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Original article

Synthetic, structural and biochemical studies of polynuclear platinum(II) complexes with heterocyclic ligands

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Abstract

''Non-classical'' di- and trinuclear Pt(II) complexes with polydentate nitrogen ligands; ionic $[(PtCl_2)_2(tptz)_2(\mu\text{-}PtClNCPh)]Cl$ (**1**) [tptz = 2,4,6-tris(2-pyridyl)-1,3,5-triazine], $[(PtCl_2)_2(bptz)_2(\mu\text{-}Pt)]Cl_2$ (**2**) [bptz = 3,6-bis(2-pyridyl)-1,2,4,5-tetrazine] and neutral $[(PtCl_2)_2(tptz)_2(\mu\text{-}PtCl_2)](H_2O)_4$ (**3**), $[(PtCl_2)_2(\mu\text{-}tppz)](CHCl_3)$ (**4**) [tppz = 2,3,5,6-tetra(2-pyridyl)pyrazine] complexes, have been prepared and structurally characterized. The neutral tptz and tppz complexes present three and two separate $PtCl_2$ moieties, respectively, in a *cis* position, presumably acting in a bifunctional mode towards DNA; the cationic tptz and bptz complexes contain monofunctional and bifunctional bridging Pt(II) moieties, respectively, (other Pt(II) moieties in the complexes are bifunctional). All complexes were tested for their biological activity. Both tptz complexes, neutral and ionic, show a potent cytotoxic activity and reduced cell viability in a concentration-dependent manner that was evaluated in a panel of different cancer cell lines: human HT29 colon–rectal carcinoma, HepG2 hepatoma, MDA-MB-231 breast cancer and MG63 osteosarcoma cells; their activity was higher than cisplatin, IC50 values have been calculated for the active compounds and flow cytometric analysis for the tptz complexes performed. Therefore, these new platinum drugs warrant further investigation into their antitumor activity against different types of tumors.

Keywords: Polydentate nitrogen ligands; Polynuclear platinum complexes; Cytotoxicity

1. Introduction

Cisplatin is one of the most effective agents used to treat various types of human cancer (bladder, testicular, ovarian, and head and neck tumors) [1,2], but its clinical effectiveness has been limited by significant undesiderable side effects, such as dose-dependent nephrotoxicity and neurotoxicity. Moreover, the use of cispaltin is limited by lack of activity against tumors with neutral or acquired resistance to this drug [3]. Pt(II) compounds have also an extensive history exhibiting virucidal activity, including a recent report of anti HIV-1 activity [4]. In this context there is a clear evidence that the carrier ligand influences the antiviral activity and modifications of the carrier ligand in cisplatin may broaden the range of antitumor and antiviral activities, therefore, there is still a need to synthesize platinum(II) complexes with novel ligands and to test them for antitumor activity, in the hope of overcoming the above mentioned limitations.

Polypyridyl complexes of metal ions have received considerable attention because of their interesting photophysical and photochemical properties, possible use as photochemical molecular devices and as light sensitive probes in biological systems [5]. The polynuclear charged platinum complexes developed by Farrell and Kelland [6] are endowed with excellent cytotoxic activity as is their ability to overcome both natural and acquired drug resistances.

Polypyridyl ligands have been used by our groups in the synthesis of mono-, bis-, and polynuclear complexes of platinum(II) and their cytotoxic activity investigated as they represent a new class of ''non-classical'' anticancer agents which

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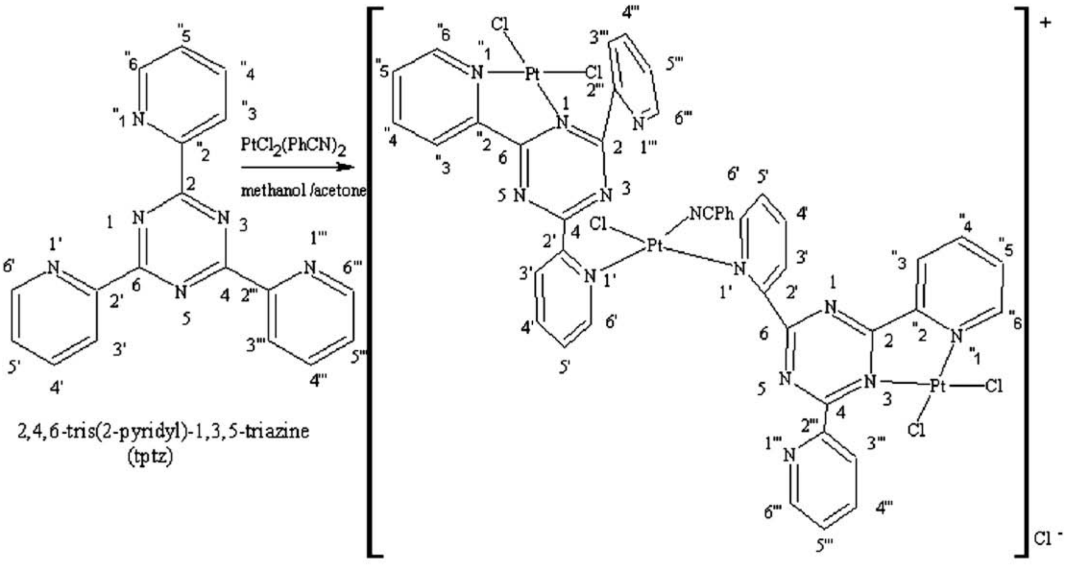


2,4,6-tris(2-pyridyl)-1,3,5-triazine (tptz)

$[(PtCl_2)_2(tptz)_2(\mu\text{-}PtClNCPh)]Cl$ (**1**)

Fig. 1. Structure of $[(PtCl_2)_2(tptz)_2(\mu\text{-}PtClNCPh)]Cl$ complex (**1**) and numbering scheme of atoms.

posses potent and distinct biological activities from cisplatin [7]. The complexes were effective death inducers in human colon–rectal carcinoma HT29 cells and their apoptotic cytotoxic activity was higher than that exerted by cisplatin.

