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In vitro cytotoxicity study on platinum (II) complexes with epoxysuccinates as leaving groups

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Abstract—A series of novel cisplatin-type platinum complexes were designed, characteristic of epoxysuccinates as leaving groups. The pertinent compounds were prepared and characterized by IR, ^{1}H NMR, and ESI-MS spectra with elementary analyses. The in vitro cytotoxic activities of compounds toward SPC-A1 human lung adenocarcinoma cell line and BGC823 human stomach adenocarcinoma cell line were determined. Biological tests have confirmed that complexes containing 4*R*,5*R*-DMID [abbreviation of (4*R*,5*R*)-4,5-bis (aminomethyl)-2-isopropyl-1,3-dioxolane] as carrier ligands have greater cytotoxicity toward tumor cells than the corresponding compounds with other carrier ligands. Most platinum complexes with *trans*-epoxysuccinates usually have higher cytotoxicity than those with *cis*-epoxysuccinates. Complex **4a** shows the most effective among those tested platinum complexes in both cell lines, and its cytotoxicity approached that of cisplatin.

Cisplatin is one of the most widely used antitumor drugs in the treatment of various tumors at present,[1–9] however, the clinical shortcoming of cisplatin is obvious, such as its toxicity,[10–12] narrow range of activity, both intrinsic and acquired resistance, and low aqueous solubility.[13–21] Up till now, much effort has been dedicated to developing cisplatin analogues with expectation to possess broader spectra of activity, improved clinical efficacy, and reduced toxicity. Thousands of platinum compounds have been synthesized and biologically evaluated, but only a small number of them have been considered to be promising for human clinical trials. This situation is due to the limitation of cisplatin mentioned above. One major approach to achieve this goal is to alternate the chloride anions of cisplatin to appropriate leaving groups. In our previous study, we have replaced the chloride anion with alkoxyacetate as carboxylato ligands to promote the aqueous solubility of the related Pt(II) complexes of the type $[Pt(A_2)(OCOCH_2OR)_2]$ (A_2 is a diamine or two monoamines). Experiments indicated that most of them showed better in vitro cytotoxicity than carboplatin against the selected cell lines.[22] However, further investigation showed that these complexes were easily disassociated into ion pairs in their aqueous solutions, due to the weak coordination bond between the monodentate carboxylato ligand and the Pt(II) species. In addition, a number of complexes with seven-membered rings were found to exhibit high antitumor activity.[23–26] Thus, dicarboxylate containing an epoxy linkage is selected as a leaving ligand to prepare the corresponding Pt(II) complex. It is expected that such resulting Pt(II) complexes with dicarboxylate as a bidentate ligand may be less disassociated in water with chelating seven-membered rings, and show high in vitro cytotoxicity. Furthermore, the effect of the *cis*- and *trans*-configuration of epoxysuccinates on the cytotoxicity of these complexes will be studied.

cis- and *trans*-Epoxysuccinic acids were prepared by oxidizing the corresponding maleic acid and fumaric acid, respectively. A general method was applied to prepare the related platinum complexes containing epoxysuccinate anions by treatment of $[Pt(A_2)I_2]$[27,28] (A_2 is a diamine or two monoamines) with silver carboxylate.[29,30] *cis*-Epoxysuccinates served as leaving groups in complexes **1a**–**5a**, whereas *trans*-epoxysuccinates as leaving groups in compounds **1b**–**5b**. All compounds were spectrally characterized by IR, ^{1}H NMR, and ESI-MS spectra together with microanalyses.[34] The elemental analysis for each compound agreed well with the empirical formula proposed. In their IR spectra, the shifts of νNH_2 and δNH_2 frequencies comparing with the free amino group demonstrated the participation of amino

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groups in binding with Pt(II) due to Pt(II)–NH_2 coordination. The shifts of the C=O absorption from free carboxylic acids near 1700 cm^{-1} to a band near 1662–1614 cm^{-1} proved that the carboxylate anion was combined with the metal atom in each case.[31] The C–O–C absorptions of epoxy groups were clearly observed near 950 cm^{-1}, but the presence of C–O–C vibrations near 1250 cm^{-1} were feeble and concealed. Most of the compounds had a peak of $[M+H]^+$ in their positive ESI mass spectra, and several compounds gave $[M+Na]^+$ peaks, which are in agreement with their molecular formula weights. Because of three isotopes of Pt element, all the mass spectra of the platinum complexes were found with three protonated ion isotopic peaks. The molecular structures of all compounds shown in Figure 1 were also confirmed by their related ^1H NMR spectral data.

