Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Inorganic Biochemistry 100 (2006) 152–157
Journal of Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

Synthesis and characterization of new palladium–clotrimazole and palladium–chloroquine complexes showing cytotoxicity for tumor cell lines in vitro

Maribel Navarro [a,*], Nayarit Prieto Peña [a], Ibis Colmenares [a], Teresa González [a], Miriam Arsenak [b], Peter Taylor [b]

[a] Centro de Química, Instituto Venezolano de Investigaciones Científicas, IVIC Apartado 21827, Caracas 1020-A, Venezuela
[b] Centro de Medicinal Experimental, Instituto Venezolano de Investigaciones Científicas, IVIC Apartado 21827, Caracas 1020-A, Venezuela

Received 3 June 2005; received in revised form 27 September 2005; accepted 27 October 2005
Available online 15 December 2005

Abstract

New palladium complexes of chloroquine (CQ) and clotrimazole (CTZ) have been prepared, characterized, and evaluated against four tumor cell lines in vitro. [Pd $(CQ)_2Cl_2$] (**1**) was synthesized by the reaction of $PdCl_2(CH_3CN)_2$ with CQ, and the [Pd $(CTZ)_2Cl_2$] (**2**) complex by a similar reaction. The new compounds were characterized by a combination of FAB-MS (fast atom bombardment-mass spectrum), elemental analysis, molar conductivity, IR, and NMR spectroscopy. The solid-state structure of **2** has been determined by X-ray crystallography. **2** crystallizes in the monoclinic space group $P(2_1/c)$, with $a = 21.100(4)$ Å, $b = 13.408(3)$ Å, $c = 22.642(5)$ Å. The structure refinement converged at $R_1 = 0.0728$, $wR_2 = 0.1918$. The cytotoxicity of these two complexes for the tumor cell lines, PANC-1, SKBR-3, MDA-MB231 and HT-29, was compared with that of the original ligands. Ligation of palladium to CTZ led to an increase in the IC_{50}, although a three-fold reduction in the IC_{50} of CQ was observed on ligation to the metal when tested against the MDA-MB231 cell line.

Keywords: Palladium; Clotrimazole; Chloroquine; Tumor; Cytotoxicity

1. Introduction

As a part of our research program, we have been using a strategy based on the fact that coordination of organic drugs, such as chloroquine and clotrimazole, with known biological activities to transition metals may enhance their biological activity. For these studies, we chose chloroquine (CQ), which has been used as an antimalarial [1] and anti-inflammatory drug [2], and clotrimazole (CTZ, Fig. 1), clinically important as an antifungal drug [3], but also showing activity against parasites, Leishmania [4], trypanosomes [5] and *Plasmodium falciparum* [6], rheumatoid arthritis [7] and tumor cells [8]. These organic compounds, attached to different transition metals, specifically gold, ruthenium and rhodium, are active against tropical diseases such as malaria and Chagas disease [9–11], but their potential activity as antineoplastic agents has been little explored [12,13]. However, there are reports of the ability of both chloroquine [14] and clotrimazole [15] to enhance the sensitivity of tumor cells to other drugs. Thus, there exists the possibility of enhancing the efficacy of transition metals with known antitumoral activity, by combining them with these two organic ligands, as we have already shown for Chagas disease and malaria [9–11,16]. We have initiated these studies with palladium due to the structural similarities between platinum (II) and palladium (II). There have been reports of some promising results obtained with palladium complexes,

* Corresponding author. Tel.: +58 212 504 1642; fax: +58 212 504 1350.
E-mail address: mnavarro@ivic.ve (M. Navarro).