In this work the tptz ligand was again used, along with tppz and bptz, to afford the ionic complexes **1**, **2** and the neutral **3**, **4** complexes. Their cytotoxic activity was tested using a panel of different cancer cell lines (hepatoma cells HepG2, breast cancer cells MDA-MB-231, osteosarcoma cells MG63 and human colon–rectal carcinoma HT29 cells). The biological activity of the complexes was investigated by means of MTT test and cytofluorimetric analysis. Both tptz complexes **1**, **3** showed a marked cytotoxic activity which was higher than cisplatin. Several rounded cells appeared isolated and detached from culture substrate indicating the commitment of cells to apoptotic death. These results, although preliminary, suggest that these complexes may represent a new class of potential lead compounds for development of chemiotherapeutic agents.

2. Results and discussion

2.1. Chemistry

A general procedure for the synthesis of the complexes was adopted: separate solutions of the ligands in the appropriate organic solvent and K_2PtCl_4 and $PtCl_2(PhCN)_2$ solutions in water and chloroform, respectively, were prepared. The latter solutions were added dropwise to the former under stirring in the dark. The solids were characterized by IR spectroscopy for solutions, NMR spectra on freshly prepared samples in DMSO-d_6 were studied by 1D and 2D ^{1}H and ^{13}C {^{1}H} NMR. Conductivity measurements were performed in nitromethane (or DMSO for complex **3**) showing evidence of significant conductivity only for the ionic complexes **1** and **2**. The scheme of the syntheses with the numbering of protons is reported in Figs. 1–4.

2.1.1. IR spectra of $[(PtCl_2)_2(tptz)_2(\mu\text{-}PtClNCPh)]Cl$, $[(PtCl_2)_2(tptz)_2(\mu\text{-}PtCl_2)](H_2O)_4$, $[(PtCl_2)_2(bptz)_2(\mu\text{-}Pt)]Cl_2$, $[(PtCl_2)_2(\mu\text{-}tppz)](CHCl_3)$ complexes

In all complexes, the absorption bands in the high frequency region which originate, in the heterocyclic ring of the ligands, appear not to be metal sensitive [8].

The IR spectrum of trinuclear complex **1** showed a stretching vibration at 2282 cm^{-1} which is assigned to ν(Pt–NC) of platinum bonded benzonitrile which is very close to the reported value of 2285 cm^{-1} [8].

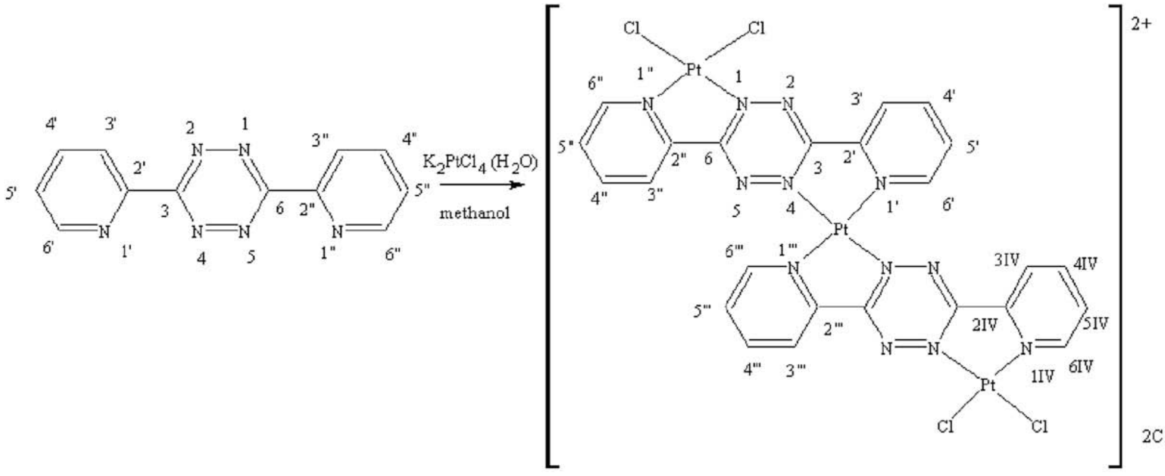
In all complexes two broad ν(Pt–Cl) vibrations in the region 320–350 cm^{-1} which are due to juxtaposition of asymmetric and symmetric stretching vibrations of $PtCl_2$ groups arranged in a *cis*-square planar stereochemistry (for the corresponding *trans* stereochemistry only one band is expected). Bands in the region 540–550 cm^{-1} are assigned to the ν(Pt–N) vibration.

2.1.2. NMR spectra

In order to avoid any complication arising from extensive solvolysis of the complexes in DMSO, the NMR solutions were made up at room temperature just prior to recording the spectra. The sharp NMR resonances for polynuclear complexes rule out oligomerization or fragmentation of the complexes. The solvated complex **4** presented a dismally low solubility even in DMSO-d_6 which prevented the acquisition of ^{13}C NMR spectra.

2.1.3. ^{1}H and ^{13}C NMR of trinuclear $[(PtCl_2)_2(tptz)_2(\mu\text{-}PtClNCPh)]Cl$ and $[(PtCl_2)_2(tptz)_2(\mu\text{-}PtCl_2)](H_2O)_4$ complexes

Trinuclear ionic complex **1** and neutral **3** are potentially present in two configurations. In fact for the ligand itself two



3,6-bis(2-pyridyl)-1,2,4,5-tetrazine (bptz)

$[(PtCl_2)(bptz)_2(\mu\text{-}Pt)]Cl_2$ (**2**)