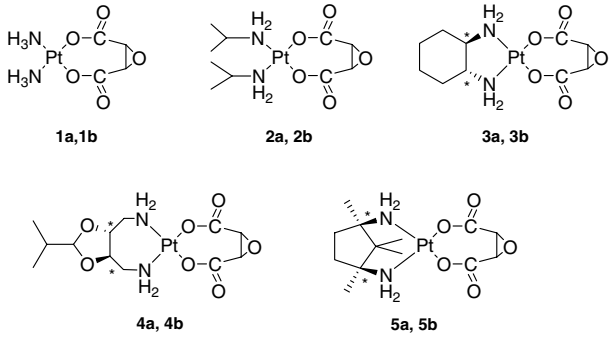

Figure 1. Structures of platinum(II) complexes (a = *cis*-epoxysuccinates; b = *trans*-epoxysuccinates).

The in vitro cytotoxicities of the platinum compounds against SPC-A1 human lung adenocarcinoma and BGC823 human stomach adenocarcinoma cell lines were screened by the School of Medicine, Nanjing University. Cytotoxicities of forerunner complexes **1a**–**5b** toward SPC-A1 human lung adenocarcinoma and BGC823 human stomach adenocarcinoma cell lines were determined by PI-FCM assay.[32,33] The cytotoxicities of these compounds were compared with those of cisplatin. In this study, cells were continuously exposed to test compounds **1a**–**5b** for 24 h. The results are given in Tables 1 and 2, and illustrated in Figures 2 and 3, respectively.

From the above biological results, it is concluded that human lung adenocarcinoma cell was more sensitive to those platinum analogues treatment. The majority of compounds **1a**–**5b** showed cytotoxicity against SPC-A1. Several of those complexes showed cytotoxicity against BGC823. Complex **4a** displayed the highest cytotoxicity against both cell lines. Its cytotoxicity was comparable to cisplatin toward both SPC-A1cell line and BGC823 cell line. As seen in Figures 2 and 3, the order of the cytotoxicities in SPC-A1 is cisplatin **>4a > 4b > 5b > 1b > 5a**, **3b > 3a**, in BGC823 is cisplatin **>4a > 4b**, **5b > 3b**.

Table 1. In vitro cytotoxicity against SPC-A1 human lung adenocarcinoma cell line of **1a**–**5b**

Complexes	The percentages of cell death after being treated with those complexes of five different concentrations (μg/mL)				
	50	20	10	5	1
1a	/	/	/	/	/
2a	/	/	/	/	/
3a	14.3 (113.9 μM)	/	/	/	/
4a	74.3 (100.2 μM)	60.2 (40.1 μM)	28.7 (20.0 μM)	14.8 (10.0 μM)	5.1 (2.0 μM)
5a	30.3 (107.1 μM)	8.0 (42.8 μM)	/	/	/
1b	40.6 (139.3 μM)	22.0 (55.7 μM)	15.5 (27.9 μM)	5.2 (13.9 μM)	/
2b	/	/	/	/	/
3b	24.2 (113.9 μM)	10.5 (45.6 μM)	3.8 (22.8 μM)	/	/
4b	78.6 (100.2 μM)	33.6 (40.1 μM)	18.8 (20.0 μM)	7.1 (10.0 μM)	2.5 (2.0 μM)
5b	66.3 (107.1 μM)	27.7 (42.8 μM)	7.2 (21.4 μM)	3.2 (10.7 μM)	2.4 (2.1 μM)
Cisplatin	74.8 (166.7 μM)	71.0 (66.7 μM)	64.0 (33.3 μM)	26.8 (16.7 μM)	2.8 (3.3 μM)

Table 2. In vitro cytotoxicity against BGC823 human stomach adenocarcinoma cell line of **1a**–**5b**