0162-0134/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2005.10.013

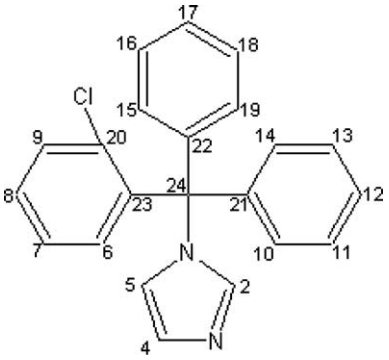

Fig. 1. Structure of CTZ.

such as *trans*-palladium complexed to a bulky amine ligand, which showed higher activity against the L929 cell line than carboplatin [17]. Also, *trans* $Pd(L)_2Cl_2$ where L is a highly substituted pyrazole ligand was more cytotoxic than *cis*-platinum with the same ligand, although less than cisplatin [18]. Additionally, the cytotoxic activity of the thiosemicarbazones and their palladium complexes was tested against human tumor cell lines with encouraging results [19,20].

These results form part of an ongoing project to synthesize and characterize *trans* palladium and *trans* platinum complexes of chloroquine and clotrimazole, and investigate their possible biomedical applications. We present here the results for the palladium complexes, which have proved to be somewhat easier to prepare, and the evaluation of their cytotoxicity against four tumor cell lines.

2. Materials and methods

All manipulations were routinely carried out under N_2 using common Schlenck techniques [21]. Solvents of analytical grade were distilled from appropriate drying agents prior to use. The extraction of chloroquine base was carried out as described previously [9]. $Pd(PhCN)_2(Cl)_2$ was synthesized by a previously reported method [22]. All other commercial reagents were used without further purification. NMR spectra were recorded on a Bruker AVANCE 300 spectrometer. IR spectra were obtained with a Nicolet 5DCX FT instrument, while UV–Vis spectra were recorded on a HP 8453 diode array instrument. High performance liquid chromatography (HPLC) analyses were done using a Hewlett Packard Series 1100, on a C_{18} hp ODS hypersil 5 μm (125 × 4 mm) column for CTZ and its metal derivatives, with 10% (phosphate buffer pH 3.5)/40% (MeOH)/50% (CH_3CN) at 1.0 mL/min for the mobile phase, and 10% (ammonium acetate buffer pH 4.9)/40% (MeOH)/50% (CH_3CN) at 1.5 mL/min, for chloroquine and its derivatives. Conductivity measurements were performed with a LaMotte CDS 5000 conductimeter. Positive ion FAB-MS (fast atom bombardment-mass spectra) were obtained in matrices of methanol–nitrobenzyl alcohol (NBA) from the analytical services of the University of California Riverside mass spectrometry facility.

2.1. Preparation of metal derivatives

*$PdCQ_2Cl_2$(**1**)*. A solution of $Pd(C_6H_5CN)_2Cl_2$ (103 mg; 0.27 mmol) in dichloromethane (20 mL) was stirred until dissolved. Chloroquine (215 mg; 0.67 mmol) was added and the mixture was stirred for 22 h at room temperature. The volume of the solvent was reduced under vacuum and a yellow product precipitated. It was filtered off, washed with dichloromethane, and dried under vacuum (yield: 77%). It was 98% pure by HPLC at 340 nm, with a melting point of 223–225 °C. Anal. Calc. for ($PdC_{36}H_{52}N_6Cl_4 \times H_2O$): C, 51.8; H, 6.5; N, 10.1. Found: C, 52.0; H, 6.5; N, 10.0%. FAB-MS $(M-H)^+ = 817$, [CQ] = 320. IR (KBr, cm^{-1}): *ν*(C=C) 1614, *ν*(C=N) 1589, *ν*(N–H) 3352. ^1H NMR ($CDCl_3$) (ppm) 9.84 (br, 1H, H8); 8.79 (br, 1H, H2); 7.81 (d, $J = 9$ Hz, 1H, H5); 7.39 (m, $J = 9$ Hz, 1H, H6); 6.46 (m, 2H, H3, NH); 3.74 (m, 1H, H1′); 2.65 (m, 4H, H5′); 2.55 (m, 2H, H4′), 1.69 (m, 4H, H2′, H3′), 1.36 (d, $J = 6$ Hz, 3H, H1′′), 1.10 (t, 6H, H6′). ^{13}C[H] NMR, C2 150.3, C3 100.0, C5 125.5, C6 121.5, C8 154.1, C10 117.7, C1′ 48.4, C1″ 19.4 C2′, 33.7, C3′ 23.0, C4′ 51.7, C5′ 46.4, C6′ 10.5. $\Lambda_{M/DMSO} = 8.0$ ohm^{-1} cm^2 mol^{-1}.