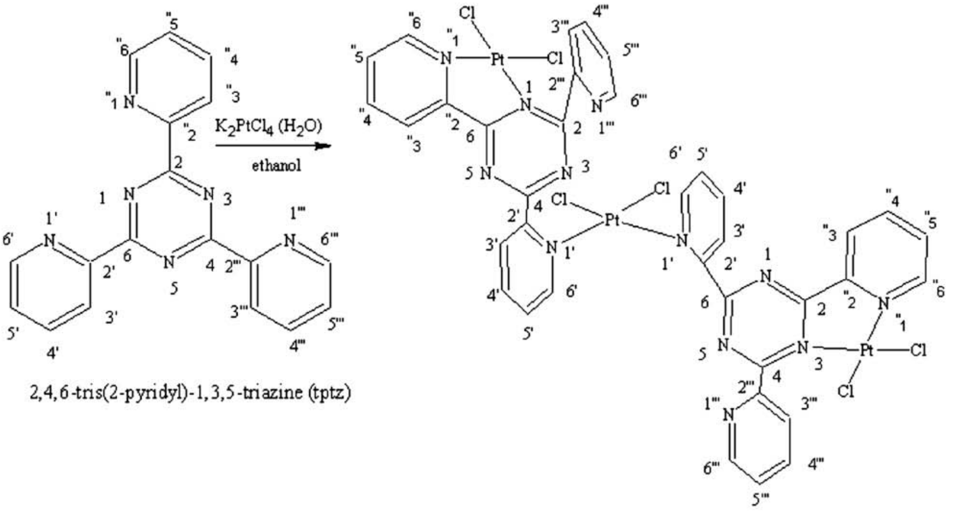
Fig. 2. Structure of $[(PtCl_2)_2(bptz)_2(\mu\text{-}Pt)]Cl_2$ complex (**2**) and numbering scheme of atoms.

possible configurations depending on the relative positions of N atoms in the pyridine rings were reported (along with the X-ray structure) [9].

In a configuration, all three cavities in the structure contain different numbers of N atoms (one, two and three) while in the other all cavities contain two N atoms [10]. The latter is preferred because in the former one, Pt atom would be triply coordinated by N atoms. The second configuration is confirmed in the complex **1** by conductivity measurements which are in agreement with a 1:1 electrolyte, being Pt—NCPh bonded to the remaining N donor atom. The proposed configuration strongly reduced any steric interaction between H_3 atoms and $PtCl_2$ or PtCl—NCPh groups for both complexes [11].

The coordination mode of the complexes were determined by using ^1H and ^{13}C NMR, ^1H—^1H COSY and assignments of resonances were based on the literature data [11] and are in good agreement with the proposed structures Figs. 1 and 3. A typical ^1H—^1H COSY spectrum for **1** is shown in Fig. 5.

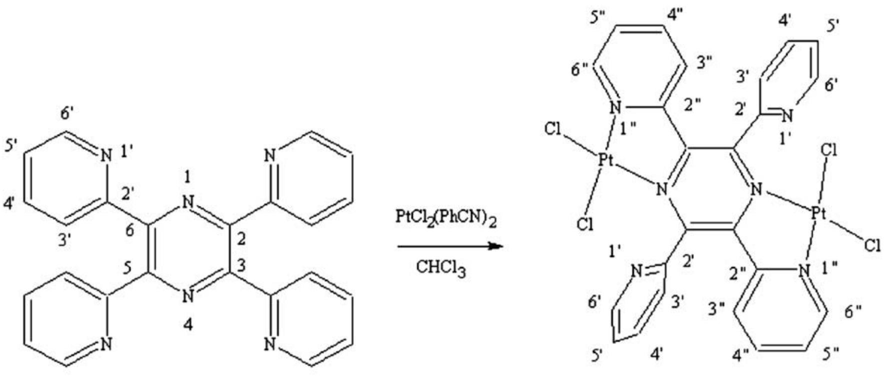
^1H NMR spectrum for the free ligand (δ ppm): 8.96 (d, 3H, $H_{6'+6''+6'''}$); 8.80 (d, 3H, $H_{3'+3''+3'''}$), 7.84 (dt, 3H, $H_{4'+4''+4'''}$); 7.42 (dt, 3H, $H_{5'+5''+5'''}$) which closely agrees with the spectra in $(CDCl_2)_2$ reported in the literature [12]. ^{13}C {^1H} NMR spectrum for the free ligand (δ ppm): 171.8 (C_{2+4+6}); 153.0 ($C_{2'+2''+2'''}$); 150.3 ($C_{3'+3''+3'''}$); 137.6 ($C_{6'+6''+6'''}$); 126.9 ($C_{5'+5''+5'''}$); 125.0 ($C_{4'+4''+4'''}$). ^1H NMR spectrum for complex **1** (δ ppm): 9.10 (d, 2H, $H_{6'+6''}$); 8.93 (d, 2H, $H_{3'+3''}$); 8.84 (d, 2H, $H_{6'''+3'''}$); 8.66 (dt, 2H, $H_{4'+4''}$); 8.25 (t, 1H, $H_{4'''}$), 8.18 (dt, 2H, $H_{5'+5''}$); 7.81 (d, 1H, $H_{5'''}$), 7.78 (m, 5H, benzonitrile). Coupling constants for the complex are: $J^3_{H6',H6''\text{-}H5',H5''} = 5.5$; $J^3_{H3',H3''\text{-}H4',H4''} = 7.2$; $J^3_{H6''',H3'''\text{-}H5''',H4'''} = 7.2$ Hz which are very similar to those found for the ligand, helping the assignments of resonances. ^{13}C {^1H} NMR for the complex (δ ppm): 172.00 (C_{4+6}), 169.9 (C_2),



2,4,6-tris(2-pyridyl)-1,3,5-triazine (tptz)

$[(PtCl_2)_2(tptz)_2(\mu\text{-}PtCl_2)](H_2O)_4$ (**3**)

Fig. 3. Structure of $[(PtCl_2)_2(tptz)_2(\mu\text{-}PtCl_2)](H_2O)_4$ complex (**3**) and numbering scheme of atoms.



2,3,5,6-tetra(2-pyridyl)pyrazine (tppz)

[(PtCl$_2$)$_2$(μ-tppz)](CHCl$_3$) (**4**)

Fig. 4. Structure of [(PtCl$_2$)$_2$(μ-tppz)](CHCl$_3$) complex (**4**) and numbering scheme of atoms.

