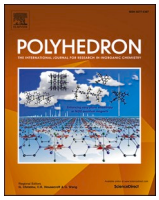
Polyhedron 248 (2024) 116756

Contents lists available at ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly



Synthesis, characterization, crystal structures and cytotoxic activity of Pt (II) complexes with N,N-donor ligands in tumor cell lines

Elena Fernández-Delgado [a], Samuel Estirado [a], Ana B. Rodríguez [a], Emilio Viñuelas-Zahínos [b,*], Francisco Luna-Giles [b], Javier Espino [a,*], José A. Pariente [a]

[a] Departamento de Fisiología, Grupo de Investigación Neuroinmunofisiología y Crononutrición, Facultad de Ciencias, Universidad de Extremadura, Avenida de Elvas s/n, 06006-Badajoz, España

[b] Departamento de Química Orgánica e Inorgánica, Grupo de Investigación Química de Coordinación, Facultad de Ciencias, Universidad de Extremadura, Avenida de Elvas s/n, 06006-Badajoz, España

ARTICLE INFO

Keywords:
Pt(II) complexes
Cytotoxicity
Thiazoline
Thiazine
Pyrazole

ABSTRACT

Cisplatin is one of the most used chemotherapeutic agents nowadays. However, it presents several and severe side effects. For that, the synthesis of new analogues of cisplatin is crucial to improve the current therapies. In this work, two squared-planar Pt(II) analogues of cisplatin were presented. These analogues included a bidentate ligand molecule with a 3,5-dimethyl-pyrazole ring and a thiazine (DMPzTz) or thiazoline (DMPzTn) ring. The chemical characterization covered single-crystal X-ray diffraction, infrared spectroscopy (IR) and elemental analysis. Their potential anticarcinogenic ability was studied in three different human tumor cell lines, i.e., histiocytic lymphoma (U-937), promyelocytic leukemia (HL-60) and epithelial cervix carcinoma (HeLa) via cytotoxicity assay. The results showed moderate cytotoxic effect of the complexes on leukemic cell lines, with scarce effect on solid tumor cell line HeLa. Lower IC_{50} values were found for U-937 cell line, being 32.3 ± 1.2 μmol/dm^3 for PtDMPzTn and 43.0 ± 1.4 μmol/dm^3 for PtDMPzTz. The comparison with previously synthesized analogues with phenyl substitutions or no substitutions in the pyrazole ring, allows to conclude that the presence of ligands with methyl substituted-pyrazole ring did not improve the effect of the complexes. In addition, the main effect of these potential chemotherapeutic agents is produced by the presence of platinum(II) as metal center, since the free ligands do not show any significant activity.

1. Introduction

According to the World Health Organization, cancer is the main reason of death along the world, reaching approximately 10 million deaths (one out of six registered patients) in 2020 [1]. One of the main procedures for cancer treatment is chemotherapy. Drugs employed to that purpose generally induce apoptosis, not only in cancer cells but also in non-tumor cells. Platinum-based drugs such as cisplatin, carboplatin, and oxaliplatin have been extensively used as chemotherapeutic agents against cancer [2]. Nevertheless, the drawbacks of these drugs, such as lack of selectivity, toxicity, or drug resistance, truly limit their clinical usage [3,4]. Some strategies to improve the effectivity of these drugs relied on modifying the structure of the existing platinum drugs. To that aim, heterocycles containing S,N or N,N donor atoms like happens with pyrazole, thiazoline or thiazine have been proved to display pharmacological potential [5–10].

Taking into account the importance of the structures of ligands in the complexes, since 2006 our group has synthesized and published a bunch of complexes containing ligands with those heterocycles [11–17] coordinated to different transition metals. In recent works using Pt(II) and Pd (II) as metal centers, it was seeing that phenyl substituents on the pyrazole ring improved the cytotoxic effects of the complexes, and that Pt (II) had, in general, strongly better results than Pd(II) complexes with the same ligands [14,15]. On the other hand, the presence of methyl substituents on the pyrazole ring has been also checked for Pd(II) complexes with no cytotoxic effects for any of the three cell lines tested [14]. Based on the previous results and the improvement showed in complexes with Pt(II) as metal center, in this article the synthesis and characterization of two Pt(II) complexes with formula $[PtCl_2(L)]$ (where L = methyl-substituted pyrazole ligand) has been reported. The final goal was to verify their hypothetical cytotoxic capability in HeLa, HL-60 and U-937 tumor cell lines and check whether this capacity was also

* Corresponding authors.
E-mail addresses: emilvin@unex.es (E. Viñuelas-Zahínos), jespino@unex.es (J. Espino).

https://doi.org/10.1016/j.poly.2023.116756
Received 25 May 2023; Accepted 25 November 2023
Available online 28 November 2023

better than that of the already published complexes with Pd(II) [14].

2. Experimental methods

2.1. General procedures

European Collection of Authenticated Cell Cultures (ECACC) (Dorset, U.K.) provided the tumor cell lines used, while the culture media and supplements were purchased from ThermoFisher Scientific (Barcelona, Spain). On the other hand, Promega (Madrid, Spain) supplied the Cell-Titer 96 AQueous One Solution Cell Proliferation Assay. The remaining reagents were of commercial grade.

Following the synthesis methods described in the literature [12,13], ligands 2-(3,5-dimethyl-1-pyrazolyl)-2-thiazoline (DMPzTn) and 2-(3,5-dimethyl-1-pyrazolyl)-1,3-thiazine (DMPzTz) were produced.

For chemical characterization, a Leco CHNS-932 microanalyzer was used for the elemental analysis and a Perkin-Elmer 100 FTIR spectrophotometer from KBr pellets in the 4000–400 cm^{-1} range was used for IR spectra of PtDMPzTn and PtDMPzTz. Particularly, for the IR of the corresponding ligands, the oily sample was placed between two NaCl discs. Melting points were determined on an Electrothermal IA 9000 apparatus and are uncorrected. ^1H NMR data could not be obtained for any of the complexes neither in DMF-d_7 nor in DMSO-d_6 due to their low solubility.