*$Pd(CTZ)_2Cl_2 \cdot 2/3CH_2Cl_2$(**2**)*. A solution of $Pd(C_6H_5CN)_2Cl_2$ (102 mg; 0.27 mmol) in dichloromethane (15 mL) was stirred until dissolved. Clotrimazole (225 mg; 0.652 mmol) was added and the mixture was stirred for 12 h at room temperature. The volume of the solvent was reduced under vacuum and diethyl ether was added until the solution became turbid. The mixture then left overnight in a refrigerator. A light yellow product precipitated. The solvent was filtered off, and the solid was washed with copious amounts of diethyl ether, and dried under vacuum (yield: 85%). It was 96% pure by HPLC. at 229 nm, with a melting point of 239–241 °C. Anal. Calc. for ($PdC_{44.6}H_{35.3}N_4Cl_{5.3}$): C, 58.1; H, 3.8; N, 6.1. Found: C, 58.3; H, 4.2; N, 5.9%. IR (KBr, cm^{-1}), *ν*(C=C) 1492, *ν*(C=N) 1445. ^1H NMR ($CDCl_3$), *δ* ppm) 7.95 (t, 1H); 7.35 (m, 9H); 7.23 (dd, $J^1 = 7.9$ Hz, $J^2 = 1.6$ Hz, 1H); 7.09 (m, 4H); 6.84 (dd, $J^1 = 7.9$ Hz, $J^2 = 1.6$ Hz, 1H); 6.57 (t, 1H); 5.28 (s, CH_2Cl_2); ^{13}C[H] NMR C2 140.7, C4 128.6, C5 120.3, C6 129.9, C7 129.7, C8 126.7, C9 131.9, (C10, C14, C15, C19), 129.6, (C11, C13, C16, C18) 127.8, (C12, C17) 128.1, C20 139.1, (C21, C22) 138.8, C23 135.0 $\Lambda_{M/DMSO} =$ 9.2 ohm$_{-1}$ cm^2 mol^{-1}.

2.1.1. X-ray structure determinations for complex **2**

Yellow crystals of complex **2**, obtained by slow evaporation from CH_2Cl_2 with dimensions 0.50 × 0.15 × 0.10 mm, were mounted on a Rigaku AFC7S diffractometer equiped with a graphite monochromator and Mo Kα ($\lambda = 0.71073$ Å) radiation. The data were registered at room temperature and were collected using the *ω*-2*θ* scan technique to a maximum 2*θ* value of 50°. The cell was refined using 25 centered reflections by full-matrix least squares techniques. During the data collection three standards reflections were monitored to check any significant variation in their intensities. It was not necessary to

apply any correction for decay. The data were corrected for Lorentz and polarization effects [23]. Absorption effects were corrected using the φ-scan empirical method [24]. The structure was solved by the direct method (SHELXTL-PLUS NT v. 5.10) and refined by full-matrix least squares techniques. The full-matrix least-squares refinement was based on F^2 [25]. All non-hydrogen atoms were refined anisotropically, except those involved in disorder. After non-hydrogen atoms were found, two residual peaks with electron densities of 7.1 and 3.5 e Å^{-3} were located to distances of 1.72 and 1.67 Å from the *ortho* C44 and C60 atoms, respectively. Both electron densities were modeled taking into account a positional disorder from the chlorophenyl group in a second orientation of its corresponding substituent –C(Ph)$_2$(Cl–Ph). After initial refinement, the partial occupations for Cl5 and Cl5a were set to 0.60:0.40, whereas 0.75:0.25 occupations were included for Cl6 and Cl6a, respectively. A similar disorder has been observed in the crystal structure of the complex RhCl(COD)(CTZ). The disorder of the solvent was modeled in two sets of positions with partial occupation of 0.50:0.50 and their atoms were refined with isotropic displacement parameters. The C–Cl distances were refined including a restrained initial value of 1.740(1) Å. All the hydrogen atoms were included in their calculated positions, except those of the disordered solvent molecule. Crystallography details are given Table 1, and selected bond distances and angles are compiled in Table 2. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 265953.