153.4 ($C_{3'+3''}$), 153.3 ($C_{3'''}$), 151.4 ($C_{2'+2''}$), 150.8 ($C_{2'''}$), 143.0 ($C_{6'+6''}$), 138.0 ($C_{6'''}$); 133.6 ($C_{5'+5''}$); 133.2 ($C_{4'+4''}$); 129.8 ($C_{5'''}$); 128.9 ($C_{4'''}$) (triazyl and pyridyl carbons), 132.5, 129.1, 127.1, 119.2 (benzonitrile).

^{1}H NMR spectrum for complex **3** (δ ppm): 9.07 (d, 2H, $H_{6'+6''}$); 8.92 (d, 2H, $H_{6'''+3'''}$); 8.83(d, 2H, $H_{3'+3''}$); 8.63 (dt, 2H, $H_{4'+4''}$); 8.23 (t, 1H, $H_{4'''}$), 8.18 (dt, 2H, $H_{5'+5''}$); 7.80 (d, 1H, $H_{5'''}$). Coupling constants for the complex are: $^3J_{H6',H6''\text{-}H5',H5''} = 5.4$; $^3J_{H3',H3''\text{-}H4',H4''} = 7.8$; $^3J_{H6''',H3'''\text{-}H5''',H4'''} = 7.8$ Hz which are very similar to those found for the ligand. ^{13}C {^{1}H} NMR for the complex (δ ppm):152.9 (C_{4+6}), 152.6 (C_2), 152.2 ($C_{3'+3''}$), 151.9 ($C_{3'''}$), 139.6 ($C_{2'+2''}$), 138.2 ($C_{2'''}$), 137.9 ($C_{6'+6''}$), 137.5 ($C_{6'''}$), 126.5 ($C_{5'+5''}$), 126.2 ($C_{4'+4''}$), 125.9 ($C_{5'''}$), 125.5 ($C_{4'''}$). Noticeably coordinated triazine and coordinated pyridyl resonances are significantly shifted to higher frequencies compared with resonances in the free ligand as reported in the literature [13].

Like for the X-ray structure of ruthenium(II) with tptz [14] complex, the triazine and coordinated pyridyl rings are expected to be co-planar, while the uncoordinated pyridyl groups are tilted out of the coordination plane.

In our complexes the proposed structure is the one, among several configurations, which minimizes steric interactions, essentially of the central bridging Pt–NCPh or PtCl$_2$ groups, with the two ligand moieties; all platinum groups adopting a square planar geometry, triazine and coordinated pyridyl rings being co-planar and uncoordinated pyridyl groups out of the above plane while the overall geometry is consistent with a transoid configurations. Although tptz has the potential to coordinate three metal ions, no structures of tptz coordinating three metals can be found in the latest version of the CCDC database. The driving force for the synthesis of trinuclear tptz complexes necessitates the concurrence of a second ligand moiety.

2.1.4. *^{1}H and ^{13}C NMR spectra of trinuclear [(PtCl$_2$)$_2$(bptz)$_2$(μ-Pt)]Cl$_2$ complex*

The metal-assisted assembly of cyclic molecular arrays plays an integral part in the development of engineering molecular architectures. Bptz, a polydentate ligand, has been used for the synthesis of metallo-ligands/synthons based on organometallics systems which could be employed in the synthesis of homo/hetero bi- or polynuclear complexes [14]. The ionic trinuclear complex **2** of Pt (II) with bptz reported in this work offers a clear indication of the multitopic complexing ability of this ligand. Assignment of the coordination mode was obtained by use of ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY spectra, resonances were identified on the basis of the literature data [15] and are in good agreement with the proposed structure Fig. 2.

^{1}H NMR spectrum for free ligand (δ ppm): 8.97 (dd, 2H, $H_{6'+6''}$), 8.68 (dd, 2H, $H_{3'+3''}$), 7.87 (dt, 2H, $H_{4'+4''}$), 7.45 (q, 2H, $H_{5'+5''}$). ^{13}C {^{1}H} NMR spectrum for the free ligand (δ ppm): 164.6 (C_3), 151.3 (C_6), 150.4 ($C_{6'}$), 150.0 ($C_{6''}$), 149.6 ($C_{2'+2''}$), 136.1 ($C_{5'}$), 135.7 ($C_{5''}$), 135.4 ($C_{3'}$), 124.0 ($C_{3''}$), 123.7 ($C_{4'}$), 123.4 ($C_{4''}$).

^{1}H NMR spectrum for complex **2** (δ ppm): 9.77 (d, 2H, $H_{6'+6''}$), 8.64 (d, 2H, $H_{6'''+6IV}$), 8.47 (d, 2H, $H_{3'+3''}$), 8.10

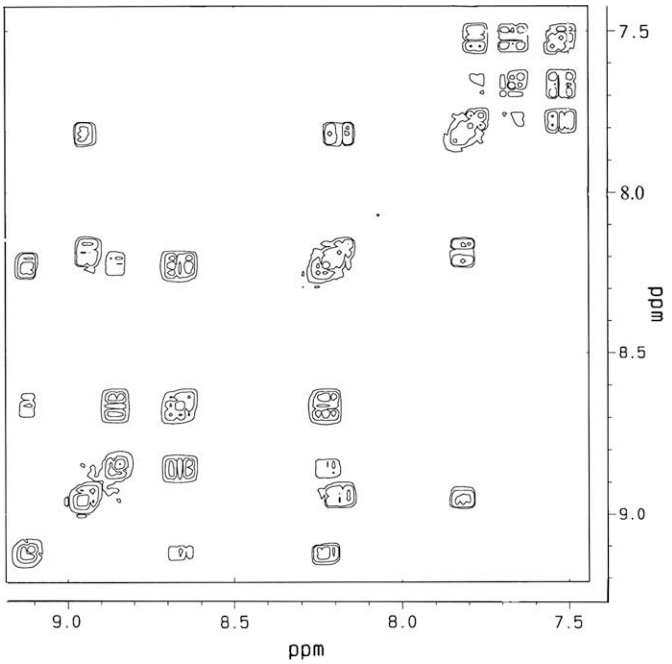


Fig. 5. ^{1}H–^{1}H COSY spectrum for [(PtCl$_2$)$_2$(tptz)$_2$(μ-PtClNCPh)]Cl complex (**1**).