2.2. Synthesis of [$PtCl_2L$]

A reaction of [$PtCl_2(DMSO)_2$] and the corresponding ligand DMPzTn or DMPzTz, both previously dissolved in ethanol (40 and 20 mL respectively), was maintained under reflux for 24 h. The solid obtained was strained and rinsed with distilled water and dried with cold diethyl ether. Then, the recrystallization of the powder was made in DMSO, which provide suitable crystals for X-ray diffraction analysis.

2.2.1. Preparation and characterization of [$PtCl_2$(DMPzTn)] (PtDMPzTn)

For PtDMPzTn synthesis, DMPzTn (100.0 mg, 0.6 mmol) and [$PtCl_2(DMSO)_2$] (232.9 mg, 0.6 mmol) were used. Yield 176.5 mg (62.17 %). Mp 340 °C (dec.). Anal. Calc. (%) for $C_8H_{11}Cl_2PtN_3S$: C, 23.43; H, 2.85; N, 9.12; S, 6.95 %. Found: C, 23.61; H, 2.85; N, 8.83; S, 7.20 %. IR (KBr): thiazoline ring vibrations 1600 [ν(C═N)]; pyrazole ring vibrations 1568, 1417, 1381, 977 cm^{-1}.

2.2.2. Preparation and characterization of [$PtCl_2$(DMPzTz)] (PtDMPzTz)

For PtDMPzTz synthesis, DMPzTz (100.0 mg, 0.5 mmol) and [$PtCl_2(DMSO)_2$] (216.2 mg, 0.5 mmol) were used. Yield 191.0 mg (94.23 %). Mp 258 °C (dec.). Anal. Calc. (%) for $C_9H_{13}Cl_2PtN_3S$: C, 21.48; H, 2.48; N, 9.40; S, 7.17 %. Found: C, 21.57; H, 2.51; N, 9.22; S, 7.19 %. IR (KBr): thiazine ring vibrations 1592 [ν(C═N)]; pyrazole ring vibrations 1562, 1405, 1387, 1346, 995 cm^{-1}.

2.3. Crystal structure data

Crystal structure information of PtDMPzTn and PtDMPzTz, along with structure determination, data collection, crystal data, and refinement are summarized in Table 1.

A Bruker D8 VENTURE PHOTON III-14 diffractometer at 100 K using graphite monochromated Mo Kα (λ = 0.71073 Å) radiation were used to collect X-ray data. The Bruker SAINT [18] software with a wide-frame algorithm was used to integrate the collected frames, while the correction of the data was made by absorption effects through the Multi-Scan method (SADABS) [19]. The structures were resolved by direct method for PtDMPzTn and Patterson method for PtDMPzTz using the SHELXS-14 [20] program and refined with SHELXL-18 [21], as implemented in the WinGX [22] software platform. Anisotropic refinement was applied to all non-hydrogen atoms and the refinement of the hydrogen atom locations was made with a riding model. It is significant to point out that the crystals made available for PtDMPzTn were all twinned and refined as a 2-component perfect twin. A twin integration was made combining both domains in one hkl5 data file. Thus, the two crystallographically different molecules of [$PtCl_2$(DMPzTn)] are comprised in the asymmetric unit. Minor variations in bond lengths and bond and torsion angles can be seen. EADP constraint instruction was applied for C1A, C2A y N3A atoms in PtDMPzTn and for N3 atom in PtDMPzTz.

Table 1
Crystal data, data collection and refinement details for PtDMPzTn and PtDMPzTz.

	PtDMPzTn	PtDMPzTz
Crystal shape	Needle	Plate
Color	Yellow	Yellow
Size (mm)	0.07 × 0.03 × 0.02	0.06 × 0.04 × 0.02
Chemical formula	$C_8H_{11}Cl_2N_3PtS$	$C_9H_{13}Cl_2N_3PtS$
Formula weight	447.25	461.27
Crystal system	Monoclinic	Orthorhombic
Space group	P 21/c	P bca
Unit cell dimensions		
a (Å)	17.9683(9)	7.4526(3)
b (Å)	7.3231(4)	17.5917(7)
c (Å)	17.3899(8)	18.3383(8)
α (°)	90	90
β (°)	91.123(2)	90
γ (°)	90	90
Cell volume (Å^3)	2287.8(2)	2404.22(17)
Z	8	8
D_{calc} (g cm^{-3})	2.597	2.549
μ (mm^{-1})	12.884	12.265
F(0 0 0)	1664	1728
θ range	2.583–26.494	2.315–28.279
Index ranges	$-22 \le h \le 22, 0 \le k \le 9, 0 \le l \le 21$	$-9 \le h \le 9, -23 \le k \le 23, -24 \le l \le 24$
Temperature	100	100
Independent reflections	8082	2979
Observed reflections	6475	2228
No. of refined parameters	253	141
R [F > 4.0 σ(F)]	0.0597	0.0290
w*R* [F > 4.0 σ(F)]	0.14	0.0520
GOF	1.051	1.027
ρ_{max}, ρ_{min} (e Å^{-3})	4.442, −3.043	1.090, −1.209

At CCDC (Cambridge Crystallographic Data Center; copies of the data can be obtained, e-mail: deposit@ccdc.cam.ac.uk or www: https://www.ccdc.cam.ac.uk/structures/) can be found the crystallographic data for PtDMPzTn and PtDMPzTz, which deposited numbers are 2260597 and 2260598, respectively.

For Powder X-ray diffraction, the powders of samples were gently ground in an agate mortar and then deposited with care in the hollow of an aluminum holder equipped with a zero-background plate. Diffraction data (Cu Kα, λ = 1.5418 Å) were collected on a Bruker D8 Advance diffractometer equipped with a Ge 111 monochromator. The generator was operated at 30 kV and 40 mA. Optics used are the following: linear detector with 6° aperture, divergence slit 0.6 mm, monochromator slit 2.0 mm. A long scan was performed with $5 < 2\theta < 25°$ with t = 5 s and $\Delta 2\theta = 0.016°$.

