{13}C-NMR spectroscopy have been used. The aim of our study was to investigate the influence of those bulky carriers on the initial cytotoxic properties of the *trans*-palladium(II) complexes against human head and neck sqamous carcinoma cells.

2. Results and discussion

2.1. Chemistry

Reaction of 5-hydrazino-1,3-dimethyl-4-nitro-1*H*-pyrazole (**1**) with one equivalent of the desired arylaldehyde (**2**–**5**) in EtOH at 25 °C readily yields the hydrazone Schiff base ligands **6**–**9** in a moderate to high yield (Scheme 1). IR- and ^{1}H-NMR spectral features agree with the formula assignments. The IR spectra of these ligands (**6**–**9**) indicate the formation of the Schiff base product by the absence of the carbonyl group (1700 cm^{-1}) band and the appearance of a strong band in the region of 1614–1622 cm^{-1}, assignable to the ν(C═N)$_{imine}$ group [13].

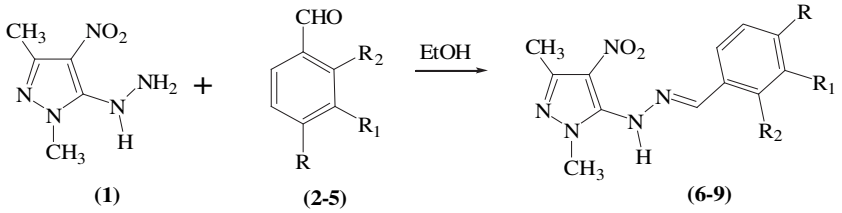
The ^{1}H-NMR spectra (Table 1) of the ligands in DMSO-d_6 showed a singlet in the range between 8.30 and 8.50 ppm which can be ascribed to the imine protone (HC═N) [13]. In addition, a singlets at around 2.4, 4.1, and 11.0 ppm have also been observed which can be attributed to the methyl groups of the pyrazol ring, C–CH_3 and N–CH_3, and NH, respectively. The ^{13}C-NMR spectra of the ligands exhibit a singlet at about 142 ppm which can be assigned to the imine (HC═N) carbon that is close to the phenyl ring.

The mass spectra of the Schiff base ligands showed the base peaks at m/z 259, 319, 293, and 275 which correspond to the original molecular weight of the ligands **6**, **7**, **8**, and **9**, respectively.

The palladium(II) complexes (**10**–**13**) were prepared by treating the starting material, [$PdCl_2$(NCPh)$_2$] with two equivalents of the corresponding ligand in acetone at room temperature (Scheme 2). The reaction with one equivalent of the ligand led also to the formation of the palladium complexes but with lower yields. The isolated compounds are microcrystalline or powder-like and stable at atmospheric conditions. A variety of techniques including elemental analysis, IR-, ^{1}H- and ^{13}C-NMR-spectroscopy have been used to characterize the new complexes. Attempts to analyze the complexes by mass spectroscopy (EI) were not successful. This may be due to the non-volatility of the palladium(II) complexes. They showed a decomposition behavior at around 290 °C.

Elemental analyses of the complexes (**10**–**13**) showed that the metal to the pyrazole ligand ratio in the dichloro complexes is 1:2. The presence of the ligands in the complexes was confirmed by IR-analyses. An insignificant shift of the peak due to the imine bond (υ(C═N)) was observed indicating complexation of the ligands with palladium. Due to geometrical reasons and the bulkiness of the ligand, we believe that the *trans* geometrical isomer is more favorable than the *cis*. This was indicated by the presence of the IR-band at 628 $cm^{-}1$ which can be assigned to υ(Pd–N) [16,17]. If they were *cis* complexes, two well defined bands should appear. The self-arrangement of such bulky monodentate ligands towards the *trans* isomer has also been observed in many palladium(II) complexes containing bulky monodentate ligands [5,7].