Table 1
Crystal data collection and refinement of complex **2**

Crystal parameters	(2)
Empirical formula	$C_{44}H_{34}Cl_4N_4Pd$. 2/3(CH_2Cl_2)
Molecular weight	923.57
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	21.100(4)
b (Å)	13.408(3)
c (Å)	22.642(5)
α (°)	90.00
β (°)	103.29(3)
γ (°)	90.00
V (Å^3), Z	6234(2), 6
Crystal color, habit	Pale yellow, prism
Crystal size (mm)	0.50 × 0.15 × 0.10
D_c (g cm^{-3})	1.476
F(000)	2808
μ (mm^{-1})	0.827
Reflections collected	10,970
Independent reflections	6620
Goodness-of-fit (GOOF)	1.016
R_1,wR_2 [$I > 2\sigma(I)$]	0.0727, 0.1919
R_1,wR_2 (all data)	248, 0.2267
Largest features in final difference map (max./min. e Å^{-3})	1.374, −1.413

Table 2
Selected bond distances (Å) and angles (°) for **2**

Bond length ranges		Angle ranges	
Pd(1)–N(1)	2.025 (6)	N(1)–Pd(1)–N(3)	180.0 (4)
Pd(1)–N(3)	2.025 (6)	N(1)–Pd(1)–Cl(1)	89.76 (18)
Pd(1)–Cl(1)	2.299 (19)	N(1)–Pd(1)–Cl(1A)	90.24 (18)
Pd(1)–Cl(1A)	2.299 (19)	Cl(1)–Pd(1)–Cl(1A)	180.00 (10)
N(1)–C(3)	1.326 (9)		
N(1)–C(1)	1.370 (9)		

2.2. Cell lines and culture

The four human tumor cell lines were the kind gift of the Tissue Culture Unit at Astra Zeneca, UK. They were grown and maintained in the following media: MDA-MB231 (breast) and PANC-1 (pancreas) in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS); SKBR-3 (breast) in DMEM with 10% FBS and 1mM glutamine; HT-29 (colon) in DMEM with 10% FBS and 0.45% glucose. All cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

2.3. Cytotoxicity assay

Tumor cells were plated at 2.5–5 × 10^4 cells/well in 100 μL of culture medium in flat-bottomed 96 well plates and allowed to attach for 24 h. Different concentrations of the complexes and ligands in 100 μL culture medium were then added. After a further 24 h, the number of viable cells was assessed using the MTS/PMS chromogenic assay (tetrazolium compound MTS/phenazine methosulfate, Promega Corp., USA) according to the manufacturer's instructions.

Cytotoxicity is expressed as the percentage of dead cells calculated from the following formula:

$$\text{Cytotoxicity}(\%) = 100 - [(\text{OD}_{\text{T24-com}} - \text{OD}_{\text{T0-com}}) / (\text{OD}_{\text{T24-control}} - \text{OD}_{\text{T0-control}}) \times 100],$$

where $\text{OD}_{\text{T0-control}}$ and $\text{OD}_{\text{T0-com}}$ correspond to the measurement of optical density at time zero immediately after the addition of the reactant medium for the assay under control or complex-treated conditions, respectively. The values $\text{OD}_{\text{T24-control}}$ and $\text{OD}_{\text{T24-com}}$ correspond to those measured after 24 h incubation.