(d, 2H, $H_{3'''+3IV}$), 7.67(dt, 2H, $H_{4'+4''}$), 7.57 (dt, 2H, $H_{4'''+4IV}$), 7.20 (m, 4H, $H_{5'+5''+5'''+5IV}$).

$^{13}C\ \{^1H\}$ NMR spectrum for the complex: 150.0 (C_3), 149.9 (C_6), 149.1 ($C_{6'}$), 148.7 ($C_{6''}$), 148.3 ($C_{6'''}$), 147.9 (C_{6IV}), 136.5 ($C_{2'+2''}$) 136.4 ($C_{2'''+2IV}$), 135.3 ($C_{5'}$), 134.9 ($C_{5''}$), 134.6 ($C_{5'''}$), 134.2 (C_{5IV}), 133.9 ($C_{3'}$), 125.6 ($C_{3''}$), 125.3 ($C_{3'''}$), 123.7 (C_{3IV}), 123.1 ($C_{4'}$), 122.8 ($C_{4''}$), 122.5 ($C_{4'''}$), 122.2 (C_{4IV}).

Coupling constants for the complex are: $J^3_{H6', H6''-H5', H5''} = 5.6$, $J^3_{H6''', H6IV-H5''',H5IV} = 5.6$, $J^3_{H4'',H5''} = 3.9$; $J^3_{H3', H3'',-H4', H4''} = 8.7$; $J^3_{H3''',H3IV-H4''', H4IV} = 7.1$.

The four proton atoms of the coordinated pyridyl rings give four doublet signals for H_3 and H_6 positions, two double triplets for H_4 positions and a multiplet for H_5. It is interesting that one ^1H NMR signal at $H_{6',6''}$ position was shifted downfield and the other six ^1H NMR signals were shifted to the upfield region relative to those of the free tptz ligand. A similar behaviour has been reported for iridium(III) complexes with bptz [15]. For our complex the explanation being that the upfield shift based on the ring current effect of the coordinated pyridyl is compensated by the downfield shift based on the coordination shift of the Pt atom to the pyridyl ring, resulting in an apparent decrease of the coordination shift to the upfield region. The central Pt–N_4 group is bridging by four N atoms of both bptz ligand moieties. Coordinated pyridyl groups and the central tetrazine are expected to be co-planar and the overall geometry of the complex is in transoid configuration.

2.1.5. *^1H and ^{13}C NMR spectra of binuclear $[(PtCl_2)_2(\mu\text{-}tppz)](CHCl_3)$*

The relevant potential of tppz as a versatile multi-site component for the design and the construction of complex structures have been demonstrated [16]. In this paper we report the synthesis of the neutral binuclear Pt(II) complex. The coordination mode of the complex was determined by using ^1H NMR, ^1H–^1H COSY spectra and assignments of resonances were based on the literature data [17] and are in good agreement with the proposed structure (Fig. 4). ^1H NMR spectrum for the free ligand (δ ppm): 8.39 (d, 4H, $H_{6'+6''}$), 8.05 (d, 4H, $H_{3'+3''}$), 7.80 (dt, 4H, $H_{4'+4''}$), 7.42 (dt, 4H, $H_{5'+5''}$). ^1H NMR spectrum for the complex (δ ppm): 8.92 (d, 2H, $H_{6''}$), 8.57 (t, 2H, $H_{5''}$), 8.28 (d, 2H, $H_{3''}$), 8.05 (t, 2H, $H_{4''}$), 7.83 (d, 2H, $H_{6'}$), 7.73 (t, 2H, $H_{4'}$), 7.59 (t, 2H, $H_{2'}$), 7.46 (d, 2H, $H_{3'}$). Coupling constants for the complex are: $J^3_{H6'',H5''} = 4,8$; $J^3_{H4'',H5''} = 3.9$; $J^3_{H3'',H4''} = 7.5$; $J^3_{H4',H5'} = 6.9$, $J^3_{H6'H5'} = 7.5$; $J^3_{H3',H4'} = 6.6$. The singlet signal at 8.3 is due to the proton of $CHCl_3$.

In our complex **4**, two sets of resonances are present for uncoordinated and coordinated pyridyl groups, the former being upfield and the latter downfield relative to the corresponding free ligand resonances. The group of resonances that shifts downfield suggests a decrease in electron density on the ring protons following coordination, while $^3J_{H\text{-}H}$ coupling constants for the complex are very similar to those of the free ligand. Apparently the resonances due to the pyridine groups are sharp and different for coordinated and non-coordinated moieties which give an indication that neither oligomerization of complexes nor fluxionality is present. The stability of the complex is in contrast with the case of dinuclear iridium(I) hydrido complex that shows broad ^1H NMR signals at room temperature and a sharp signal with lower temperature [16] given the presence of fluxional behaviour in mono- and dinuclear complexes with tppz.

In rhenium(I) complex with tppz [18], the two free pyridyl rings are rotated at angles of 61.7° and 57.6° with respect to the pyrazine ring to minimize the steric interactions with the coordination spheres. The proposed structure (Fig. 4) reflects the above reported structure and an overall transoid configuration is indicated for the complex.

