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Synthesis, Crystal Structures and Anticancer Activity of Pt(II) Complexes with Two Sterically Hindered 2-Methylpyridine Groups as The Carrier

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A series of novel platinum(II) complexes with two sterically hindered 2-methyl- pyridine groups as the carrier, *cis*-[Pt(2-methylpyridine)$_2$X$_2$] (X = Cl$^-$ (**1**), oxalate (**2**), malonate (**3**), 1,1-cyclobutane dicarboxylate (**4**), 3-hydroxy-1,1-cyclobutane dicarboxylate (**5**), were synthesized and characterized by elemental analysis, IR, mass spectra and ^{1}H NMR spectra. The crystal structures of complexes **3-5** were further determined by X-ray single crystal diffraction. The two pyridine moieties adopts an almost perpendicular orientation relation to the platinum coordination plane and the two CH_3 groups are located above the coordination plane, approaching Z axis. These structural arrangements produce greater steric hindrance for ligand substitution reactions. Unfortunately the complexes do not show any significant anticancer activity against A549, A549/ ATCC and SGC-7901 cancer cell lines, possibly due to the fact that they lack hydrogen atoms on N-donors.

Key Words: Platinum(II), Sterically hindered, Crystal structure, Anticancer activity.

INTRODUCTION

Cisplatin is one of the most widely used and most active chemotherapeutic agents available for the treatment of a variety of malignancies, especially testicular and ovarian carcinoma. However, the drug's clinical utility is restricted by both toxicities and tumor resistance. In attempt to overcome these limitations of cisplatin, numerous analogues have been prepared and evaluated in a search for alternative active agents[1-5]. As of yet, there has been little success in developing a Pt drug capable of overcoming either innate or acquired drug resistance.

In recent years, much has been elucidated concerning the mechanisms underlying tumor resistance to cisplatin. Studies have revealed that a combination of reduced platinum transport, increased cytoplasmic detoxification *via* elevated glutathione and/or metallothionein level, enhanced DNA repair and increased cellular tolerance to Pt-DNA adducts are the major mechanisms underlying resistance[6-8]. Picoplatin,

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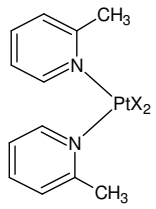
cis-amminedichloro (2-methylpyridine) platinum(II), currently undergoing phase II clinical trials[9], was rationally designed to circumvent resistance by sterically hindering cellular detoxification by glutathione and other cellular thiols. It is well-known that Pt(II) complexes are square planner configuration and the substitution reactions follow SN^2 pathway. The crystal structure of picoplatin revealed that the pyridine ring is tilted by 103° with respect to the Pt square plane. The tilt of the pyridine ring placed the 2-methyl group directly over the Pt square plane and leading to decreases the rate of substitution reactions by which detoxification of glutathione and other cellular thiols are produced[10,11]. However, the picoplatin contains only one sterically hindered group above Z axis and its water-solubility is poor. So, it is presumed that the steric hindrance would be improved if the complexes contain two sterically hindered groups located above and below the coordination plane, respectively. Based on this assumption[12-14], a series of hydrophilic platinum(II) complexes containing two 2-methylpyridine carriers are synthesized (Fig. 1).



1: X_2=2Cl$^-$, **2**: X_2=oxalate, **3**: X_2=malonate, **4**: X_2=CBDCA, **5**: X_2= HO-CBDCA

Fig. 1. Chemical structure of *cis-bis*(2-methylpyridine)platinum(II) complexes

EXPERIMENTAL

2-Methylpyridine and 1,1-cyclobutanedicarboxylate (CBDCA) were purchased from Aldrich companies. All the reagents and solvents used as received. 3-Hydroxy-1,1-cyclo-butane dicarboxylic acid (HO-CBDCA) was prepared according to the reported method[15]. Elemental analysis for C, H and N was performed with a Perkin-Elemer 240 Instrument, whereas platinum was determined according to the method in USP24. Mass spectral studies were carried out on a VG-AutoSpec3000 spectrometry in the FAB^+ mode using glycerine as matrix. IR spectra were recorded in the 4000-400 cm^{-1} regions on a Perkin-Elmer 880 spectrometer with KBr pellets. ^1H NMR was performed on Brucker DRX-500 (500.13 MHz) in DMSO.