The solution behavior of the complexes was determined by NMR spectroscopy in DMSO-d_6 at room temperature (Table 1). The coordination of the bulky Schiff base ligands in the complexes most probably takes place *via* N-pyrazole, as this lone pair of electrons is more available for coordination than that of N═CH or NH due to geometrical and steric reasons [7a]. The ^{1}H-NMR experiments showed that the δ (HC═N) and δ (N–H) of the free ligands (8.5 and 11.0, respectively) remains almost unchanged upon coordination with palladium. Furthermore, only a minor shift for the singlet due to methyl group of the pyrazole ring (4.0 ppm) could be observed compared with the free ligand. This was also confirmed by ^{13}C-NMR; no change in the chemical shift for the carbon atom in the imine HC═N (∼142.5–142.9 ppm) for free ligand was observed upon complexation (Table 1), which indicate that the imine nitrogen is not involved in coordination. On the contrary, a slight



2, 6 : R, R_1, R_2 = H
3, 7 : R = H, R_1, R_2 = OCH_3
4, 8 : R = Cl, R_1, R_2 = H
5, 9 : R = OH, R_1, R_2 = H

Scheme 1. Synthesis of the hydrazino pyrazole ligands (**6**–**9**).

Table 1
NMR data of compounds **6–13**.

Compound	^{1}H-NMR[a]	^{13}C-NMR[a]
6	2.38 (s, 3H C–CH_3), 4.13 (s, 3H, N–CH3), 8.44 (s, 1H, N=CH), 11.1 (s, 1H, NH)	14.6 (N=C–CH_3), 142.9 (N=CH), 145.9 (N–C–NH)
7	2.38 (s, 3H, C–CH_3), 4.14 (s, 3H, N–CH_3), 8.50 (s, 1H, N=CH), 11.0 (s, 1H, NH)	14.4 (N=C–CH_3), 142.4 (N=CH), 143.5 (N=C–CH_3), 145.4 (N–C–NH), 153.1 (C–OCH_3)
8	2.35 (s, 3H C–CH_3), 4.0 (s, 3H, N–CH_3), 8.49 (s, 1H, N=CH), 11.04 (s, 1H, NH)	14.4 (N=C–CH_3), 142.7 (N=CH), 143.6 (N=C–CH_3), 145.6 (N–C–NH)
9	2.37 (s, 3H C–CH_3), 4.11 (s, 3H, N–CH_3), 8.33 (s, 1H, N=CH), 11.05 (s, 1H, NH)	14.4 (N=C–CH_3), 142.7 (N=CH), 143.6 (N=C–CH_3), 147.3 (N=C–NH), 159.7 (C–OH)
10	2.35 (s, 3H C–CH_3), 4.02 (s, 3H, N–CH_3), 8.51 (s, 1H, N=CH), 11.03 (s, 1H, NH)	n.d.[b]
11	2.35 (s, 3H C–CH_3), 4.0 (s, 3H, N–CH_3), 8.50 (s, 1H, N=CH), 11.1 (s, 1H, NH)	14.6 (N=C–CH_3), 142.9 (N=CH), 143.8 (N=C–CH_3), 145.6 (N–C–NH), 153.3 (C–OCH_3)
12	2.35 (s, 3H C–CH_3), 4.0 (s, 3H, N–CH_3), 8.50 (s, 1H, N=CH), 11.1 (s, 1H, NH)	14.4 (N=C–CH_3), 142.5 (N=CH), 143.9 (N=C–CH_3), 145.6 (N–C–NH)
13	2.37 (s, 3H C–CH_3), 4.11 (s, 3H, N–CH_3), 8.47 (s, 1H, N=CH), 11.1 (s, 1H, NH)	14.5 (N=C–CH_3), 142.9 (N=CH), 143.8 (N=C–CH_3), 147.5 (N–C–NH), 159.8 (C–OH)

[a] 25 °C, in DMSO-d_6, δ [ppm].
[b] n.d. not determined due to low solubility of the Pd(II) complex.

shift to the signal due to the imin carbon of the pyrazole ring (N=C–CH_3) was observed (143.8 ppm).

2.2. Biological investigations

The effect of palladium(II) complexes on human head and neck squamous carcinoma cell survival, SQ20B and SCC has been studied and compared with commercial drug, cisplatin. The cells were treated with two different doses (0.2, 0.5 μM) for 24 h and analyzed for clonogenic survival. The results showed that at 0.2 μM, palladium(II) complexes (**11**, **12**, and **13**) or cisplatin did not significantly alter SCC cell survival compared to control (Fig. 1A). The maximum cell killing was observed with complex (**11**). Further increasing the dose of cisplatin to 0.5 μM caused significant cell killing compared to control or palladium(II) complexes (Fig. 1B).