2.2. *Biology*

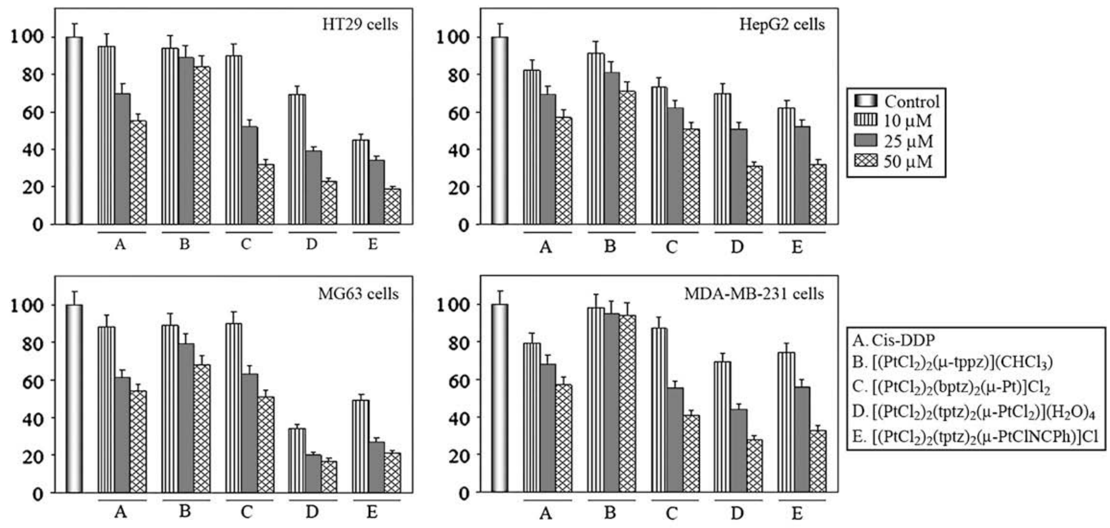
2.2.1. *The effect of platinum complexes on viability of cancer cells*

The aim of the biological study was to evaluate the effects exerted by the novel polynuclear complexes of Pt(II), in comparison with cisplatin, on monolayer cultures of a panel of different cancer cell lines, including human HT29 colon–rectal carcinoma, HepG2 hepatoma, MDA-MB-231 breast cancer and MG63 osteosarcoma cells. For these experiments, monolayer cultures were treated with the compounds at various concentrations and cell viability was studied by MTT assay as reported in Section 4.1. Fig. 6A shows the results obtained at 24 h treatment. All the compounds except complex **4**, clearly reduced cell viability in a dose-dependent manner showing a cytoxicity always higher than that exerted by cisplatin. The comparison of IC50 values evidenced that tptz complexes **1** and **3** were particularly efficacious in all the cell line tested (Fig. 6B). For these compounds the maximum reduction in cell viability (70–85%) was reached at the concentration of 50 μM. These results were confirmed by microscopic and cytofluorimetric analyses. Fig. 7A shows the morphological changes induced by treatment for 24 h with **1** and **3** in HT29 cells. Many rounded cells appeared isolated and detached from culture substrate indicating the commitment of cells to apoptotic death. The effects of cisplatin were much less evident. Similar results were obtained in the other cell lines employed (not shown). Experiments were also performed by means of flow cytometric analysis after staining of DNA with propidium iodide. The percentage of cells with subdiploid DNA confined to the sub-G0/G1 phase of the cell cycle was assumed as apoptosis index. Fig. 7B shows that tptz-treatment for 24 h induced an higher percentage of HT29 cells in the sub-G0/G1 phase in comparison to cisplatin-treated cells.

Experiments performed to assay the possible activity of free ligands used to synthesize the Pt(II) complexes demonstrated that these ligands were not able to exert any inhibitory activity (not shown).

3. Conclusions

In this paper we report the synthesis, structural characterization and cytotoxic activity of ‘‘non-classical’’ platinum complexes **1**, **2**, **3** and **4** which represent an array of tri-, and



IC50 (μM)

	HT29	HepG2	MG63	MDA-MB-231
Cis-DDP	58	65	60	66
$[(PtCl_2)_2(bptz)_2(\mu\text{-}Pt)]Cl_2$	29	53	54	36
$[(PtCl_2)_2(tptz)_2(\mu\text{-}PtCl_2)](H_2O)_4$	21	28	8	23
$[(PtCl_2)_2(tptz)_2(\mu\text{-}PtClNCPh)]Cl$	9	26	10	28

Fig. 6. The effects of Pt(II) complexes or *cis*-DDP on the viability of different cancer cell lines. Cells were treated for 24 h with the compounds at the indicated concentrations. Cell viability was estimated by MTT assay, as reported in Section 4.1. Data, expressed as the percentage of viable cells with respect to untreated controls, represent the means ± SEM of four separate experiments performed in triplicate. (B) Table shows IC50 values relative to the indicated compounds.

dinuclear complexes, which include both ionic and neutral species. The neutral triazine complex **3** presents three separate $PtCl_2$ moieties in a *cis* position, presumably acting in a bifunctional mode towards DNA. The cationic complex **1** contains monofunctional and bifunctional Pt(II) moieties; complex **2** contains bifunctional $PtCl_2$ groups and a bridging Pt(II) atom binding the bptz ligand moieties; further in complex **4** bifunctional Pt(II) moieties are present. Two of the novel