2.4. Cell culture and treatments

Two cultured media supplemented with 2 mM L-glutamine, 10 % heat-inactivated FBS, 100 U/mL penicillin, and 10 μg/mL streptomycin were used to maintain the cells, being DMEM for HeLa and RPMI-1640 for U-937 and HL-60. Density selected for plating the different cell lines into 25 cm^2 culture flasks was as follows: 7×10^5 cells/ml for HeLa; and 3×10^5 cells/ml for HL-60 and U-937. Cells were maintained under an atmosphere of 95 % humidity air and 5 % CO_2 at 37 °C. Trypan-blue exclusion method was used to regularly test viability of cultures. Cells were challenged with PtDMPzTn, PtDMPzTz or vehicle for 24 h in concentrations from 1 to 100 μmol/dm^3. Their respective ligands

(DMPzTn and DMPzTz) were examined in previous studies [14]. To prepare the complexes solutions, dimethyl sulfoxide (DMSO) was used, obtaining suspensions due to their low solubility. To consider the solvent effect, a control with DMSO (vehicle) was used, its final concentration not being higher than 0.5 % (v/v).

2.5. In vitro cytotoxicity assay

To evaluate the cytotoxic properties of PtDMPzTn and PtDMPzTz on all three cell lines, an assay focused on the reduction of an MTS tetrazolium compound was used (CellTiter 96 AQueous One Solution Cell Proliferation Assay). For that, cells were seeded in 96-well plates at a density of 8×10^3 cells/ml (HeLa), 1.5×10^4 cells/ml (HL-60) or 2.5×10^4 cells/ml (U-937) into 25 cm^2 culture flasks, and treated with the Pt (II) complexes for 24 h. Then, cells were incubated at 37 °C with 10 μl of the CellTiter 96 AQueous One Solution Reagent during 15 min for HeLa cells, 1 h for U-937 cells or 2 h for HL-60 cells. Finally, the absorbance was recorded at 490 nm (test wavelength) and 650 nm (reference wavelength) on a microplate reader (Infinite M200; Tecan). All analyses were run in triplicate. The cell viability was calculated as percentage of control values (untreated samples).

2.6. Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical significance to compare treatments was calculated by one-way analysis of variance (ANOVA) followed by Dunnett or Tukey's test, as applicable. The dose–response curves of each compound were used to calculate IC_{50} values by fitting the curve to the data using nonlinear regression. GraphPad Prism 7.04 statistics software for Windows was used for these calculations. $P < 0.05$ was considered to indicate a statistically significant difference.

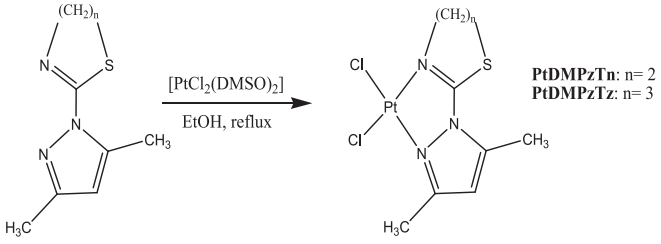
3. Results and discussion

3.1. Chemical synthesis

The synthesis procedure of PtDMPzTn and PtDMPzTz is described in Scheme 1. The Pt(II) complexes were synthesized making react the Pt(II) precursor *cis*-[$PtCl_2(DMSO)_2$] with the previous mentioned ligands, being both previously dissolved in ethanol and maintaining them under reflux for 24 h. Finally, the powder obtained was recrystallized in DMSO. The complexes structures were then characterized by single-crystal X-ray diffraction analysis, elemental analysis and IR spectroscopy.

3.2. Crystal structures

As can be seen in the molecular structure presented in Fig. 1, monomeric units of [$PtCl_2L$] (L = DMPzTn or DMPzTz) compose the crystal structures of PtDMPzTn and PtDMPzTz. In the case of PtDMPzTn, there are two types of independent molecules in the unit cell. A slightly distorted square-planar geometry coordination around Pt(II) is found for both complexes, which is indicated by the value of the dihedral angles



Scheme 1. Synthesis of PtDMPzTn and PtDMPzTz.

between Cl(1)-Pt-Cl(2) and N(1)-Pt-N(3), being in both cases lower than 5° (Table S1). The calculated τ_4 values [23], 0.07 for Pt(II) centers in both complexes, confirm the geometry. Likewise, the Pt(II) centers are perfectly placed on the mean plane calculated between the four donor atoms [Pt···mean l.s. plane: PtDMPzTn: 0.010 and 0.005 Å; PtDMPzTz: 0.016 Å]. DMPzTn and DMPzTz act as bidentate ligands and coordinate Pt(II) ion through the nitrogen of 2-thiazoline or 1,3-thiazine ring and the nitrogen of pyrazole cycle, forming a chelate ring of five members. As can be checked in Fig. 1, two chlorines in *cis* dispositions are also joined to Pt(II) ion. The selected bond lengths and angles are listed in Table 2.

Pt-Cl bond lengths are similar for both complexes and slightly longer than Pt-N bond lengths (Table 2). Furthermore, after an exhaustive research on Cambridge Structural Database (CSD, Version v5.42, Nov 2022) [24], it was checked that all the Pt-Cl distances are in the same order as the average values for squared-planar *cis*-complexes with a Cl_2N_2 coordination environment around Pt(II) [2.299(18) Å for 669Pt (II) complexes]. This is also true for Pt-$N_{pyrazole}$ bond lengths, which are similar to the mean value calculated for squared-planar complexes with Pt(II) in a Cl_2N_2 coordination environment [2.007(23) Å for 38Pt(II) complexes] in CSD [24] specially for PtDMPzTz, being slightly larger in the case of PtDMPzTn. As to the Pt-$N_{thiazoline}$ bond distance is a bit shorter to the mean values calculated for this type of bonds in CSD [24]: 2.038(48) Å for 7Pt(II) complexes, occurring the same way for Pt-$N_{thiazine}$ bond distance, which is also a little shorter than the average value calculated in CSD [24]: 2.077(35) Å for 13Pt(II) complexes.