3. Results and discussion

The new complexes *trans* [Pd(CQ)$_2$Cl$_2$] (**1**) and *trans* [Pd(CTZ)$_2$Cl$_2$] (**2**) were synthesized at room temperature by the reaction of Pd(C$_6$H$_5$CN)$_2$Cl$_2$ with chloroquine or clotrimazole in dichloromethane, respectively. The organic ligands displaced the labile ligand (C$_6$H$_5$CN) from the starting complex Pd(C$_6$H$_5$CN)$_2$Cl$_2$. They were characterized by elemental analysis, fast atom bombardment (FAB) mass, infrared (IR), and ^{1}H NMR spectroscopy as

described in Section 2. It was only possible to obtain suitable crystals for structural determination in the case of complex **2**. However, from the results of the techniques employed, it is possible to propose the structure of complex **1**.

Elemental analysis of the two complexes is in agreement with the molecular formulas proposed. The IR spectra of the complexes displayed peaks clearly associated with the presence of the coordinated ligands. The molar conductivity values obtained for these complexes are in the range for a non-electrolyte [26]. Complex **1** was characterized by FAB-MS because it was not possible to obtain suitable crystals for structural determination; the FAB-MS spectrum displayed parent peaks of high intensity corresponding to its molecular ion $(M - H)^+$ at *m/z* 817. All ^1H NMR resonances could be unequivocally assigned on the basis of 1D- and 2D correlated spectroscopy (COSY) and heteronuclear correlation (HETCOR) experiments (for complete ^1H and ^{13}C NMR data, and for the atom numbering used, see Section 2). We have used the ^1H chemical shift variation of each signal with respect to those of the free ligand ($\Delta\delta$) as a parameter to deduce the mode of bonding of CQ and CTZ to the metal. It has been previously shown by us [9–11] and by others [12] that the largest variations are always observed for the protons located in the vicinity of the N-atom attached to the metal. In complex **1**, the largest shift with respect to the free ligand was observed for H8 ($\Delta\delta = 1.87$ ppm). All other chloroquine protons shifted by <0.26 ppm, except NH which moved 1.15 ppm downfield, indicating that CQ is bound to the metal through the N1 atom of the quinoline moiety, a good donor site of this molecule. In complex **2**, the imidazole protons were displaced downfield with respect to the free ligand, H2 ($\Delta\delta = 0.30$ ppm), H4 ($\Delta\delta = 0.24$ ppm), H5 ($\Delta\delta = 0.22$ ppm), and the other crotrimazole protons were

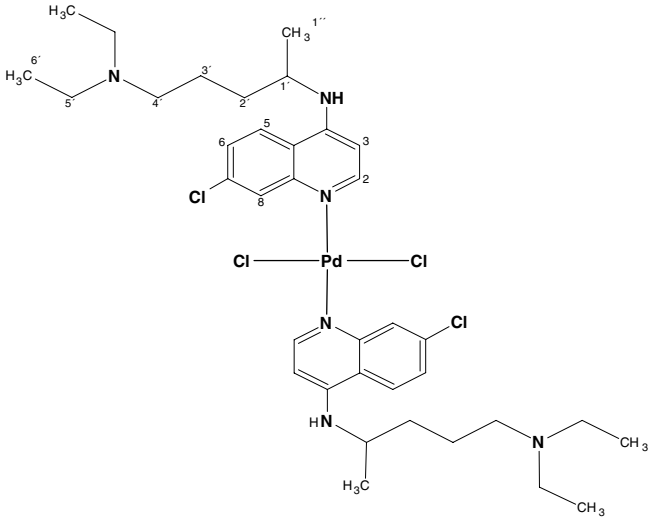

Fig. 2. Proposed structure of $PdCl_2(CQ)_2$ (**1**).

shifted by <0.09 ppm. This indicates that CTZ is coordinated to palladium through the imidazole nitrogen atom. According to the data available, the formulation for **1** represented in Fig. 2 corresponds to a 16-electron Pd(II) configuration, most probably in the usual square planar coordination geometry [27].