The single crystal data were collected on a SMART APEX II CCD diffractometer at room temperature. For extracting intensities from CCD images the program Nonius was used. The structure were solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were calculated and allowed to ride. Computer programs: structure solution, SHELXS-97[16], refinement, SHELXS-97[17], molecular diagrams, ORTEP[18].

Synthesis: *cis*-[Pt(2-Methylpyridine)$_2$Cl$_2$] and *cis*-[Pt(2-methylpyridine)$_2$ (C_2O_4)] were prepared by using an extension of Dhara's method[19,20], due to less solubility of these two complexes in water.

***cis*-[Pt(2-Methylpyridine)$_2$Cl$_2$] (1):** Anal. calcd.(%) for $C_{12}H_{14}N_2Cl_2Pt$: C 31.86, H 3.13, N 6.19, Pt 43.13; Found (%): C 31.84, H 3.16, N 6.18, Pt 43.11. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 3.11 (m, 6H, 2CH_3), 7.25-7.36 (m, 2H, 2(C_5-H)), 7.49-7.55 (m, 2H, 2(C_3-H)), 7.78-7.81 (m, 2H, 2(C_4-H)), 8.92 (s, 2H, 2(C_6-H)). IR (KBr, ν_{max}, cm^{-1}): 3076 (C-H) m, 1609 (C=C) s, 1566 (C=C) m, 1515 (C=C) m. MS-FAB$^+$ m/z (%): 453 ((M+1)$^+$, 25), 380 ((M$^+$-2Cl), 80).

***cis*-[Pt(2-Methylpyridine)$_2$(C_2O_4)] (2):** Anal. calcd. (%) for $C_{14}H_{14}N_2O_4Pt$: C 35.82, H 3.01, N 5.97, Pt 41.56; Found (%): C 35.79, H 3.05, N 5.99, Pt 41.57. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 3.06 (m, 6H, 2CH_3), 7.31-7.34 (m, 2H, 2(C_5-H)), 7.58-7.59 (m, 2H, 2(C_3-H)), 7.91-7.93 (m, 2H, 2(C_4-H)), 8.91 (s, 2H, 2(C_6-H)). IR (KBr, ν_{max}, cm^{-1}): 3071 (C-H) m, 1672 asym.(COO$^-$) s, 1610 (C=C) m, 1566 (C=C) m, 1482 (C=C) m, 1436 (C=C) m, 1368 sym.(COO$^-$) m. MS-FAB$^+$ m/z (%): 470 ((M+1)$^+$, 100), 380 ((M$^+$-C_2O_4), 80).

For the preparation of more water-soluble *cis*-[Pt(2-methylpyridine)$_2$(malonate)], *cis*-[Pt(2-methylpyridine)$_2$(CBDCA)] and *cis*-[Pt(2-methylpyridine)$_2$(HO-CBDCA)], to a suspension of *cis*-[Pt(2-methylpyridine)$_2$I$_2$] (3.0 g, 4.72 mmol) in 80 mL water was added to the disilver salt of corresponding dicarboxylic acid. The reaction mixture was stirred at 40 ºC for 36 h. After AgI formed was filtrated off, the complexes were collected by freeze-drying.

***cis*-[Pt(2-Methylpyridine)$_2$(malonate)] (3):** Anal. calcd. (%) for $C_{15}H_{16}N_2O_4Pt$: C 37.27, H 3.34, N 5.80, Pt 40.36; Found (%): C 37.29, H 3.37, N 5.87, Pt 40.40 ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 3.09 (m, 6H, 2CH_3), 3.50 (s, 2H, CH_2) 7.32-7.35 (m, 2H, 2(C_5-H)), 7.54-7.56 (m, 2H, 2(C_3-H)), 7.87-7.90 (m, 2H, 2(C_4-H)), 8.96-8.97 (m, 2H, 2(C_6-H)). IR (KBr, ν_{max}, cm^{-1}): 3070 (C-H) m, 1646 asym.(COO$^-$) m, 1610 (C=C) m, 1567 (C=C) m, 1484 (C=C) m, 1347 sym.(COO$^-$) m. MS-FAB$^+$ m/z (%): 484 ((M+1)$^+$, 100), 380 ((M$^+$-$C_3H_2O_4$), 95).