Considering the values of torsion angles showed in Table S2, it can be deduced that N(1) and N(3) atoms are simultaneously coordinated to Pt (II) ions, since thiazoline and thiazine rings rotate around C(1)-N(2) bond.

Respect to the intermolecular interactions produced in these complexes, both PtDMPzTn and PtDMPzTz showed π -π stacking interactions between pyrazole rings. The values and a representation of these interactions could be found in the supplementary material (Fig. S1 and Table S3).

With the Powder X-ray Diffraction data, we established that the samples were pure when comparing the X-ray diffractograms of simulated and experimental polycrystalline powder of complexes (see Figs. S2 and S3). The similarity of the profile of both diffractograms indicates that the compound obtained were the same. However, small deviations were observed in the position of the diffraction peaks, which are attributable to the temperature difference in the realization of the X-ray diffractograms. The simulated powder diffractogram obtained from the single-crystal X-ray diffraction data was acquired at low temperature while the experimental diffractogram was recorded at room temperature. This causes a difference in the interplanar spacing of the different families of planes of the two samples, resulting in a shift of the diffraction peaks at lower values of 2θ in the sample recorded at room temperature.

3.3. IR analyses

The IR spectra data of the ligands DMPzTn and DMPzTz and the complexes PtDMPzTn and PtDMPzTz can be found in Table S4 and Figs. S4-S7. The values indicated that the bands corresponding to $\Psi_1[\upsilon(C{=}N)]$ vibration in thiazine were moved to lower wavenumber ($\Delta = 47$ cm^{-1}) in PtDMPzTz (1592 cm^{-1}) in comparison to its respective ligand (1639 cm^{-1}). Same trend occurs for $W_1[\upsilon(C{=}N)]$ vibration in thiazoline, which were also shifted to lower wavenumber ($\Delta = 35$ cm^{-1}) in PtDMPzTn (1600 cm^{-1}) in comparison to DMPzTn (1635 cm^{-1}). This displacement seems to be related with the compensation of electron density loss on the nitrogen atom by the back-coordination from the platinum ion to the ligand molecule [25]. On the other hand, the bands belonging to pyrazole ring vibrations did not show a well define patron in any of the complexes, with little shifts to higher or lower wavenumber for PtDMPzTn respect to its ligand (Δ = 6–7 cm^{-1}) and higher shift

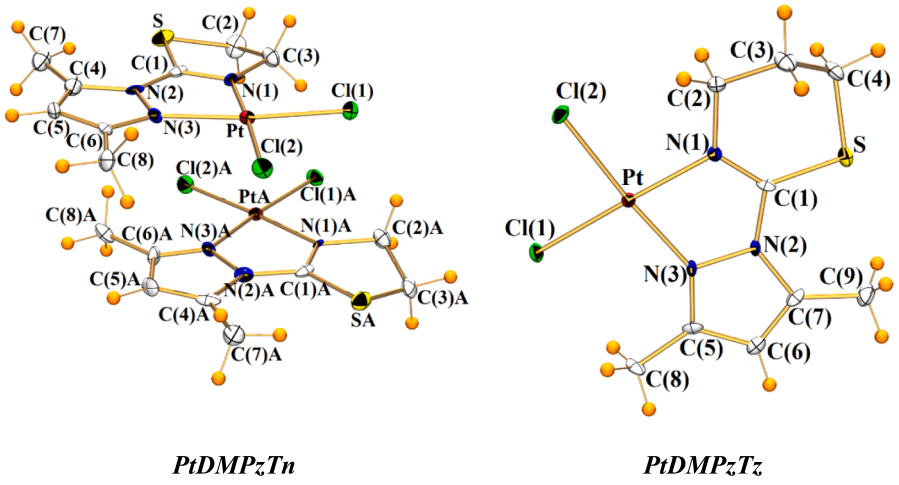


Fig. 1. Crystal structures of PtDMPzTn and PtDMPzTz.

Table 2
Selected bond lengths (Å) and angles (°) for complexes.

	PtDMPzTn	PtDMPzTz
Pt-Cl(1)	2.287(4)	2.296(1)
PtA-Cl(1)A	2.303(4)	
Pt-Cl(2)	2.299(3)	2.298(1)
PtA-Cl(2)A	2.288(3)	
Pt-N(1)	1.984(14)	2.022(4)
PtA-N(1)A	1.992(13)	
Pt-N(3)	2.022(11)	2.003(4)
PtA-N(3)A	2.024(11)	
Cl(1)-Pt-Cl(2)	89.3(1)	87.5(1)
Cl(2)A-PtA-Cl(1)A	87.9(1)	
N(1)-Pt-Cl(1)	176.9(3)	175.4(1)
N(1)A-PtA-Cl(1)A	177.6(3)	
N(1)-Pt-Cl(2)	93.6(3)	95.3(1)
N(1)A-PtA-Cl(2)A	94.2(3)	
N(1)-Pt-N(3)	79.6(5)	79.8(2)
N(1)A-PtA-N(3)A	78.9(5)	
N(3)-Pt-Cl(1)	97.6(4)	97.5(1)
N(3)A-PtA-Cl(1)A	99.1(4)	
N(3)-Pt-Cl(2)	172.8(4)	174.7(1)
N(3)A-PtA-Cl(2)A	172.8(4)	

values for PtDMPzTz respect to DMPzTz (Δ = 4–31 cm^{-1}). This undefined patron may be justified by the presence of the methyl substituents, as also happened for other complexes with same substituents [14]. For all that, the frequency shifts observed on the complexes respect to the ligands confirmed the coordination of Pt ion to both heterocycles through the nitrogen atoms.