SQ20B cells were more sensitive to palladium(II) complexes treatment compared to SCC, with significant cell killing observed at the dose of 0.2 and 0.5 μM with 20 and 60% killing respectively as compared to either control or cisplatin (Figs. 2A,B). Furthermore, SQ20B cells were more sensitive to cisplatin treatment at 0.5 μM consistent with previous results [18]. At this concentration, the palladium(II) complexes **11** and **12** showed similar cytotoxic effect but fairly higher than that observed for **13**.

These results showed that the cytotoxic effect of the palladium(II) complexes (**11**, **12**, and **13**) on SQ20B head and neck cancer cells is dose dependent and varies by cell type. The slight differences among the cytotoxic influence of the complexes under investigation indicate that different substituents on the ligands (OMe (**7**), Cl (**8**), and OH (**9**)) had insignificant influence on the coordinated nitrogen donor atom and consequently on the electronic properties of the palladium metal center. This observation is coincide with the results observed in the IR-analysis of the palladium(II) complexes. Only slight difference was observed in the shift for the peak due to the imine bond (ν(C=N) in the complexes.

3. Experimental

3.1. Chemistry

3.1.1. Materials and physical measurements

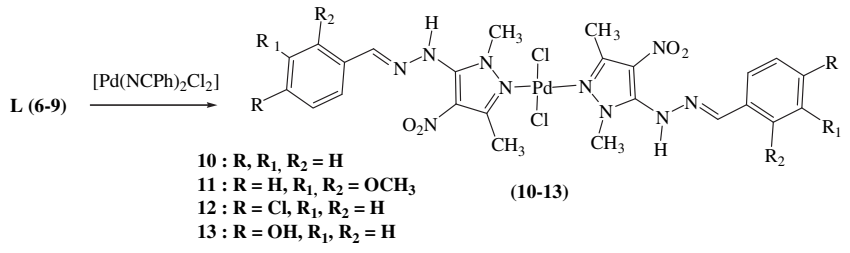
Reagent grade chemicals were used as received unless otherwise stated. $[PdCl_2(PhCN)_2]$ was prepared according to a literature procedure [19].

Elemental analyses were performed using a (EURO EA 3000 instrument). ^{1}H-NMR spectra were recorded on a Bruker spectrometer operating at 300 MHz using DMSO-d_6 as a solvent with TMS as an internal standard. ^{13}C-NMR spectra were obtained on a Bruker spectrometer operating at 75 MHz using DMSO-d_6 as a solvent. Infrared spectra (KBr discs) were measured on a Nicolet-Magna-IR 560 Spectrophotometer. Mass spectra (EI) were acquired using a Shimadzu-QP5050A. Melting points were measured by a Stuart Scientific melting Apparatus (uncorrected ±0.1 °C).

3.1.2. General procedure for the synthesis of ligands

To a solution of 5-hydrazino-1,3-dimethyl-4-nitro-1*H*-pyrazole (**1**) (0.34 g, 2.0 mmol) in absolute ethanol (100 ml), arylaldehydes (**2–5**) (2.0 mmol) was added. The solution was refluxed for 3 h. Ethanol was evaporated, and the solid product was collected to afford the desired products (**6–9**) as yellowish to orange solids.

*3.1.2.1. H-BDH (**6**).* Yield, 0.52 g (83%). M.p. (dec.) 246 °C. Found: C, 55.36; H, 5.21; N, 27.50. Anal. Calc. for $C_{12}H_{13}N_5O_2$: C, 55.59; H, 5.05; N, 27.01. IR (KBr, cm^{-1}): ν = 3421 (m), 3255 (m), 1619 (s). ^{1}H-NMR (DMSO-d^6, ppm): δ = 11.1 (s, NH), 8.44 (s, HC=N), 7.94–7.74



Scheme 2. Synthesis and suggested structure of the palladium(II) complexes (**10–13**).