Complexes	The percentages of cell death after being treated with those complexes of five different concentration (μg/mL)				
	50	20	10	5	1
1a	/	/	/	/	/
2a	/	/	/	/	/
3a	/	/	/	/	/
4a	39.6 (100.2 μM)	20.4 (40.1 μM)	7.8 (20.0 μM)	6.0 (10.0 μM)	3.0 (2.0 μM)
5a	/	/	/	/	/
1b	/	/	/	/	/
2b	/	/	/	/	/
3b	16.4 (113.9 μM)	6.4 (45.6 μM)	/	/	/
4b	17.8 (100.2 μM)	11.7 (40.1 μM)	/	/	/
5b	24.6 (107.1 μM)	9.6 (42.8 μM)	4.2 (21.4 μM)	/	/
Cisplatin	45.9 (166.7 μM)	34.4 (66.7 μM)	32.8 (33.3 μM)	27.8 (16.7 μM)	18.8 (3.3 μM)

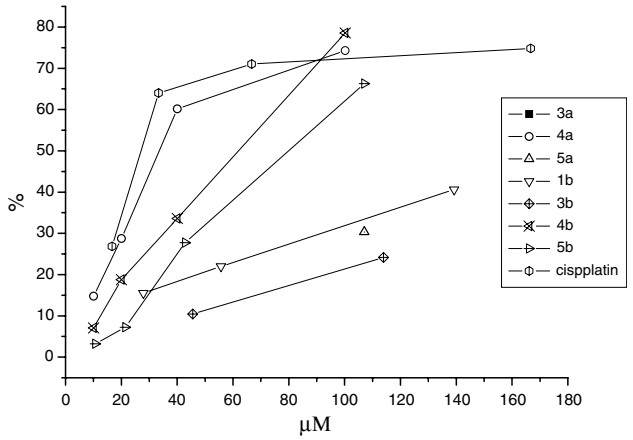

Figure 2. The percentages of cell death of human lung adenocarcinoma cell after being treated with those complexes of five different concentrations (μM).

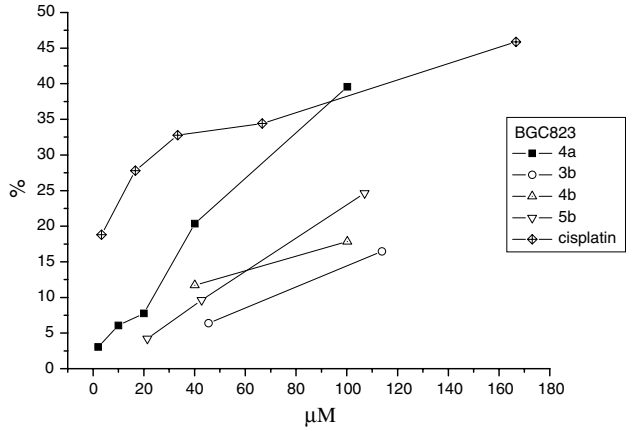

Figure 3. The percentages of cell death of human stomach adenocarcinoma cell after being treated with those complexes of five different concentrations (μM).

Based on the percentages of cell death, the structure–activity relationship revealed that the structure of the amino ligand was very important to cytotoxic activity. In general, it has been demonstrated that most of the platinum complexes with *trans*-*R*,*R*-bidentate diamine, such as **3a**–**5a**, and **5b**, are more active than those with the monoamine carrier ligand when the leaving group is the same. However, among these compounds, there is a reversing case, **1b** > **3b** in SPC-A1 cell. The activities of the complexes with isopropylamine seemed lowest in both selected cell lines.

The cytotoxicities of the resulting platinum complexes are also related to the nature of the leaving group. It has been observed from Figures 2 and 3 that nearly all platinum complexes with *trans*-epoxysuccinate moieties have higher cytotoxicity than those with *cis*-epoxysuccinate groups when the amine carrier ligand is the same, that is **5b** > **5a**; **3b** > **3a** and **1b** > **1a** in both selected cells. However, there is an exception, such as **4b** < **4a** in both SPC-A1 and BGC823.

The 3D models of complexes **4a** and **4b** are shown in Figure 4 by chemoffice.[35] It is observed that the seven-membered chelating ring in the complexes with *cis*-epoxysuccinates is less distorted than that of those with *trans*-epoxysuccinates. This hints that complexes *b* series may exhibit higher in vitro cytotoxicity than complexes *a* series, when these compounds have the same carrier ligand. This is probably due to the fact that the big tension of the chelating ring with *trans*-epoxysuccinates is liable to leave in contrast to those with *cis*-epoxysuccinates, which has been proved by experimental data only with an exception of complex **4a**.