3.4. Biological studies

To determine the cytotoxic effect of PtDMPzTn and PtDMPzTz, both complexes were tested during 24 h on the selected human tumour cell lines (HL-60, U-937 and HeLa) at concentrations from 1 to 100 μmol/dm^3 (Fig. 2). Data obtained indicated that PtDMPzTn and PtDMPzTz showed moderate cytotoxicity on non-solid tumor HL-60 and U-937 cell lines, showing lower IC_{50} values for PtDMPzTn in both cases (Fig. 2A-B and Table 3). On the other hand, these two complexes had any cytotoxic effect on solid-tumor HeLa cell line (Fig. 2C and Table 3). The lowest IC_{50} values for both complexes were found on U-937 cell line, being 32.6 ± 1.2 μmol/dm^3 for PtDMPzTn and 43.0 ± 1.4 μmol/dm^3 for PtDMPzTz. (Table 3). Nevertheless, any of them presented similar cytotoxicity to CisPt values, whose IC_{50} value was lower in the three cell lines elected. Otherwise, as it can be observed in Table 3, the complex with the thiazoline ring demonstrated lower IC_{50} values for all the three lines. In fact, significant differences were found between this complex and the one with the thiazine ring. Similar differences were already observed in previously studied Pt(II) complexes with high structural similarity [15] (40–43, Table S6, 9–12, Table S7 and 12–15, Table S8) where the complexes with thiazoline ring ligands were more active than those containing thiazine. In that case, it was found a higher accumulation into the cells of the complexes with thiazoline than the ones with thiazine. This could be the cause for the difference observed in PtDMPzTn and PtDMPzTz.

On the other hand, it was previously verified that the free ligands DMPzTn and DMPzTz, did not produce significant effects on cell viability in any of the tumor cell lines selected [14]. This seems to indicate that the cytotoxic effect of the PtDMPzTn and PtDMPzTz is produced by the presence of platinum(II) as metal center. In Table S5 it can be found the IC_{50} of complexes and ligands.

Taking into account other *cis*-complexes with a Cl_2N_2 coordination environment around Pt(II) tested on the same tumoral cell lines, several of them displayed higher cytotoxicity than our complexes on HeLa (1–15, 22, 25, 28–36, Table S6) [26–29] and U-937 (1–9, Table S8) [30], but in general with quite different chemical structures and with variable incubation times (48 h-7 days). On the other hand, PtDMPzTn and PtDMPzTz showed lower IC_{50} values than some of the complexes found in the literature at the same, or even at shorter, incubation times (24 h) in HeLa (16–21, Table S6) [31,32] and HL-60 (1–6, Table S7) [31,32], and similar values than some complexes in HeLa cells at shorter incubation times (23, 24, 26, 27, Table S6) [33,34]. Nevertheless, it is important to highlight that some of the comparisons are not accurate because some authors measured cell proliferation by other methods different from MTS assay [26].

As already mentioned, the chemical structures of the complexes found in the literature are quite distinct from ours. Hence, to compare to closer structures, we have used several complexes containing similar ligands that were previously synthesized by our research group [14–17,35,36]. Respect to these Pt(II) complexes, we could not improve the results obtained for the complexes showed in [15,17,35,36] in any of the three lines selected (39–45, Table S.6, 9–12, Table S.7, 10–15 Table S8), although similar values were achieved comparing PtDMPzTn and complex PtPyTz in HL-60 (8, Table S7). These results indicate that the methyl substitution in the ligand is not as effective as the phenyl substitution or the absence of extra substituents in the pyrazole ring of the ligand. In fact, Segapelo and coworkers [27] also demonstrated that the aryl substitution of pyrazole ring in their Pt(II) complexes (6 and 8, Table S6) improved their cytotoxicity in comparison with alkyl substitutions (5 and 7, Table S6). The same conclusion was obtained by