The structure of complex **2** was determined by X-ray diffraction. The asymmetric unit of this crystal structures contains one and a half $Pd(Cl)_2(CTZ)_2$ complexes and one CH_2Cl_2 molecule. One complex lies with the Pd ion located on the center of symmetry, and the second complex occupies a general position. The molecular diagram corresponding to the center symmetrical complex is shown in Fig. 3, and selected bond distances and angles are shown in Table 2. The coordination geometry of both around

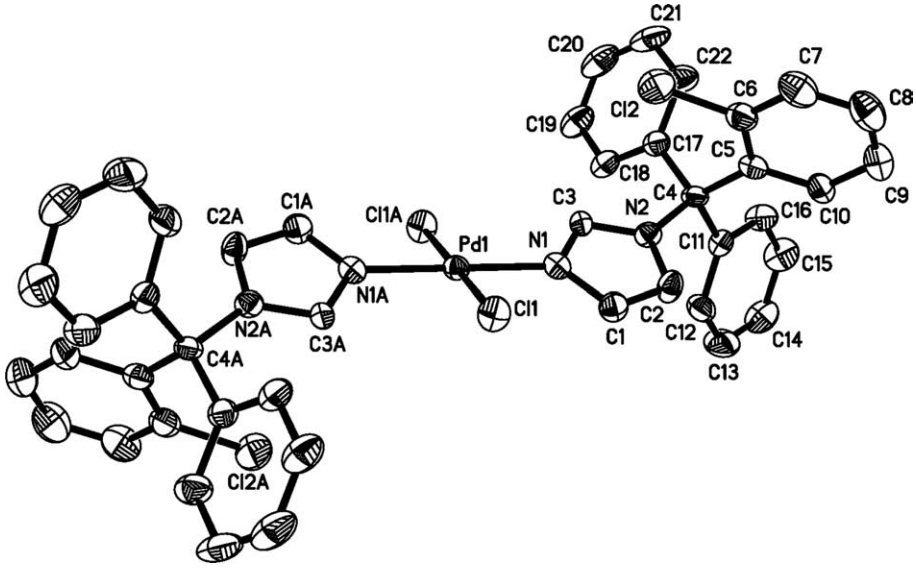

Fig. 3. Diagram of the molecular structure of $[Pd(CTZ)_2Cl_2]_2 \cdot 2/3[CH_2Cl_2]$ (**2**) with thermal ellipsoids drawn at the 35% probability level. H atoms omitted for clarity.

the palladium ion can be described as a square-planar arrangement with two clotrimazole ligands coordinated through one nitrogen atom of the imidazole ring. The remaining coordination positions are occupied by two chloride anion *trans* to each other. Both complexes adopt statistically equal metal–ligand distances [average: Pd–N = 2.022(6) Å; Pd-Cl = 2.303(2) Å]. In the center symmetrical complex (Pd1), the planes of the imidazole rings form a dihedral angle of 26.8° with that defining the coordination geometry (N1–Cl1–Pd–N1a–Cl1a). Intramolecular C–H···Cl hydrogen bonds help to sustain these conformations around the M–N bonds (see Fig. S1 in the supplementary material). All the structural features of the complex Pd2 are similar to complex Pd1 (details of the crystallographic data are available in the Cambridge Crystallographic Data Center, CCDC No. 265953). These structural features are similar to those previously reported for other metal-clotrimazole complexes [11,28].

The two palladium compounds were tested for cytotoxicity on four human tumor cell lines, PANC-1, SKBR-3, MDA-MB 231 and HT-29, representing tumors of three different origins, pancreas, breast and colon. The results are shown in Fig. 3 and the calculated IC_{50} values in Table 3. Of the two ligands, CTZ was the more active, showing an $IC_{50}$of 27 μM for the pancreatic tumor cell line, PANC-1, whereas CQ was much less cytotoxic, not reaching 50% inhibition for three lines at 200 μM, the highest concentration used here. The palladium–CTZ complex showed very poor cytotoxicity against the 4 cell lines, even taking into account the fact that each complex contains two ligand groups. On the other hand, the results shown in Fig. 4 indicate that complexing of palladium to CQ did increase cytotoxicity compared to the free ligand for three out of the four cell lines (PANC-1, SKBR-3 and MDA-MB 231). The IC_{50} of the free ligand fell from 158 to 49 μM when complexed with palladium when tested with the MDA-MB231 cell line. Obviously the cytotoxicity observed here is probably attributable to the metal, but it is interesting to note the much greater sensitivity of MDA-MB231 to the CQ complex than to the CTZ complex. The results suggest that further syntheses should be undertaken to determine the possible potentiating effect of CQ ligated to antineoplastic metals, and other ligands to palladium.