***cis*-[Pt(2-Methylpyridine)$_2$(CBDCA)] (4):** Anal. calcd. (%) for $C_{18}H_{20}N_2O_4Pt$: C 41.29, H 3.86, N 5.35, Pt 37.26; Found (%): C 41.33, H 3.88, N 5.41, Pt 37.31 ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 1.69-1.75 (m, 2H, CBDCA), 2.74-2.77 (t, 4H, CBDCA), 3.02 (m, 6H, 2CH_3), 7.33-7.38 (m, 2H, 2(C_5-H)), 7.53-7.55 (m, 2H, 2(C_3-H)), 7.88-7.91 (m, 2H, 2(C_4-H)), 8.97-8.98 (m, 2H, 2(C_6-H)). IR (KBr, ν_{max}, cm^{-1}): 3029 (C-H) m, 1634 asym.(COO$^-$) m, 1611 (C=C) m, 1567 (C=C) m, 1483 (C=C) m, 1456 (C=C) m, 1349 sym.(COO$^-$) m. MS-FAB$^+$ m/z (%): 524 ((M+1)$^+$, 16), 431 ((M$^+$-C_6H_7N), 10), 380 ((M$^+$-CBDCA), 38).

***cis*-[Pt(2-Methylpyridine)$_2$(HO-CBDCA)] (5):** Anal. calcd. (%) for $C_{18}H_{20}N_2O_5Pt$: C 40.07, H 3.74, N 5.19, Pt 36.16; Found (%): C 40.11, H 3.79, N 5.23, Pt 36.21. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 2.42-2.44 (t, 4H, CBDCA-CH_2), 3.01 (m, 6H, 2CH_3), 3.86-3.89 (m, 1H, CBDCA-CH) 5.06 (m, 1H, CBDCA-OH)

7.32-7.36 (m, 2H, 2(C_5-H)), 7.54-7.55 (m, 2H, 2(C_3-H)), 7.88-7.91 (m, 2H, 2(C_4-H)), 8.93-8.94 (m, 2H, 2(C_6-H)). IR (KBr, ν_{max}, cm^{-1}): 3071(C-H) m, 1623 asym.(COO^-) m, 1568 (C=C) m, 1484 (C=C) m, 1456 (C=C) m, 1353 sym.(COO^-) m. MS-FAB$^+$ m/z (%): 540 ($(M+1)^+$, 55), 381 ($(M^+$-CBDCA), 52).

Cytotoxicity assay: The *in vitro* cytotoxicities of the platinum complexes were assessed by sulforhodamine B (SRB) colorimetric assay as described in the literature using A549 lung carcinomas, A549/ATCC lung carcinomas(resistant to several anti-cancer drugs) and SGC-7901 stomach carcinomas. Cells were continuously exposed to test compounds **1-5** and picoplatin for 72 h. The IC_{50} values were calculated from curves constructed by plotting cell survival (%) *versus* compound concentration.

RESULTS AND DISCUSSION

Compounds **1-5** were prepared by using an extension of Dhara's method or by the direct reaction between *cis*-[Pt(2-methylpyridine)$_2$I$_2$] and the disilver salt of corresponding dicarboxylic acid. All these platinum compounds were characterized by chemical analysis and spectroscopic data along with X-ray crystal structure for compounds **3-5** (Table-1). The elemental analysis data for each complex were in good agreement with the calculated values. The complexes showed $[M+1]^+$ and $[M-L]^+$ (L = leaving group) corresponding to their molecular ion and relative fragmental peaks. The mass spectra also exhibited typical three protonated molecular ion peaks because of the isotopes ^{194}Pt (33 %), ^{195}Pt (34 %) and ^{196}Pt (25 %). The dicarboxylatoplatinum(II) compounds showed the $\nu_{asym.}$(COO)-$\nu_{sym.}$(COO) values are more than 200 cm^{-1}, suggesting that carboxylate groups act as monodentate ligands. The ^{1}H NMR spectra of the compounds were all consistent with their corresponding protons both in the chemical shifts and the number of hydrogen. Complexes **3-5** have good solubility (> 20 mg/mL) and are stable in water.