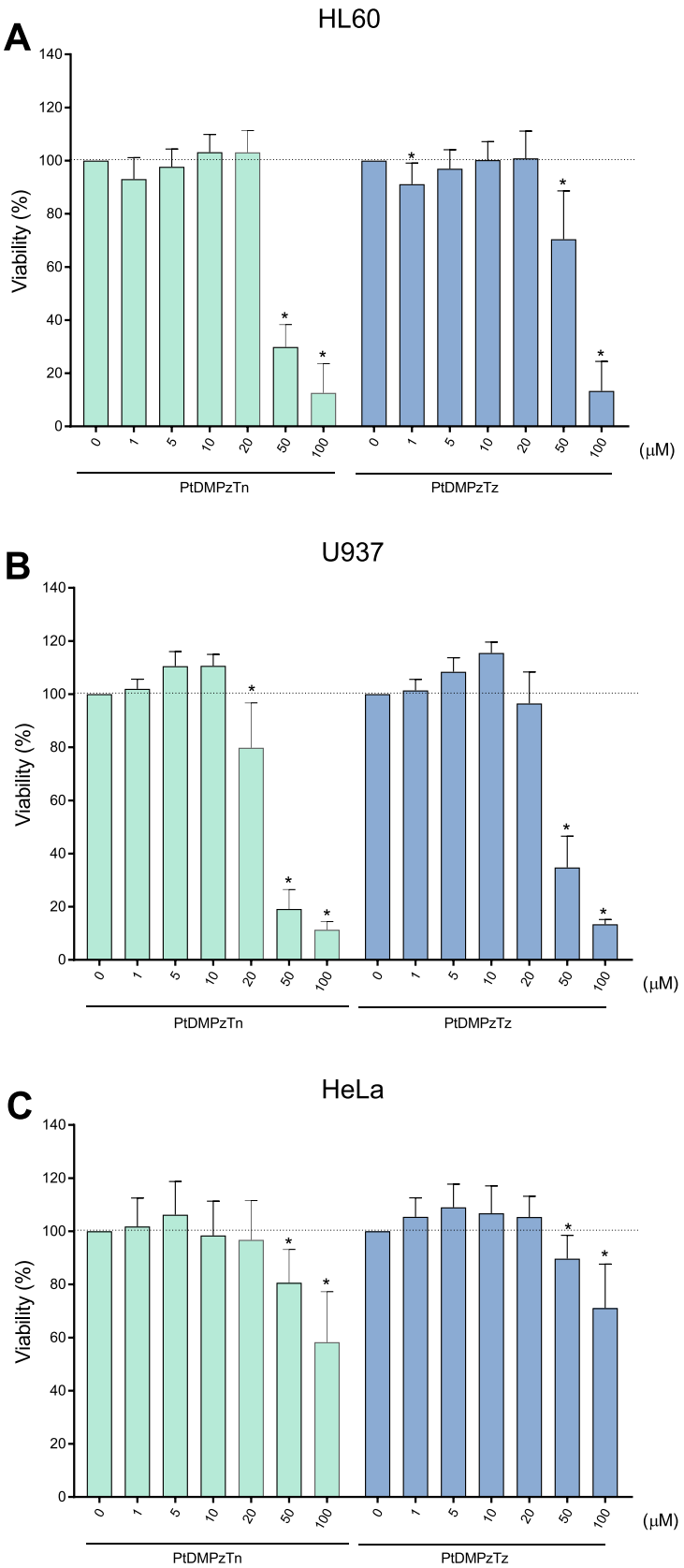


Fig. 2. Pt(II) complexes cytotoxicity. HL-60 (A), U-937 (B) and HeLa (C) cells were challenged for 24 h with 1, 5, 10, 20, 50 and 100 μmol/dm^3 of PtDMPzTn and PtDMPzTz, or the vehicle (DMSO). Data are stated as percentage of control values and correspond to means ± S.D. of 7 independent experiments. *$P < 0.05$ compared to the corresponding values of their controls.

Table 3
Cytotoxicity ($IC_{50} \pm SD$, μmol/dm^3) of CisPt, PtDMPzTn and PtDMPzTz towards selected tumour cell lines.

	HeLa	HL-60	U-937
CisPt	16.1 ± 1.0^a	11.3 ± 1.0^a	7.9 ± 0.5^a
PtDMPzTn	$>100^b$	42.8 ± 1.8^b	32.6 ± 1.2^b
PtDMPzTz	$>100^b$	62.3 ± 1.9^c	43.0 ± 1.4^c

[a, b, c] Within each column, values followed by a diverse lowercase letter are significantly different ($P < 0.0001$; Tukey's test).

Zafar and coworkers [37], who also improved cytotoxicity with benzyl instead of methyl substitutions of their Pd(II) complexes, but in this case making the substitution on the pyridine ring. When comparing to Pd(II) complexes with the same type of ligands, which were also synthesized by our research group, all of them improved the cytotoxic potential of the Pt(II) complexes showed herein (i.e., PtDMPzTn and PtDMPzTz) [14,17] in HL-60 and U-937 cell lines. In fact, the Pd(II) complexes with the same ligands (PdDMPzTn and PdDMPzTz) did not show cytotoxic effect on any of the three cell lines [14], which indicates that the presence of platinum as metal center is the main reason of the cytotoxic effect of the complexes with these methyl-substituent ligands.

4. Conclusions

In this work, we presented the synthesis and characterization of two new complexes with Pt(II) as metal center and with a ligand containing not only a pyrazole but also a thiazine/thiazoline heterocycle. It was checked the cytotoxicity produce by these complexes on three tumor cell lines, one from solid tumors (HeLa) and two from non-solid tumors (such as the leukemic lines HL-60 and U-937). The results presented in this work indicated no cytotoxic effect on the solid tumor cell line and a moderate induction of cell death on leukemic cell lines, obtaining the lowest IC_{50} on U-937 cell line (32.6 ± 1.2 μmol/dm^3 for PtDMPzTn and 43.0 ± 1.4 μmol/dm^3 for PtDMPzTz). The results showed an improvement of the effect of the complexes with respect to their respective ligands and a significant difference on the cytotoxicity between the complex with thiazoline ring and the one with thiazine, with PtDMPzTn displaying better results in all the three lines. On the other hand, Pt(II) complexes showed better cytotoxic effect than their Pd(II) counterparts (PdDMPzTn and PdDMPzTz) on HL-60 and U-937 cell lines. Altogether, it can be concluded that the methyl substitution on the ligand did not improve the effect of the complex and that the main effect of these potential chemotherapeutic agents was produced by the presence of Pt(II) as metal center.

CRediT authorship contribution statement

Elena Fernández-Delgado: Investigation, Data curation, Writing – original draft. **Samuel Estirado:** Investigation, Data curation. **Ana B. Rodríguez:** Writing – original draft, Writing – review & editing. **Emilio Viñuelas-Zahínos:** Data curation, Writing – original draft, Writing – review & editing, Supervision. **Francisco Luna-Giles:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision, Funding acquisition. **Javier Espino:** Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **José A. Pariente:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

The authors appreciate the technical and human support provided by the facilities of Bioscience Applied Techniques and Elemental and Molecular Analysis Service of SAIUEx (financed by UEx, Junta de Extremadura, MICINN, FEDER, and FSE). X-Ray intensity measurements were performed at the Unidade de Raios X (RIAIDT, University of Santiago de Compostela, Spain). This work was supported by Junta de Extremadura grants (ref. GR21042 and GR21075). E. Fernández-Delgado holds a research pre-doctoral fellowship from Junta de Extremadura (jointly financed by European Social Fund, ref. PD18020).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.poly.2023.116756.

