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Synthesis and in vitro cytotoxicity of novel lipophilic (diamine)platinum(II) complexes of salicylate derivatives

Qing-Song Ye,[a] Li-Guang Lou,[b] Wei-Ping Liu,[a,*] Yao Yu,[a] Xi-Zhu Chen,[a] Shu-Qian Hou,[a] Wen-Qui Gao[a] and Yang Liu[a]

[a]*Platinum-Based Drug Lab, Kunming Institute of Precious Metals, Kunming 650021, China*
[b]*Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai, China*

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Abstract—Novel lipophilic (diamine)platinum(II) complexes of salicylate derivatives as the leaving groups were synthesized and characterized by elemental analysis, FAB^+-MS, FT-IR, and ^1H NMR spectroscopy. Most of the resulting platinum complexes had high solubility in organic solvents such as ethanol, acetone, and ether, and had right partition coefficient suited to be encapsulated in liposomes. The pertinent complexes were evaluated for their in vitro cytotoxicity against A549 human lung carcinoma and SGC-7901 human gastric carcinoma cell lines. They showed better cytotoxic activity than carboplatin and oxaliplatin.

Cisplatin, *cis*-diamminedichloroplatinum(II), is one of the most successful drugs currently used in clinical cancer therapy and active in a series of solid tumors, especially in metastatic testicular germ-cell cancer.[1,2] However, its clinical use is frequently limited by severe toxic side effects such as nephrotoxicity, neurotoxicity, and emetogenic as well as drug resistance.[3,4] One of the most intriguing strategies to overcome the drawbacks of cisplatin is to encapsulate the agent in a liposome,[5,6] and some anticancer drugs such as doxorubicin, the liposomal formulation (doxil), have been approved for the treatment of AIDS-related Kaposi's sarcoma and relapsed ovarian cancer in America and Europe.[7] A few different liposomal formulations of cisplatin have also been prepared and biologically evaluated since the introduction of cisplatin.[8–10] Among them, SPI-77 and lipoplatin (two liposomal cisplatin formulations) are currently in Phase I and II clinical trials.[11–13] Nevertheless, so far none of liposomal formulations of cisplatin have been approved for the clinical use in the world. The key reasons are the poor water solubility and low lipophilicity of cisplatin, which make it difficult to efficiently encapsulate the drug in a liposome.[5] An alternative approach is to synthesize lipophilic platinum complexes. The lipophilic platinum complex NDDP (*cis*-bis-neodecanoato-*trans*-*R,R*-1,2-diaminocyclohexane platinum(II)) is an example, and the liposomal NDDP (L-NDDP) has entered Phase II clinical trials.[14,15] Unfortunately, the complex is intraliposomally instable due to two monodentate carboxylate as the leaving groups.[16] Furthermore, in order to improve liposolubility, highly branched aliphatic carboxylate were used in NDDP, which greatly increased the molecular weight, leading to difficult passive diffusion through the cell membrane. Therefore, it is important to design and synthesize lipophilic platinum complexes using chelating bidentate ligands with small molecular weight.

Most of the platinum complexes reported to date have dicarboxylate as leaving groups. Recently, there have been some reports that platinum complexes with α-hydroxylcarboxylate as leaving groups had high antitumor activity,[17,18] and the α-hydroxylcarboxylatoplatinum drugs, nedaplatin (*cis*-diammine (glycolato-*O*,*O*)platinum (II)) and lobaplatin (*cis*-[*trans*-1,2-cyclobutanebis(methylamine)][(*S*)-lactato-*O*,*O*]platinum(II)), were approved for clinical cancer therapy in Japan and China, respectively.[3,19] But, to the best of our knowledge, the synthesis of platinum complexes with lipophilic α-hydroxylcarboxylate especially salicylates were not reported.

On the basis of these findings, we designed and synthesized a series of novel lipophilic platinum(II) complexes

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* Corresponding author. Tel.: +86 871 5133680; fax: +86 871 5133648; e-mail: liuweiping0917@sohu.com

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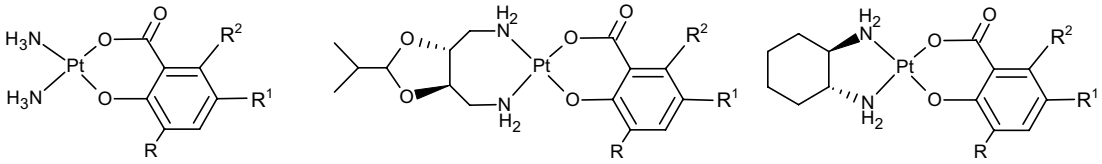

1 R = R^1 = I, R^2 = H **2** R = R^1 = I, R^2 = H **3** R = R^1 = I, R^2 = H

4 R= $CH(CH_3)_2$, R^1 = H, R^2 = CH_3 **5** R= $CH(CH_3)_2$, R^1 = H, R^2 = CH_3 **6** R = $CH(CH_3)_2$, R^1 = H, R^2 = CH_3

Figure 1. Structures of platinum(II) complexes **1**–**6**.