The ORTEP drawing of complexes **3-5** depicted along with their atomic numbering scheme are shown in Figs. 2-4, respectively. The Pt(II), except complex **4**, has the expected square planar geometry exhibiting the usual structure parameters. The Pt atom of complex **4** is coordinated in a square-pyramidal environment because of the steric effect. As shown in Table-2, the average Pt-N bond lengths (2.015 Å) is slightly longer than average Pt-O bond lengths (2.003 Å). The six-membered chelate ring which leaving groups formed with the Pt(II) atom adopt the boat conformation and cyclobutane ring is nearly perpendicular to Pt(II) coordination plane. The most notable feature of the structure is the orientation of the two picoline ring with respect to the Pt square plane. The two pyridine moieties adopt an almost perpendicular orientation relation to the platinum coordination plane and the two CH_3 groups are located above the coordination plane instead of located above and below the coordination plane, respectively. The distances of Pt···CH_3 and the dihedral angle are listed in Table-3.

TABLE-1
CRYSTAL DATA AND STRUCTURE REFINEMENT FOR COMPLEXES **3**, **4** AND **5**

Complexes	**3**	**4**	**5**
E.f.	$C_{15}H_{16}N_2O_4Pt$	$C_{18}H_{20}N_2O_4Pt$	$C_{18}H_{20}N_2O_5Pt$
F_w	483.39	523.45	539.45
Temperature (K)	293(2)	293(2)	293(2)
Wavelength (nm)	0.71073	0.71073	0.71073
Crystal system	Monoclinic	Orthorhombic	Orthorhombic
Space group	P2(1)/n	Pnma	P2(1)2(1)2(1)
Unit cell dimensions (Å, º)	a = 8.5790(6)	a = 12.7339(7)	a = 9.5157(7)
	b = 15.8079(11)	b = 14.5313(8)	b = 13.1417(9)
	c = 11.5595(8)	c = 9.7716(6)	c = 15.2884(11)
	β = 107.7480(10)	β = 90	β = 90
Volume ($Å^{-3}$)	1493.04(18)	1808.14(18)	1911.9(2)
Z	4	4	4
Density (calc.)(gcm^{-3})	2.150	1.923	1.874
μ (mm^{-1})	9.417	7.785	7.369
F (000)	920	1008	1040
Crystal size (mm^3)	0.24 x 0.19 x 0.16	0.26 x 0.22 x 0.12	0.24 x 0.22 x 0.17
θ range (º)	2.25 to 28.26	2.51 to 28.29	2.04 to 28.33
Limiting indices	-11< = h< = 11, -20< = k< = 20, -15< = l< = 15	-16< = h< = 16, -19< = k< = 19, -12< = l< = 12	-12< = h< = 12, -17< = k< = 16, -20< = l< = 20
Reflection collected	12687	14744	16189
Independent reflection	3532[R(int) = 0.0288]	2278[R(int) = 0.0278]	4511 [R(int) = 0.0535]
Max. and min. Transmission	0.3142 and 0.2109	0.4552 and 0.2367	0.3672 and 0.2708
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/ parameters	3532 / 0 / 201	2278 / 0 / 119	4511 / 0 / 238
Goodness-of-fit on F^2	0.950	0.839	1.098
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0206, wR_2 = 0.0429	R_1 = 0.0192, wR_2 = 0.0527	R_1 = 0.0529, wR_2 = 0.1455
R indices (all data)	R_1 = 0.0282, wR_2 = 0.0458	R_1 = 0.0237, wR_2 = 0.0564	R_1 = 0.0600, wR_2 = 0.1505
Largest diff. peak and hole/e·$Å^{-3}$	0.525 and -0.756	0.952 and -1.042	4.964 and -2.215