References

[1] World Health Organization, World Health Organization, (n.d.). https://www.who.int/es/news-room/fact-sheets/detail/cancer (accessed March 27, 2023).
[2] S. Dilruba, G.V. Kalayda, Platinum-based drugs: past, present and future, Cancer Chemother. Pharmacol. 2016 776. 77 (2016) 1103–1124. https://doi.org/10.1007/S00280-016-2976-Z.
[3] R. Oun, Y.E. Moussa, N.J. Wheate, The side effects of platinum-based chemotherapy drugs: a review for chemists, Dalton Trans. 47 (2018) 6645–6653, https://doi.org/10.1039/C8DT00838H.
[4] S. Gupta, I. Portales-Castillo, A. Daher, A. Kitchlu, Conventional chemotherapy nephrotoxicity, Adv. Chronic Kidney Dis. 28 (2021) 402–414.e1, https://doi.org/10.1053/J.ACKD.2021.08.001.
[5] D. Havrylyuk, B. Zimenkovsky, O. Vasylenko, L. Zaprutko, A. Gzella, R. Lesyk, Synthesis of novel thiazolone-based compounds containing pyrazoline moiety and evaluation of their anticancer activity, Eur. J. Med. Chem. 44 (2009) 1396–1404, https://doi.org/10.1016/J.EJMECH.2008.09.032.
[6] A.Y. Shaw, H.H. Liau, P.J. Lu, C.N. Yang, C.H. Lee, J.Y. Chen, Z. Xu, G. Flynn, 3,5-Diaryl-1H-pyrazole as a molecular scaffold for the synthesis of apoptosis-inducing agents, Bioorg. Med. Chem. 18 (2010) 3270–3278, https://doi.org/10.1016/J.BMC.2010.03.016.
[7] J.V. Faria, P.F. Vegi, A.G.C. Miguita, M.S. dos Santos, N. Boechat, A.M. R. Bernardino, Recently reported biological activities of pyrazole compounds, Bioorg. Med. Chem. 25 (2017) 5891–5903, https://doi.org/10.1016/J.BMC.2017.09.035.
[8] F.S. Han, H. Osajima, M. Cheung, H. Tokuyama, T. Fukuyama, Novel structural motifs consisting of chiral thiazolines: synthesis, molecular recognition, and anticancer activity, Chem. – A Eur. J. 13 (2007) 3026–3038, https://doi.org/10.1002/CHEM.200601446.
[9] M. Ferreira, L.S. Assunção, F.B. Filippin-Monteiro, T.B. Creczynski-Pasa, M.M. Sá, Synthesis of 1,3-thiazine-2,4-diones with potential anticancer activity, Eur. J. Med. Chem. 70 (2013) 411–418, https://doi.org/10.1016/J.EJMECH.2013.10.017.
[10] F.K. Keter, J. Darkwa, Perspective: the potential of pyrazole-based compounds in medicine, BioMetals 2011 251. 25 (2011) 9–21. https://doi.org/10.1007/S10534-011-9496-4.
[11] A. Bernalte-García, A.M. Lozano-Vila, F. Luna-Giles, R. Pedrero-Marín, Structural characterization of a thiazoline-pyrazole ligand and its complexes with cobalt(II) and copper(II), Polyhedron 25 (2006) 1399–1407, https://doi.org/10.1016/J.POLY.2005.09.028.
[12] P. Torres-García, R. Pedrero-Marín, F. Luna-Giles, A.V. Huertas-Sánchez, E. Viñuelas-Zahínos, Influence of steric strain of S, N-heterocycles derivative ligands on the coordination geometry in cadmium(II) nitrato complexes, Polyhedron 31 (2012) 307–318, https://doi.org/10.1016/J.POLY.2011.09.033.
[13] P. Torres-García, E. Viñuelas-Zahínos, F. Luna-Giles, J. Espino, F.J. Barros-García, Zinc(II) complexes with novel 1,3-thiazine/pyrazole derivative ligands: synthesis, structural characterization and effect of coordination on the phagocytic activity of human neutrophils, Polyhedron 30 (2011) 2627–2636, https://doi.org/10.1016/J.POLY.2011.07.014.
[14] E. Fernández-Delgado, S. Estirado, A.B. Rodríguez, F. Luna-Giles, E. Viñuelas-Zahínos, J. Espino, J.A. Pariente, Cytotoxic effects of new palladium(II) complexes with thiazine or thiazoline derivative ligands in tumor cell lines, Pharmaceutics 15 (2023) 696, https://doi.org/10.3390/PHARMACEUTICS15020696/S1.
[15] E. Fernández-Delgado, S. Estirado, J. Espino, E. Viñuelas-Zahínos, F. Luna-Giles, A. B. Rodríguez Moratinos, J.A. Pariente, Influence of ligand lipophilicity in Pt(II) complexes on their antiproliferative and apoptotic activities in tumour cell lines, J. Inorg. Biochem. 227 (2022), 111688, https://doi.org/10.1016/J.JINORGBIO.2021.111688.