In conclusion, a part of compounds exhibit better cytotoxic activity against tested cell lines. Complex **4a**, *cis*-(*cis*-epoxysuccinato)[(4*R*,5*R*)-4,5-diaminomethyl-2-isopropyl-1,3-dioxolane]platinum(II), displays the highest cytotoxicity against both tested cell lines. Since **4a** has lower solubility in both water and organic solvents, much work needs to be done to improve physicochemical properties of this series of complexes.

Acknowledgments

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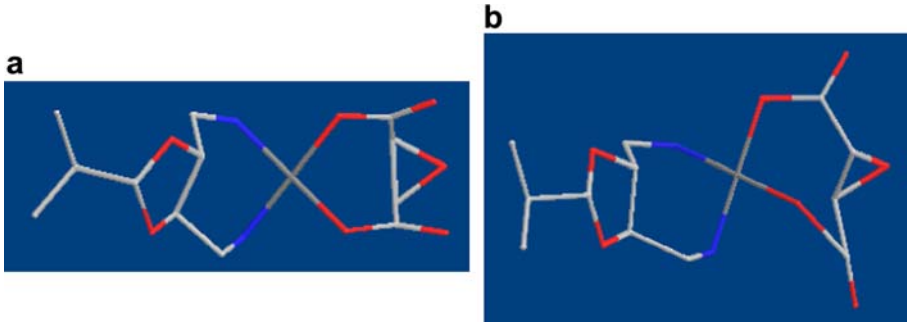

Figure 4. 3D models for **4a** and **4b**.