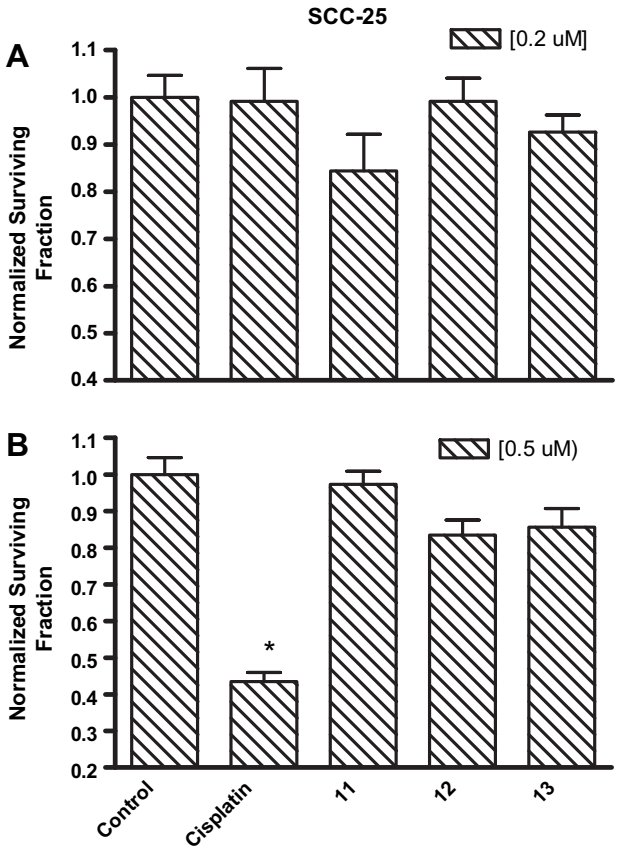


Fig. 1. Effect of palladium(II) complexes (**11**–**13**) compared to cisplatin on survival of SCC-25 cells. The cells were treated with 0.2 (A) and 0.5 (B) μM of the palladium(II) complexes and cisplatin for 24 h and then plated for clonogenic survival. Clonogenic cell survival data were normalized to control. Error bars represent ± standard error of the mean of $N = 3$ experiments performed with at least three cloning dishes taken from one treatment dish. $^{*}p < 0.05$ versus respective control.

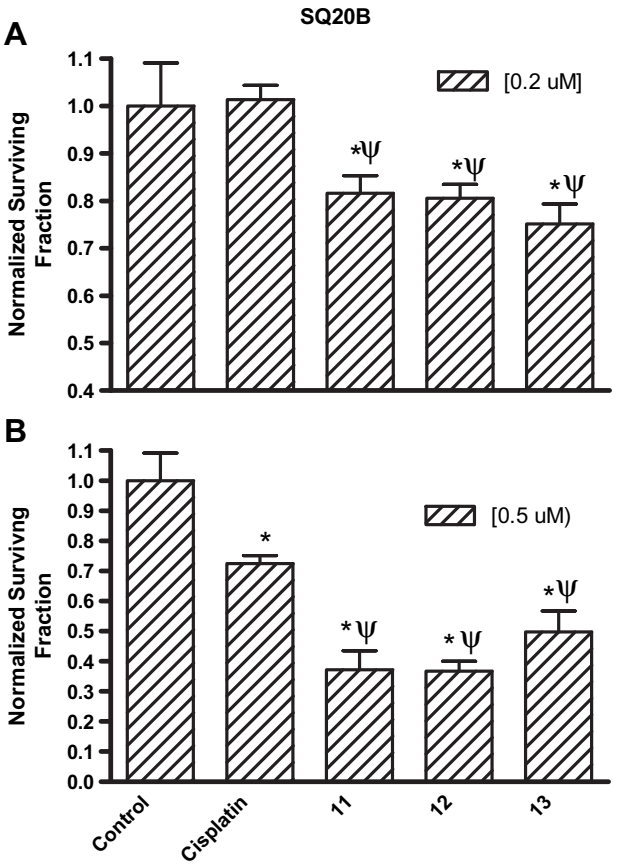


Fig. 2. Effect of palladium(II) complexes (**11**–**13**) compared to cisplatin on survival of SQ20B cells. The cells were treated with 0.2 (A) and 0.5 (B) μM palladium(II) complexes and cisplatin for 24 h and then plated for clonogenic survival. Clonogenic cell survival data were normalized to control. Error bars represent ± standard error of the mean of $N = 3$ experiments performed with at least three cloning dishes taken from one treatment dish. $^{*}p < 0.05$ versus respective control; $\psi p < 0.05$ versus cisplatin.

(m, Harom), 4.13 (s, N–CH_3), 2.38 (s, C–CH_3). ^{13}C-NMR (DMSO-d^6, ppm): δ = 14.6, 40.6, 127.4, 128.9, 129.1, 129.3, 130.8, 145.9, 154.7, MS (EI) (%): 259 (M^+, 38), 207 (40), 180 (32), 131 (100).