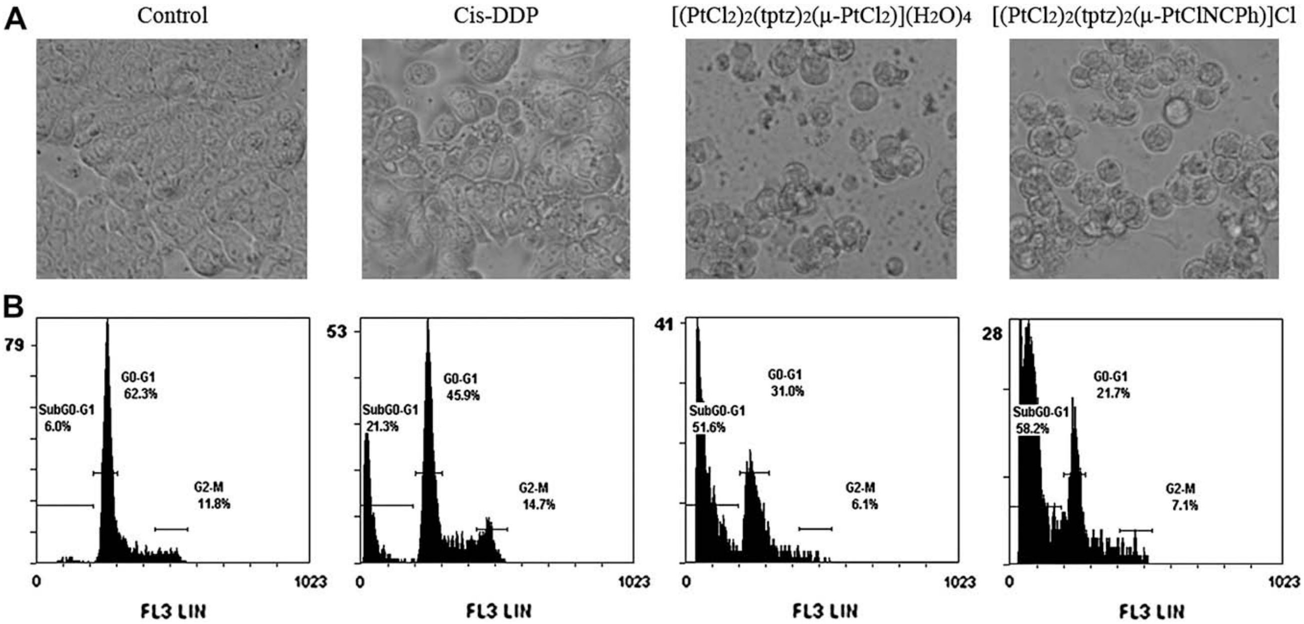


Fig. 7. Morphological and cytofluorometric analysis of HT29 cells. The cells were treated for 24 h with 25 μM tptz complexes or *cis*-DDP and observed by phase contrast microscopy (A) or analyzed by flow cytometry as reported in method (B). Data are representative of three independent experiments.

polypyridyl platinum complexes **1** and **3** synthesized in our laboratory show a potent cytotoxic activity in various cancer cell lines and, therefore, may represent a new class of potential chemotherapeutic agents.

A tentative examination of a qualitative structure–activity relationship indicates that the biological activity of Pt(II) in this series of complexes depends on the nature of the ligand moieties, and to a minor extent by the ionic character. We previously reported [7] that potentially polyfunctional dinuclear ionic platinum(II) complex with tptz was endowed with significant cytotoxic activity higher than cisplatin. Similarly among the polypyridyl Pt(II) complexes presented in this work, the most active are those containing the tptz ligand, both being trinuclear, both containing a bridging Pt(II) group, one neutral the other ionic. It appears that the number of Pt(II) moieties is not ‘‘per se’’ a prerequisite of enhanced antitumor activity, the evidence that tptz complexes is endowed with similar structures imposed by the polydentate ligand moiety geometry, which gives an indication that the latter ligand represents the driving force to form adducts with a DNA. In conclusion, tptz represents the most efficient molecular scaffold for the drug design of this family of novel Pt(II) complexes, the overall structure of the complexes being the major determinant of proapoptotic action. The relevance of ligand role in platinum(II) complexes has been stressed by Hambley [19] who correlated biological activity with steric conformation and characteristic electronic crowding of aromatic nitrogen heterocyclic ligands which modify the modes of DNA binding.

4. Experimental

4.1. Materials and methods

Potassium tetrachloro platinate(II), platinum(II) chloride and the ligands tppz, bptz and tptz were purchased from Aldrich; methanol was dried by standard methods and syntheses were performed with exclusion of moisture and direct light.

Compounds were analyzed for C, H, N at the Laboratorio di Microanalisi (Università degli Studi di Padova, Italy), chlorine was determined by potentiometric titration with standard silver nitrate after combustion in pure oxygen according to Schöninger [20].

Thermogravimetric measurements were performed from room temperature to 500 °C, with a Mettler TA-4000 system operating in a pure nitrogen atmosphere. The IR spectra were recorded, as nujol mulls, with a Model Spectrum ONE Perkin–Elmer Fourier Transform Instrument. Conductivity measurements for 10^{-3} M solutions in nitromethane were obtained with a Crison GLP 31 Model conductometer. Conductivity measurements on freshly prepared samples showed no evidence of significant irreversible solvolysis of platinum groups since only the ionic complexes **1** and **2** showed conductivity. The determination of platinum(II) content in polynuclear complexes was obtained using a flame atomic spectrometer Perkin–Elmer 372 and the reported values are the average of four independent measurements. A mechanistic study of mode of action of the complexes in water (kinetics of hydrolysis) was prevented by their insolubility in water.

The very poor solubility of all complexes made it necessary to record the NMR spectra in DMSO-d_6. 1D and 2D ^{1}H and ^{13}C {^{1}H} NMR spectra for the Pt(II) derivatives were obtained at 298 K with Bruker Avance 300 instrument operating at 300 MHz for ^{1}H and at 63 MHz for ^{13}C, in DMSO-d_6 with tetramethylsilane (TMS) as the internal standard; furthermore, to facilitate the assignments, ^{1}H–^{1}H COSY (H,H correlated spectroscopy) experiments were carried out. Abbreviations are as follows: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet.

4.2. Chemical synthesis

4.2.1. Synthesis of $[(PtCl_2)_2(tptz)_2(\mu\text{-}PtClNCPh)]Cl$ (*1*)

To a solution of tptz (0.200 g, 0.64 mmol) in dry methanol (10 mL) a yellow solution of $PtCl_2(PhCN)_2$ (0.302 g, 0.64 mmol) in acetone (50 mL) was added dropwise with stirring in the dark. The mixture was heated to 30 °C for 48 h in the dark. The colour of the resulting solution changes to orange-red. The product **1** was filtered, washed with methanol and acetone and dried over P_4O_{10}. Yield: 90%. Anal. calcd for $C_{43}H_{29}N_{13}Pt_3Cl_6$: C, 33.85; H, 1.92; N, 11.93; Cl, 13.94; Pt, 38.36%. Found: C, 33.49; H, 1.82; N, 11.90; Cl, 14.10; Pt, 38.39%. Melting point: >350 °C (decomp.). The complex is soluble in DMSO. Thermogravimetric analysis confirmed the occurrence of 1 mol of PhCN per formula unit. Conductivity measurements of the complex for 10^{-3} M solution in nitromethane afforded a value of $\Lambda_M = 84.4\ \text{ohm}^{-1}\,\text{cm}^2\,\text{mol}^{-1}$ which was consistent with uni-univalent complexes [21].