***In vitro* cytotoxic activity:** The *in vitro* anticancer activities of the complexes were assessed by SRB assay and the results are given in Table-4. Preliminary cell proliferation assays performed for complexes **1-5**, disappointingly, indicate that cytotoxic activity were poor. The poor biological activity may be attributable to the fact that the complex lacks a hydrogen substituent on a N-donor atom, for the hydrogen bonds between the carrier and biomacromolecules play a positive role in binding PtA_2 active group to DNA.

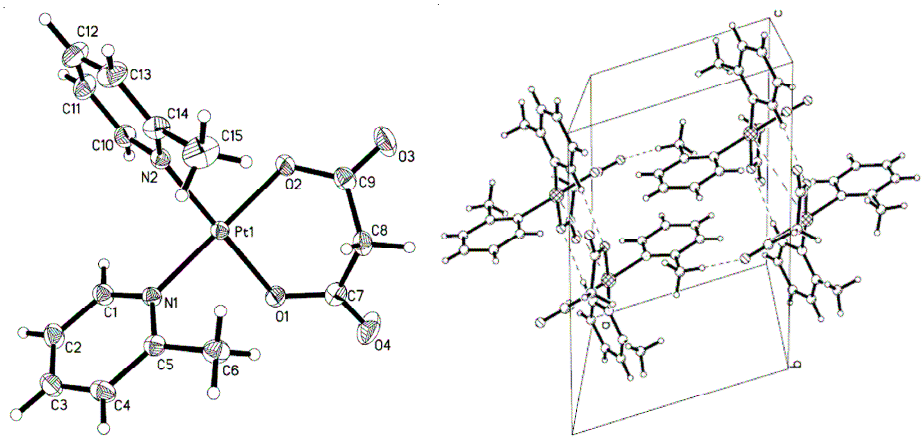


Fig. 2. Molecular structure of **3** and a packing view of **3**

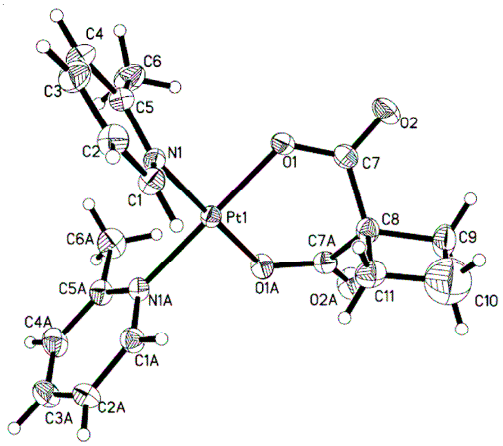


Fig. 3. Molecular structure of **4**

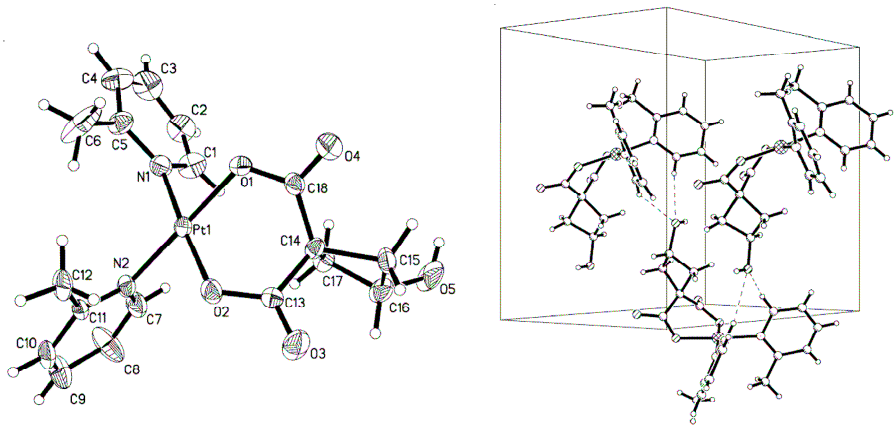


Fig. 4. Molecular structure of **5** and a packing view of **5**

TABLE-2
SELECTED BOND LENGTHS [Å] AND ANGLES [°] FOR COMPLEXES **3-5**

	3	**4**	**5**
Pt(1)-O(1)	2.013(2)	1.999(2)	1.992(8)
Pt(1)-O(2)	2.015(2)	1.999(2)	2.001(8)
Pt(1)-N(1)	2.022(3)	2.018(2)	2.005(10)
Pt(1)-N(2)	2.019(3)	2.018(2)	2.008(9)
O(1)-Pt(1)-O(2)	90.94(10)	92.21(12)	91.4(4)
O(1)-Pt(1)-N(2)	175.20(10)	178.74(9)	177.7(4)
O(2)-Pt(1)-N(2)	87.82(10)	87.12(10)	87.1(4)
O(1)-Pt(1)-N(1)	89.39(10)	87.12(10)	86.3(4)
O(2)-Pt(1)-N(1)	179.30(10)	178.74(9)	177.0(5)
N(2)-Pt(1)-N(1)	91.80(11)	93.54(14)	95.3(4)

TABLE-3
DIHEDRAL ANGLE BETWEEN THE 2-PICOLINE RING AND THE Pt SQUARE PLANE FOR COMPLEXES **3-5**, THE SHORTEST DISTANCE FROM Pt ATOM TO CH_3

3	C(1)C(5)–N(1)N(2)O(1)O(2) 68.3°	C(10)C(14)–N(1)N(2)O(1)O(2) 85.3°	Pt(1)···C(6) 3.191 Å	Pt(1)···C(15) 3.238 Å
4	C(1)C(5)–N(1)N(1A)O(1)O(1A) 76.5°	C(1A)C(5A)–N(1)N(1A)O(1)O(1A) 76.5°	Pt(1)···C(6) 3.196 Å	Pt(1)···C(6A) 3.196 Å