Table 3
Cytotoxicity of the two palladium complexes and their ligands tested against four human tumor cell lines

Compound	IC_{50} (μM)			
	PANC-1	SKBR-3	MDA-MB231	HT-29
CTZ	29	154	39	77
CQ	>200	>200	158	>200
$Pd(CTZ)_2Cl_2$	89	>200	159	155
$Pd(CQ)_2Cl_2$	170	>200	49	>200

Acknowledgements

The authors are grateful to FONACIT Grants S1-98000945 and LAB-97000821.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jinorgbio.2005.10.013.

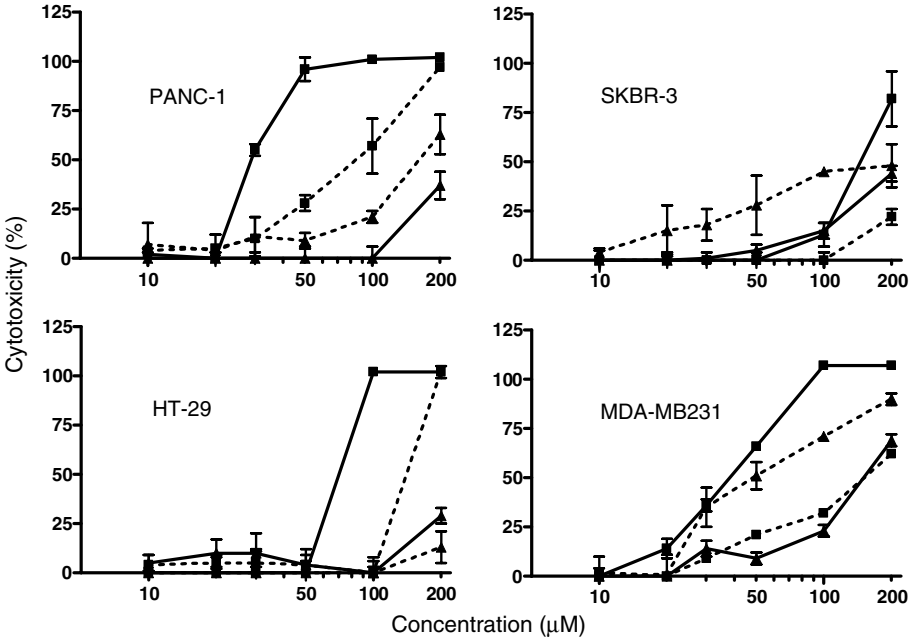

Fig. 4. Cytotoxicity of the two palladium complexes and their ligands tested against four human tumor cell lines. The results represent one experiment of 3 (mean ± SD, $N = 3$). ■— CTZ, ▲— CQ, ■--- $Pd(CTZ)_2Cl_2$, ▲--- $Pd(CQ)_2Cl_2$.