*3.1.2.2. MeO-BDH (**7**).* Yield, 0.58 g (92%). M.p. (dec.) 234 °C. Found: C, 52.41; H, 5.76; N, 21.96. Anal. Calc. for $C_{14}H_{17}N_5O_4$: C, 52.66; H, 5.37; N, 21.93. IR (KBr, cm^{-1}):ν = 3422 (m), 3240 (m), 1614 (s). ^{1}H-NMR (ppm): δ = 11.03 (s, NH), 8.50 (s, HC═N), 7.50–7.13 (m, Harom), 4.14 (s, N–CH_3), 2.38 (s, C–CH_3). ^{13}C-NMR (ppm): δ = 14.4, 41.1, 56.1, 61.6, 114.3, 117.1, 124.8, 142.4, 153.1, MS (EI) (%): 319 (M^+, 78), 307 (46), 289 (35).

*3.1.2.3. Cl-BDH (**8**).* Yield, 0.28 g (48%). M.p. (dec.) 266 °C. Found: C, 49.15; H, 4.26; N, 24.38. Anal. Calc. for $C_{12}H_{12}ClN_5O_2$: C, 49.07; H, 4.12; N, 23.84. IR (KBr, cm^{-1}):ν = 3423 (m), 3252 (w), 1623 (s). ^{1}H-NMR (ppm): δ = 11.04 (s, NH), 8.49 (s, HC═N), 7.69–7.50 (m, Harom), 4.00 (s, N–CH_3), 2.35 (s, C–CH_3). ^{13}C-NMR (ppm): δ = 14.4, 39.8, 116.1, 116.6, 125.3, 128.9, 133.4, 134.8, 142.7, 143.6, 145.6. MS (EI) (%): 293 (M^+, 64), 153 (71), 111 (37), 67 (100).

*3.1.2.4. OH-BDH (**9**).* Yield, 0.28 g (76%). M.p. (dec.) 265 °C. Found: C, 52.80; H, 4.81; N, 25.82. Anal. Calc. for $C_{12}H_{13}N_5O_3$: C, 52.36; H, 4.76; N, 25.44. IR (KBr, cm^{-1}): ν = 3247 (m), 1619 (s). ^{1}H-NMR (ppm): δ = 11.05 (s, NH), 8.33 (s, HC═N), 7.63–6.93 (m, Harom), 4.11 (s, N═CH_3), 2.37 (s, C–CH_3). ^{13}C-NMR (ppm): δ = 14.4, 39.8, 116.1, 116.6, 125.3, 128.9, 142.7, 143.6, 147.3, 159.7. MS (EI) (%): 275 (M^+, 20), 176 (100).

3.1.3. General procedure for the synthesis of complexes

A filtered solution of the desired ligand (**6**–**9**) (0.72 mmol) in acetone (30 ml) was added to a solution of [$PdCl_2$(NCPh)$_2$] (0.25 g, 0.65 mmol) in acetone (50 ml) with continuous stirring. Upon addition, a yellow solid was formed. After 5 h stirring, the precipitate (**10**–**13**) was filtered, washed with acetone (2 × 5 ml), Et_2O (2 × 10 ml) and dried in vacuum.

*3.1.3.1. $PdCl_2$(H–BDH)$_2$· H_2O (**10** · H_2O).* Yield of 0.16 g (35%). M.p. (dec.) 278 °C. Found: C, 40.64; H, 4.23; N, 20.05. Anal. Calc. for $C_{24}H_{26}N_{10}O_4PdCl_2 \cdot H_2O$: C, 40.38; H, 3.95; N, 19.62. IR (KBr, cm^{-1}):ν = 3442 (mbr), 3252 (m), 1629 (s), 1547 (m), 1455 (m), 1373 (s), 1230 (m), 1142 (w), 1086 (w), 758 (m), 628 (w), 513 (w). ^{1}H-NMR (ppm): δ = 11.03 (s, NH), 8.51 (s, HC═N), 7.70–7.45 (m, Harom), 4.02 (s, N═CH_3), 2.35 (s, C–CH_3).