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34. *Synthesis of the complexes.* A suspension of the corresponding diiododiamineplatinum (II) complex (2 mmol) and silver epoxysuccinates (2 mmol) in H_2O (100 mL) was stirred at 60 °C in the dark overnight, the resulting deposits were filtered off and washed with water. The filtrate was evaporated to nearly dryness and refrigerated to give white solids, which were filtered off and dried under vacuum.
Data for **1a**. Yield: 28%, straw yellow solids. IR(ν, cm^{-1}): 3430 m (br), 3237 m, 1626vs, 1360s, 1119s, 1061s, 940s. ^1H NMR (D_2O/TMS): δ 3.97–4.19 (m, 2H, 2H of epoxysuccinates) ESI-MS: m/z $[M+H+H_2O]^+$ = 378(100%). Anal. ($C_6H_{16}N_2O_6Pt$) C, H, N.
Data for **1b**. Yield: 40%, straw yellow solids. IR (ν, cm^{-1}): 3256m (br), 1636vs, 1363s, 1112s, 1086s, 935s. ^1H NMR (D_2O/TMS): δ 3.96–4.15 (m, 2H, 2H of epoxysuccinates). ESI-MS: m/z $[M+Na+H_2O]^+$ = 400(100%). Anal. ($C_8H_{20}N_2O_6Pt$) C, H, N.
Data for **2a**. Yield: 19%, gray solids. IR(ν, cm^{-1}): 3428 m (br), 3215m, 2976w, 1635vs, 1351s, 1117s, 931s. ^1H NMR (D_2O/TMS): δ 1.34 (m, 12H, 12H of 2CH(CH_3)$_2$), 2.97 (m, 2H, 2H of 2CH(CH_3)$_2$), 4.03–4.52 (m, 2H, 2H of epoxysuccinates). ESI-MS: m/z $[M+Na+H_2O]^+$ = 484(100%). Anal. ($C_{12}H_{28}N_2O_6Pt$) C, H, N.
Data for **2b**. Yield: 28%, gray solids. IR(ν, cm^{-1}): 3422m (br), 3217m, 2975w, 1636vs, 1371s, 1116s, 1086s, 929s, 878s. ^1H NMR (DMSO-d_6/TMS): δ 1.34 (m, 12H, 12H of 2CH(CH_3)$_2$), 2.95 (m, 2H, 2H of 2CH(CH_3)$_2$), 4.25–4.52 (m, 2H, 2H of epoxysuccinates). ESI-MS: m/z $[M+H+2H_2O]^+$ = 480(100%). Anal. ($C_{12}H_{28}N_2O_6Pt$) C, H, N.
Data for **3a**. Yield: 56%, ivory solids. IR(ν, cm^{-1}): 3425m (br), 3210m, 2940w, 1629vs, 1348s, 1120m, 1064s, 922s, 613s. ^1H NMR (D_2O/TMS): δ 1.16–2.30 (m, 10H, 10H of 4CH_2 and 2CH of DACH), 4.07–4.45 (m, 2H, 2H of epoxysuccinates). ESI-MS: m/z $[M-DACH+K]^+$ = 364(100%). Anal. ($C_{14}H_{32}N_2O_6Pt$) C, H, N.
Data for **3b**. Yield: 48%, white solids. IR(ν, cm^{-1}): 3424m (br), 3219m, 2939w, 1636vs, 1362s, 1065s, 925s, 669s. ^1H NMR (D_2O/TMS): δ 1.07–2.25 (m, 10H, 10H of 4CH_2 and 2CH of DACH), 3.98–4.35 (m, 2H, 2H of epoxysuccinates). ESI-MS: m/z $[M-DACH+K]^+$ = 364(100%). Anal. ($C_{18}H_{40}O_6Pt$) C, H, N.
Data for **4a**. Yield: 14%, ivory solids. IR(ν, cm^{-1}): 3426m (br), 3228m, 2967w, 1630vs, 1363s, 1122v, 1097s, 955s, 678s. ^1H NMR (D_2O/TMS): δ 0.93 (s, 6H, 6H of (CH_3)$_2$CH), 1.88 (s, 1H, 1H of (CH_3)$_2$$CH$), 2.85–3.57 (m, 4H, 4H of 2C$H_2$$NH_2$), 3.94–4.53 (m, 4H, 2H of 2OCHC$H_2$$NH_2$ and 2CH of epoxysuccinates), 4.99 (m, 1H, 1H of CHCH (CH_3)$_2$). ESI-MS: m/z $[M+Na+2H_2O]^+$ = 558(100%). Anal. ($C_{14}H_{28}N_2O_8$ Pt) C, H, N.
Data for **4b**. Yield: 12%, ivory solids. IR(ν, cm^{-1}): 3421m (br), 3236m, 2966w, 1635vs, 1363s, 1096s, 952s, 669s. ^1H NMR (D_2O/TMS): δ 0.80(s, 6H, 6H of (CH_3)$_2$CH), 1.72(s, 1H, 1H of (CH_3)$_2$$CH$), 2.70–3.25 (m, 4H, 4H of 2C$H_2$$NH_2$), 4.10–4.40 (m, 4H, 4H of 2OCHC$H_2$$NH_2$ and 2CH of epoxysuccinates), 4.91 (m, 1H, 1H of CHCH(CH_3)$_2$). ESI-MS: m/z $[M+Na]^+$ = 522(100%). Anal. ($C_{16}H_{32}N_2O_8Pt$) C, H, N.
Data for **5a**. Yield: 62%, ivory solids. IR(ν, cm^{-1}): 3428m (br), 3218m, 3122m, 2975w, 1718s, 1618vs, 1351s, 1102s, 1042s, 941s, 668s. ^1H NMR (D_2O/TMS): δ 0.75–1.31 (m, 9H, 9H of 3CH_3), 1.84–2.10 (m, 4H, 4H of 2CH_2), 2.49–2.66 (m, 1H, 1H of CH), 3.90–4.42 (m, 2H, 2H of epoxysuccinates). ESI-MS: m/z $[M+H+2H_2O]^+$ = 504(100%). Anal. ($C_{14}H_{28}N_2O_8Pt$) C, H, N.
Data for **5b**. Yield: 46%, ivory solids. IR(ν, cm^{-1}): 3424m (br), 3220m, 3123m, 2971w, 1635vs, 1354s, 1099s, 931s, 669s. ^1H NMR (D_2O/TMS): δ 0.75–0.77 (m, 3H, 3H of CH_3), 1.03–1.12 (m, 6H, 6H of 2CH_3), 1.86–2.09 (m, 4H, 4H of 2CH_2), 2.51 (m, 1H, 1H of CH), 4.28 (m, 2H, 2H of epoxysuccinates). ESI-MS: $m/z$$[M+H+2H_2O]^+$ = 504(100%). Anal. ($C_{16}H_{32}N_2$ O_8Pt) C, H, N.
35. ChemDraw® Ultra 8.0, Cambridge Soft Corporation.