5	C(1)C(5)–N(1)N(2)O(1)O(2) 94.6°	C(7)C(11)–N(1)N(2)O(1)O(2) 114.1°	Pt(1)···C(6) 3.217 Å	Pt(1)···C(12) 3.185 Å

TABLE-4
in vitro CYTOTOXIC ACTIVITY IC_{50} OF COMPLEXES **1-5**

Complexes	IC_{50} (μmol L^{-1})		
	SGC-7901	A549	A549/ATCC
1	>100	>100	>100
2	>100	>100	>100
3	>100	>100	>100
4	>100	>100	>100
5	>100	>100	>100
Picoplatin	59.1	26.9	>100

Conclusion

Five novel platinum(II) complexes involving two 2-methylpyridine groups as the carrier have been synthesized and characterized by elemental analysis, IR, mass spectra and ^{1}H NMR spectra. The single crystals of three hydrophilic representative platinum complexes, *cis*-[Pt($C_6H_7N)_2$(malonate)], *cis*-[Pt($C_6H_7N)_2$(CBDCA)] and *cis*-[Pt($C_6H_7N)_2$(HO-CBDCA)] have been cultivated and subjected to X-ray crystallographic analysis. The crystal structures of complexes **3** and **5** exhibit that the platinum atom achieves a typical square planar arrangement with two nitrogen from the *bis*(2-methylpyridine) and two oxygen atoms in *cis*-position. The six-membered chelate

ring which leaving groups form with the Pt(II) atom adopt the boat conformation and cyclobutane ring is nearly perpendicular to Pt(II) coordination plane. The two pyridine moieties adopt an almost perpendicular orientation relation to the platinum coordination plane and the two CH_3 groups are located above the coordination plane. All the complexes do not show any significant anticancer activity against A549, A549/ATCC and SGC-7901 cancer cell lines, possibly due to the fact that the complexes lacks hydrogen atoms on N-donors.

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