[16] E. Fernández-Delgado, F. de la Cruz-Martínez, C. Galán, L. Franco, J. Espino, E. Viñuelas-Zahínos, F. Luna-Giles, I. Bejarano, Pt(II) and Pd(II) complexes with a thiazoline derivative ligand: Synthesis, structural characterization, antiproliferative activity and evaluation of pro-apoptotic ability in tumor cell lines HT-29 and U-937, J. Inorg. Biochem. 202 (2020), 110870, https://doi.org/10.1016/j.jinorgbio.2019.110870.
[17] S. Gutiérrez-Tarriño, J. Espino, F. Luna-Giles, A.B. Rodríguez, J.A. Pariente, E. Viñuelas-Zahínos, G. Crisponi, A. Dominguez-Martin, Synthesis, characterization and antiproliferative evaluation of Pt(II) and Pd(II) complexes with a thiazine-pyridine derivative ligand, Pharmaceuticals 14 (2021) 395, https://doi.org/10.3390/ph14050395.
[18] A.X.S.I. Bruker, APEX3 and SAINT, Madison, Wisconsin, USA, 2015.
[19] A.X.S.I. Bruker, SADABS, Madison, WI, 2012.
[20] M. Sheldrick, SHELXS-14, Program for Crystal Structures Solution, University of Göttingen, (2014).
[21] G.M. Sheldrick, Crystal structure refinement with SHELXL, Acta Crystallogr. Sect. c. 71 (2015) 3–8, https://doi.org/10.1107/S2053229614024218.
[22] L.J. Farrugia, WinGX and ORTEP for Windows: an update, J. Appl. Crystallogr. 45 (2012) 849–854, https://doi.org/10.1107/S0021889812029111.
[23] L. Yang, D.R. Powell, R.P. Houser, Structural variation in copper(i) complexes with pyridylmethylamide ligands: structural analysis with a new four-coordinate geometry index{,} τ4, Dalt, Trans. (2007) 955–964, https://doi.org/10.1039/B617136B.
[24] I.J. Bruno, J.C. Cole, P.R. Edgington, M. Kessler, C.F. Macrae, P. McCabe, J. Pearson, R. Taylor, New software for searching the Cambridge Structural Database and visualizing crystal structures, Acta Crystallogr. Sect. B Struct. Sci. 58 (2002) 389–397, https://doi.org/10.1107/S0108768102003324.
[25] K. Burger, I.T. Millar, Coordination Chemistry: Experimental Methods, Butterworths, London, 1973.
[26] M. Carland, M.J. Grannas, M.J. Cairns, V.J. Roknic, W.A. Denny, W.D. McFadyen, V. Murray, Substituted 9-aminoacridine-4-carboxamides tethered to platinum(II) diamine complexes: Chemistry, cytotoxicity and DNA sequence selectivity, J. Inorg. Biochem. 104 (2010) 815–819, https://doi.org/10.1016/J.JINORGBIO.2010.03.011.
[27] T.V. Segapelo, I.A. Guzei, L.C. Spencer, W.E. Van Zyl, J. Darkwa, (Pyrazolylmethyl) pyridine platinum(II) and gold(III) complexes: Synthesis, structures and evaluation as anticancer agents, Inorg. Chim. Acta 362 (2009) 3314–3324, https://doi.org/10.1016/J.ICA.2009.02.046.
[28] G. Eren, S. Yilmaz, F. Gumus, In vitro cytotoxic activities of platinum(II) complex with 1-methyl-2-(3'- hydroxypropyl)benzimidazole and 2-(3'-hydroxypropyl) benzimidazolium hexa- and tetrachloroplatinate salts, Lett. Drug Des. Discov. 15 (2017) 65–69, https://doi.org/10.2174/1570180814666171012163325.
[29] G. Xu, Z. Yan, N. Wang, Z. Liu, Synthesis and cytotoxicity of cis-dichloroplatinum (II) complexes of (1S,3S)-1,2,3,4-tetrahydroisoquinolines, Eur. J. Med. Chem. 46 (2011) 356–363, https://doi.org/10.1016/J.EJMECH.2010.11.025.
[30] I. Würtenberger, B. Angermaier, B. Kircher, R. Gust, Synthesis and in vitro pharmacological behavior of platinum(II) complexes containing 1,2-diamino-1-(4-fluorophenyl)-2-alkanol ligands, J. Med. Chem. 56 (2013) 7951–7964, https://doi.org/10.1021/JM400967Z/SUPPL_FILE/JM400967Z_SI_001.PDF.
[31] S. Moradell, J. Lorenzo, A. Rovira, M.S. Robillard, F.X. Avilés, V. Moreno, R. De Llorens, M.A. Martinez, J. Reedijk, A. Llobet, Platinum complexes of diaminocarboxylic acids and their ethyl ester derivatives: the effect of the chelate ring size on antitumor activity and interactions with GMP and DNA, J. Inorg. Biochem. 96 (2003) 493–502, https://doi.org/10.1016/S0162-0134(03)00252-6.
[32] S. Moradell, J. Lorenzo, A. Rovira, S. Van Zutphen, F.X. Avilés, V. Moreno, R. De Llorens, M.A. Martinez, J. Reedijk, A. Llobet, Water-soluble platinum(II) complexes of diamine chelating ligands bearing amino-acid type substituents: the effect of the linked amino acid and the diamine chelate ring size on antitumor activity, and interactions with 5′-GMP and DNA, J. Inorg. Biochem. 98 (2004) 1933–1946, https://doi.org/10.1016/J.JINORGBIO.2004.08.011.
[33] C. Pérez-Arnaiz, J. Leal, N. Busto, M.C. Carrión, A.R. Rubio, I. Ortiz, G. Barone, B. Díaz De Greñu, J. Santolaya, J.M. Leal, M. Vaquero, F.A. Jalón, B.R. Manzano, B. García, Role of seroalbumin in the cytotoxicity of cis-dichloro Pt(II) complexes with (N^N)-donor ligands bearing functionalized tails, Inorg. Chem. 57 (2018) 6124–6134, https://doi.org/10.1021/acs.inorgchem.8b00713.
[34] C. Marzano, S.M. Sbovata, V. Gandin, R.A. Michelin, A. Venzo, R. Bertani, R. Seraglia, Cytotoxicity of cis-platinum(II) cycloaliphatic amidine complexes: Ring size and solvent effects on the biological activity, J. Inorg. Biochem. 103 (2009) 1113–1119, https://doi.org/10.1016/J.JINORGBIO.2009.05.009.
[35] M. Márquez Sánchez, Complejos de paladio y platino con un ligando que contiene anillos de tiazina, Bachelor's Thesis, University of Extremadura, Spain, 2022.
[36] M.L. Paisano de Lavera, Síntesis, caracterización y actividad citotóxica de complejos de platino(II) y paladio(II), Bachelors Thesis, University of Extremadura, Spain, 2022.
[37] M.N. Zafar, A.M. Butt, G.E.S. Chaudhry, F. Perveen, M.F. Nazar, S. Masood, A. F. Dalebrook, E.U. Mughal, S.H. Sumrra, Y.Y. Sung, T.S.T. Muhammad, L.J. Wright, Pd(II) complexes with chelating N-(1-alkylpyridin-4(1H)-ylidene)amide (PYA) ligands: Synthesis, characterization and evaluation of anticancer activity, J. Inorg. Biochem. 224 (2021), 111590, https://doi.org/10.1016/J.JINORGBIO.2021.111590.