*3.1.3.2. $PdCl_2$(MeO-BDH)$_2$· H_2O (**11** · H_2O).* Yield of 0.08 g (15%). M.p. (dec.) 269 °C. Found: C, 40.23; H, 4.24; N, 16.82. Anal. Calc. for $C_{28}H_{34}N_{10}O_8PdCl_2 \cdot H_2O$: C, 40.33; H, 4.35; N, 16.8. IR (KBr, cm^{-1}): ν = 3423 (mbr), 3270 (m), 1618 (s), 1532 (m), 1447 (m), 1373 (s), 1265 (m), 1241 (m), 1228 (m), 1142 (w), 1085 (w), 756 (m), 628 (w), 585 (w). ^{1}H-NMR (ppm): δ = 11.1 (s, NH), 8.50 (s, HC═N), 7.69–7.50 (m, Harom), 4.00 (s, N–CH_3), 2.35 (s, C–CH_3). ^{13}C-NMR (ppm): δ = 14.6, 99.9, 128.9, 129.5, 133.5, 142.9, 143.8, 145.6, 153.3.

*3.1.3.3. $PdCl_2$(Cl-BDH)$_2$· $3H_2O$ (**12** · $3H_2O$).* Yield of 0.21 g (39%). M.p. (dec.) 300 °C. Found: C, 34.55; H, 3.39; N, 17.30. Anal. Calc. for

$C_{24}H_{24}Cl_2N_{10}O_4PdCl_2 \cdot 3H_2O$: C, 35.21; H, 3.69; N, 17.11. IR (KBr, cm^{-1}): $\nu = 3416$ (mbr), 3250 (m), 1629 (s), 1541 (m), 1455 (m), 1373 (s), 1235 (m), 1148 (w), 1086 (w), 753 (m), 622 (w), 518 (w). ^{1}H-NMR (ppm): $\delta = 11.1$ (s, NH), 8.50 (s, HC═N), 7.95–7.51 (m, Harom), 4.00 (s, N−CH_3), 2.35 (s, C−CH_3). ^{13}C-NMR (ppm): $\delta = 14.5$, 98.0, 128.9, 129.5, 133.5, 134.7, 142.5, 143.9, 145.6.

*3.1.3.4. $PdCl_2(OH{-}BDH)_2$ (**13**).* Yield of 0.27 g (57%). M.p. (dec.) 290 °C. Found: C, 39.94; H, 3.94; N, 19.55%. Anal. Calc. for $C_{24}H_{26}N_{10}O_6PdCl_2$: C, 39.60; H, 3.60; N, 19.24. IR (KBr, $cm^{-}1$): $\nu = 3363$ (mbr), 3266 (m), 1626 (s), 1537 (m), 1450 (m), 1371 (s), 1229 (m), 1146 (w), 1081 (w), 759 (m), 629 (w), 532 (w). ^{1}H-NMR (ppm): $\delta = 11.1$ (s, NH), 8.47 (s, HC═N), 7.59, −7.48 (m, Harom), 4.00 (s, $-CH_3$), 2.34 (s, C−CH_3). ^{13}C-NMR (ppm): $\delta = 14.5$, 116.3, 125.5, 129.1, 142.9, 143.8, 147.5, 159.8.

3.2. Clonogenic cell survival experiments

SQ20B and SCC-25 human head and neck squamous carcinoma cells were used in our study. SQ20B cells were maintained in Dulbecco's modified Eagle's medium (DMEM), containing 4 mM L-glutamine, 1 mM sodium pyruvate, 1.5 g/l sodium bicarbonate, and 4.5 g/l glucose with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA). SCC cells were maintained in DMEM-Ham's F-12, 0.4 μg/ml of hydrocortisone, and 10% FBS medium. Cultures were maintained in 5% CO_2 and humidified in a 37 °C incubator.

Attached cells from experimental dishes were trypsinized with 1 ml trypsin-EDTA (CellGro, Herndon, VA, USA) and inactivated with medium containing 10% FBS (Hyclone, USA). The cells were diluted and counted using a heme cytometer. Cells were plated at low density (300–1000 per plate), and clones were allowed to grow in a humidified 5% CO_2, 37 °C environment for 14 days in complete medium, in the presence of 0.1% gentamicin. Cells were fixed with 70% ethanol and stained with Coomassie blue for analysis of clonogenic cell survival as previously described [20]. Individual assays were performed with multiple dilutions in at least three cloning dishes per data point, repeated in at least three separate experiments.

Drugs were added to cells at final concentrations of 0.2 and 0.5 μM palladium(II) complexes (**11**, **12**, and **13**) or cisplatin. All stock solutions were dissolved and diluted in PBS containing 10% DMSO, and the required volume was added directly to complete cell culture medium on cells to achieve the desired final concentration. All cells were placed in a 37 °C incubator and harvested at the time points indicated.