(Fig. 1) containing two salicylate derivatives 3,5-diiodosalicylate (DISA) and 3-isopropyl-6-methylsalicylate (thymotate) as the leaving groups. DISA is a food additive as iodine source and thymotate is derived from the genus Thymus plants. Moreover, salicylate and its derivatives are important nonsteroidal anti-inflammatory agents. It has been well-known that they can block metastasis of cancer cells by inhibiting synthesis of prostaglandin. This is another reason for selecting salicylate derivatives as leaving groups in the target platinum complexes. As for non-leaving groups, the diammines of cisplatin, oxaliplatin, and SKI-2053R were used. All the design strategies in our research are to develop platinum complexes with expectation of higher liposolubility and chemical stability, along with higher antitumor activities and lower systemic toxicity. The resulting platinum complexes were evaluated for their in vitro cytotoxicity against A549 human non-small cell lung carcinoma and SGC-7901 human gastric carcinoma cell lines.

All the complexes were synthesized as precipitates in aqueous solution by the general method owing to their low water solubility,[20,21] that is, K_2PtCl_4 was first converted to K_2PtI_4 in situ by treatment with KI, which was subsequently treated with ammine/diamine to form diam(m)inediiodoplatinum(II) complexes, then the diam(m)inediiodoplatinum(II) complexes reacted with silver nitrate and converted to $[PtA_2(H_2O)_2](NO_3)_2$, followed by mixing with sodium salicylate derivatives to produce the precipitates of target complexes. The resulting platinum complexes were characterized by elemental analysis, FT-IR, ^{1}H NMR, and FAB$^+$-MS spectra.[22] The elemental analysis data for each compound were in good agreement with the empirical formula proposed. The binding of the salicylic acid derivatives to platinum(II) atoms as a bidentate ligand was confirmed by the shift of $\nu_{C=O}$ to lower frequencies and the absence of ν_{O-H} absorption in IR spectra in the resulting complexes.[23,24] All complexes showed $[M+H]^+$ peaks, corresponding to their molecular weights. ^{1}H NMR spectral peaks were compatible to the chemical structures given in Figure 1.

The solubility of the complexes both in water and organic solvents such as ethanol, acetone, and ether was determined. All the complexes, except for complexes **1** and **4**, had low solubility in aqueous solution but high solubility in the organic solvents (>20 mg/ml). Partition coefficients for the lipophilic platinum complexes **2**, **3**, **5**, and **6** were measured in an octanol/water system according to the literature method.[25,26] The partition coefficients and solubility in water as well as their melting points are listed in Table 1. The lipophilic complexes were stable in the organic solvents for five days at room temperature indicated by the changes of their UV spectra, presumably as a result of the chelation effect of the leaving groups.

The in vitro cytotoxicities of the platinum complexes were assessed by sulforhodamine B (SRB) colorimetric assay as described in the literature using A549 and SGC-7901 cell lines.[27,28] Cells were continuously exposed to test compounds **1**–**6**, carboplatin, oxaliplatin, and SKI-2053R for 72 h, and the results are given in Table 2.

All the resulting complexes were more active against A549 cell line with lower IC_{50} values (the concentration of a compound at which cell growth was inhibited by 50%) than carboplatin, oxaliplatin, and SKI-2053R;

Table 1. Partition coefficient and solubility of platinum(II) complexes **1**–**6** as well as their melting points

Complexes	Solubility in water (mg/ml)	Partition coefficient (log *P*)	Mp (°C dec)
1	0.30		170
2	0.012	3.3	227
3	0.025	3.1	240
4	0.25		185
5	0.012	3.4	203
6	0.0073	4.3	212

Table 2. In vitro cytotoxicity against selected human tumor cell lines of complexes **1**–**6**

Complexes	Non-leaving groups	Leaving groups	IC_{50} (μM)	
			A549	SGC-7901
1	$2NH_3$	DISA[c]	1.54	2.65
2	BAMID[a]	DISA[c]	2.16	2.93
3	DACH[b]	DISA[c]	1.05	2.93
4	$2NH_3$	Thymotate[d]	0.89	1.83
5	BAMID[a]	Thymotate[d]	1.27	2.64
6	DACH[b]	Thymotate[d]	1.49	6.95
Carboplatin			9.26	16.34
Oxaliplatin			3.54	7.77
SKI-2053R			3.56	2.36

[a] BAMID: (4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane.
[b] DACH: *trans-1R,2R*-diaminocyclohexane.
[c] DISA: 3,5-diiodosalicylate.
[d] Thymotate: 3-isopropyl-6-methylsalicylate.

As for SGC-7901 human gastric carcinoma cell line, the complexes showed higher or slightly higher activity than carboplatin and oxaliplatin, but similar activity to SKI-2053R. Most of the complexes had high liposolubility, which could make them to be efficiently encapsulated in liposomes.[6] The liposomes loaded with platinum drugs were reported to possess higher antitumor activity profiles and lower systemic toxicity than the parent drugs, for the liposomes can selectively deliver more drugs to the tumor site.[11–13] The final goal of this study was to develop liposomal formulations of new platinum drugs, so the complexes are worthy of further research as potentially novel anticancer agents.