4.2.2. Synthesis of $[(PtCl_2)_2(bptz)_2(\mu\text{-}Pt)]Cl_2$ (*2*)

An aqueous solution of K_2PtCl_4 (0.832 g, 2.01 mmol) was treated with a solution of bptz (0.237 g, 1.00 mmol) in methanol (25 mL) and kept under reflux for 24 h in the dark, the colour of the solution changed to red-black. A black solid **2** was filtered and washed with water, methanol and diethyl ether and then dried over P_4O_{10}. Yield: 80%. Anal. calcd for $C_{24}H_{16}N_{12}Pt_3Cl_6$: C, 22.69; H, 1.27; N, 13.23; Cl, 16.74; Pt, 46.07%. Found: C, 23.08; H, 1.35; N, 13.60; Cl, 16.75; Pt, 45.68%. Melting point: >350 °C (decomp.). The complex is sparingly soluble in DMSO. Conductivity measurements of the complex for 10^{-3} M solution in nitromethane afford a value of $\Lambda_M = 135.1\ \text{ohm}^{-1}\,\text{cm}^2\,\text{mol}^{-1}$ which was consistent with a 1:2 electrolyte [21].

4.2.3. Synthesis of $[(PtCl_2)_2(tptz)_2(\mu\text{-}PtCl_2)](H_2O)_4$ (*3*)

An aqueous solution of K_2PtCl_4 (30 mL) (0.664 g, 1.60 mmol) was added dropwise with stirring in the dark to a solution of tptz (0.5 g, 1.60 mmol) in ethanol (50 mL). The mixture was heated to 40 °C for 48 h on argon atmosphere. The colour of the solution changes to red-yellow. A brown solid **3** was filtered and washed with water and ethanol. The solid was redissolved in 50 mL of acetonitrile and kept at 60 °C for 1 h. Upon cooling a solid was obtained which was filtered and dried over P_4O_{10}. Yield: 90%. Anal. calcd for

$C_{36}H_{32}N_{12}Pt_3Cl_6O_4$: C, 28.25; H, 2.37; N, 10.98; Cl, 13.90; Pt, 38.23%. Found: C, 27.95; H, 2.63; N, 11.04; Cl, 14.23; Pt, 38.30%. Melting point: >350 °C (decomp.). The complex is sparingly soluble in DMSO. Thermogravimetric analysis confirmed the occurrence of 4 mol of H_2O per formula unit. Conductivity measurements of the complex for 10^{-3} M solution in DMSO afforded a value of $\Lambda_M = 7.1\ \text{ohm}^{-1}\ \text{cm}^2\ \text{mol}^{-1}$associated with a neutral complex.

*4.2.4. Synthesis of [(PtCl$_2$)$_2$(μ-tppz)](CHCl$_3$) (**4**)*

To a solution containing a slight excess of tppz in chloroform (0.323 g, 0.83 mmol), a yellow solution of $PtCl_2(PhCN)_2$ (0.357 g, 0.76 mmol) in chloroform (20 mL) was added dropwise with stirring, in the dark for 24 h, without heating. The colour of the solution turned to orange-brown. A brown solid **4** was filtered, washed with chloroform and dried over P_4O_{10}. Yield: 90%. Anal. calcd for $C_{25}H_{17}N_6Pt_2Cl_7$: C, 28.88; H, 1.65; N, 8.08; Cl, 23.87; Pt, 37.52%. Found: C, 29.10; H, 1.38; N, 7.82; Cl, 23.70; Pt, 37.75%. Melting point: >350 °C (decomp.). The complex is sparingly soluble in DMSO.

Thermogravimetric analysis confirmed the occurrence of 1 mol of chloroform for formula unit.

4.3. Biological activity

4.3.1. Chemicals and reagents

Stock solutions of the compounds were prepared in DMSO and opportunely diluted in the culture medium. The final concentration of DMSO never exceeded 0.1% which is a concentration that was experimentally determined to have no discernible effect from the control in the employed cell lines.

4.3.2. Cell lines and culture conditions

Human colon–rectal carcinoma HT29 cells, hepatoma HepG2 cells and MDA-MB-231 breast cancer cells were grown as monolayers in RPMI 1640 medium. Osteosarcoma MG63 cells were grown in DMEM medium. All the culture media were supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 2.0 mM glutamine, at 37 °C in a humidified atmosphere containing 5% CO_2. HepG2 culture medium also contained 1.0 mM sodium pyruvate. For the experiments, cells were seeded on 96-well plates at a density of 10^4 cells/well. After plating, cells were allowed to adhere overnight and then treated with the compounds or vehicle alone (control cells).

4.3.3. Cell viability assay

The cytotoxic activity of the compounds was determined by the MTT quantitative colorimetric assay [22]. This method is based on the reduction of the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) into purple formazan by mitochondrial dehydrogenases of living cells. The absorbance at 570 nm (test wavelength) and at 630 nm (reference wavelength) was measured using an ELISA microplate reader (OPSYS MR, Dynex Technologies) against lysis buffer as a blank. Values reported in figures are expressed as percentage of the viability with respect to control cells.

4.3.4. Cell cycle analysis

The cell cycle phase distribution was evaluated by cytofluorimetric analysis. After exposure to the compounds, cells were harvested by trypsinization and incubated for 2–3 h at 4 °C in a hypotonic solution containing 50 μg/mL propidium iodide, 0.1% sodium citrate, 0.01% Nonidet P-40 and 10 μg/mL RNase A. The cell cycle profiles were analyzed by Epics XL flow cytometer (Beckman Coulter) using Expo32 software.

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