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References

[1] A.S. Abu-Surrah, M. Kettunen, Curr. Med. Chem. 13 (2006) 1337–1357.
[2] (a) F.Z. Wimmer, S. Wimmer, P. Castan, S. Cros, N. Johnson, E. Colacio-Rodrigez, Anticancer Res. 9 (1989) 791–794;
(b) G. Zhao, H. Lin, P. Yu, H. Sun, S. Zhu, X. Su, Y. Chen, J. Inorg, Biochem. 73 (1999) 145–149.
[3] A.S. Abu-Surrah, H.H. Al-Sa'doni, M.Y. Abdalla, Cancer Therapy 6 (2008) 1–10.
[4] H. Mansuri-Torshizi, T.S. Srivastava, H.K. Parekh, M.P. Chitnis, J. Inorg, Biochem. 45 (1992) 135–148.
[5] A.S. Abu-Surrah, T.A.K. Al-Allaf, L.J. Rashan, M. Klinga, M. Leskelä, Eur. J. Med. Chem. 37 (2002) 919–922.
[6] Y.-P. Ho, K.K.W. To, S.C.F. Au-Yeung, X. Wang, G. Lin, X. Han, J. Med, Chem. 44 (2001) 2065–2068.
[7] (a) T.A.K. Al-Allaf, L.J. Rashan, Eur. J. Med. Chem. 33 (1998) 817–820;
(b) T.A.K Al-Allaf, L.J. Rashan, M.T. Ayoub, M.H. Adday, U.K. Patent No. 2 304 712/ (1997).
[8] C. Barja-Fidalgo, I.M. Fierro, A.C.B. Lima, E.T. Da Silva, C.C. De Amorim, E.J. Barreiro, J. Pharm, Pharmacol. 51 (1999) 703–707.
[9] I.A. da Silveira, L.G. Paulo, A.L. de Miranda, S.O. Rocha, A.C.C. Freitas, E.J. Barreiro, J. Pharm, Pharmacol., 45 (1993) 646–649.
[10] A.C.C. Freitas, E.J. Barreiro, A.C.B. Lima, E.F.R. Pereira, A. Nuno, Quimica Nova 18 (1995) 138–143.
[11] (a) G.W. Bushnell, K.R. Dixon, D.T. Eadie, S.R. Stobart, Inorg. Chem. 20 (1981) 1545–1552;
(b) M.C. Carrión, A. Díaz, A. Guerrero, F.A. Jalón, B.R. Manzano, A. Rodríguez, New J. Chem. 26 (2002) 305–312;
(c) E. Budzise, M. Malecka, B. Nawrot, Tetrahedron 60 (2004) 1749–1759.
[12] K. Lappalainen, K. Yliheikkilä, A.S. Abu-Surrah, M. Kalmi, M. Polamo, M. Leskelä, T. Repo, Z. Anorg, Allgam. Chem. 631 (2005) 763–768.
[13] (a) A.S. Abu-Surrah, M. Kettunen, K. Lappalainen, U. Piironen, M. Klinga, M. Leskela, Polyhedron 21 (2002) 27–31;
(b) A.S. Abu-Surrah, Orient. J. Chem. 19 (2003) 331–336.
[14] A.S. Abu-Surrah, T.A.K. Al-Allaf, M. Klinga, M. Leskelä, Polyhedron 22 (2003) 1529–1534.
[15] (a) T.A.K. Al-Allaf, L.J. Rashan, A.S. Abu-Surrah, R. Fawzi, M. Steiman, Transit. Met. Chem. 23 (1998) 403–406;
(b) T.A.K. Al-Allaf, M.T. Ayoub, L.J. Rashan, J. Inorg, Biochem. 38 (1990) 47–56.
[16] S.M. Ben Saber, A.A. Maihub, S.S. Hudere, M.M. El-ajaily, Microchem. J., 81 (2005) 191–194.
[17] D.F. Schriver, P.W. Atkins, Inorganic Chemistry, third ed. Oxford, NY, 2001.
[18] D.M. Mattson, I.M. Ahmad, D. Dayal, A.D. Parsons, N. Aykin-Burns, L. Li, K.P. Orcutt, D.R. Spitz, K.F. Dornfeld, A.L. Simons, Free Radic. Biol. Med. 46 (2008) 232–237.
[19] J.R. Doyle, P.E. Slade, H.B. Jonassen, Inorg. Synth. 6 (1960) 216.
[20] D. Spitz, R. Malcolm, R. Robert, Biochem. J., 267(1009) 453–459.