References

[1] M.J. Miller, Amer. J. Trop. Med. Hyg. 3 (5) (1954) 458–463.
[2] A. Ferrante, B. Kelly, W. Seowy, Y. Thong, Immunology 58 (1986) 125–142.
[3] S.L. Kelly, S. Kenna, H.F.J. Bligh, P.F. Watson, I. Stanfield, S.W.Y Ellis, D.E. Kelly, Biochemistry of cell walls and membranes in fungi, in: Lanosterol to Ergosterol-Enzimology, Inhibition and Genetics, Springer-Verlag, New York, 1989, pp. 222–243 (Chapter 15).
[4] D.H. Beach, L.J.Y. Goad, G. Holtz Jr., Mol. Biochem. Parasitol. 31 (1988) 149–162.
[5] J. Urbina, K. Lazardi, E. Marchan, G. Visbal, T. Aguirre, M. Piras, R. Piras, R. Maldonado, G. Payares, W. De Souza, Antimicrob. Agents Chemother. 37 (1993) 580–591.
[6] J. Kevin, K. Saliba, Kirk, Trans. Roy. Soc. Trop. Med. Hyg. 92 (1998) 666–667.
[7] W.B. Dennison, R.F. Loeser, R.A. Turner, J.A..Y. Jonson, H.B. Wells, J. Rheumatol. 17 (1990) 1003–1007.
[8] J. Penso, R. Beitner, Eur. J. Pharmacol. 342 (1998) 113–117.
[9] R. Sánchez-Delgado, M. Navarro, H. Pérez, J. Urbina, J. Med. Chem. 5 (1996) 1095–1099.
[10] M. Navarro, F. Vasquez, R. Sánchez-Delgado, H. Pérez, V. Sinou, J. Schrevel, J. Med. Chem. 47 (2004) 5204–5209.
[11] M. Navarro, E. Cisneros-Fajardo, T. Lehmann, R. Sánchez-Delgado, R. Atencio, P. Silva, R. Lira, J. Urbina, Inorg. Chem. 40 (2001) 6879–6884.
[12] W.I. Sundquist, D.P. Bancroft, S.J. Lippard, J. Am. Chem. Soc. 112 (1990) 1590–1596.
[13] M. Strasberg Rieber, A. Anzellotti, R. Sanchez Delgado, M. Rieber, Int. J. Cancer 112 (2004) 376–384.
[14] J.M. Zamora, W.T. Beck, Biochem. Pharmacol. 23 (1986) 4303–4310.
[15] M.H. Khalid, S. Shibata, T. Hiura, J. Neurosurg. 5 (1999) 918–927.
[16] R.A. Sánchez-Delgado, K. Lazardi, L. Rincón, J.A. Urbina, A.J. Hubert, A.F. Noels, J. Med. Chem. 36 (1993) 2041–2043.
[17] A.S. Abu-Surrah, T.A.K. Al-Allaf, L.J. Rashan, M. Klinga, M. Leskela, Eur. J. Med. Chem. 37 (2002) 919–922.
[18] E. Budzisz, U. Krajewska, M. Rozalski, A. Szulawska, M. Czyz, B. Nawrot, Eur. J. Pharmacol. 502 (2004) 59–65.
[19] A.P. Rebolledo, M. Vieites, D. Gambino, O.E. Piro, E. Castellano, C.L. Zani, E.M. Souza-Fagundes, L.R. Teixeira, A.A. Batista, H. Beraldo, J. Inorg. Biochem. 99 (2005) 698–706.
[20] A. Gómez-Quiroga, C. Navarro-Ranninger, Coord. Chem. Rev. 248 (2004) 119–133.
[21] D.F. Shriver, M.A. Drezdon, The Manipulation of Air Sensitive Compounds, second ed., Willey, New York, 1986.
[22] R. Doyle, P.E. Slade, H.B. Jonassen, Inorg. Synth. 6 (1960) 216–219.
[23] TEXSAN: Single Crystal Structure Analysis Software, Version 1.6, Molecular.
[24] A.T.C. North, D.C. Phillips, F.S. Mathews, Acta Crystollogr. A 24 (1986) 351–359, Structure, The Woodlands, TX, USA, 1993.
[25] G.M. Sheldrick. SHELXTL, Release 5.03, Siemens Analytical X-ray Instruments Inc., Madison, WI, 1994.
[26] W.J. Geary, Coord. Chem. Rev. 7 (1971) 81–122.
[27] G. Wilkinson, first ed.Comprehensive Coordination Chemistry, vol. 5, Pergamon Press, Great Britain, 1987 (Chapter 51).
[28] R. Sánchez-Delgado, M. Navarro, K. Lazardi, R. Atencio, M. Capparelli, F. Vargas, J. Urbina, A. Bouillez, A.F. Noels, D. Masi, Inorg. Chem. Acta 275–276 (1998) 528–540.