From the IC_{50} values in Table 2, it can be seen that the order of cytotoxic activity of DISA-platinum complexes is **3 > 1 > 2** against A549 cell line and **3 ≈ 2 > 1** against SGC-7901 cell line. For thymotate–platinum complexes the order is **4 > 5 > 6**. There is no close structure–activity relationship among these complexes with different diammines.

In conclusion, we have synthesized a series of new platinum complexes with high cytotoxic activity and good liposolubility which are liable to be encapsulated in liposomes due to high compatibility between the lipophilic complexes and liposomes.[29] Further research to evaluate their in vivo antitumor activity and preparation for their liposomal formulations are in progress.

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22. Data for **1**. Yield: 70%. Found (calculated for $C_7H_8N_2O_3I_2Pt$) C 13.45 (13.63), H 1.28 (1.31), N 4.48 (4.54), Pt 31.16 (31.62). IR (KBr, ν, cm^{-1}): 3437 (s), 3290 (s), 3059 (m), 1621 (m), 1564 (s), 1442 (vs), 1357 (s), 1309 (m), 1266 (m), 1101 (m). ^1H NMR (DMSO, 500 MHz): 7.82–7.86 (m, 2H, 2*Ar–H*). FAB^+-MS: *m/z* $[M+H]^+ = 618$ (100%). Data for **2**. Yield: 35%. Found (calculated for $C_{15}H_{20}N_2O_5I_2Pt$) C 23.88 (23.79), H 2.60 (2.66), N 3.75 (3.70), Pt 25.65 (25.76). IR (KBr, ν, cm^{-1}): 3425 (m), 3222 (s), 2964 (s), 2875 (m), 1620 (s), 1555 (s), 1435 (vs), 1357 (s), 1248 (m), 1097 (s). ^1H NMR (DMSO, 500 MHz): 0.87 (d, 6H, $CH(CH_3)_2$), 1.73 (m, 1H, $CH(CH_3)_2$), 2.52–2.71 (m, 2H, 2$CH$$NH_2$), 3.03–3.24 (m, 2H, 2$CH$$NH_2$), 4.54–4.59 (m, 2H, O$CHCH$O), 4.81 (d, 1H, O$CH$O), 5.37–5.57 (m, 4H, NH_2), 7.85–7.89 (m, 2H, *2Ar–H*). FAB^+-MS: *m/z* $[M+H]^+ = 758$ (100%). Data for **3**. Yield: 80%. Found (calculated for $C_{13}H_{16}N_2O_3I_2Pt$) C 22.48 (22.40), H 2.25 (2.31), N 4.02 (4.02), Pt 27.58 (27.98). IR (KBr, ν, cm^{-1}): 3409 (m), 3207 (s), 3093 (s), 2936 (s), 2859 (m), 1621 (m), 1560 (s), 1433 (vs), 1355 (s), 1256 (m), 1099 (w). ^1H NMR (DMSO, 500 MHz): 0.97–1.04 (m, 4H, $CH_2CH_2CH_2CH_2$ of DACH), 1.21–1.29 (m, 2H, $CH_2CH_2CH_2CH_2$ of DACH), 1.41–1.50 (m, 2H, $CH_2CH_2CH_2CH_2$ of DACH), 1.94–2.09 (m, 2H, 2$CH$$NH_2$), 4.96–5.20 (m, 2H, NH_2), 5.58 (d, 2H, NH2), 7.84 (s, 1H, *Ar–H*), 7.88 (s, 1H, *Ar–H*). FAB^+-MS: *m/z* $[M+H]^+ = 698$ (100%). Data for **4**. Yield: 25%. Found (calculated for $C_{11}H_{18}N_2O_3Pt$) C 31.15 (31.36), H 4.20 (4.31), N 6.68 (6.65), Pt 46.02 (46.30). IR (KBr, ν, cm^{-1}): 3406 (m), 3267 (s), 3111 (s), 2958 (s), 2870 (m), 1590 (s), 1556 (s), 1478 (vs), 1430 (s), 1379 (vs), 1279 (m), 1238 (m). ^1H NMR (DMSO, 500 MHz): 1.12 (d, 6H, $CH(CH_3)_2$), 2.35 (s, 3H, CH_3–Ar), 3.13 (m, 1H, $CH(CH_3)_2$), 6.53 (d, 1H, Ar), 7.03 (d, 1H, Ar). FAB^+-MS: *m/z* $[M+H]^+ = 422$ (100%). Data for **5**. Yield: 35%. Found (calculated for $C_{19}H_{30}N_2O_5Pt$) C 40.59 (40.64), H 5.28 (5.38), N 4.81 (4.99), Pt 34.49 (34.74). IR (KBr, ν, cm^{-1}): 3422 (m), 3212 (m), 2962 (m), 2874 (m), 1591 (m), 1564 (m), 1451 (s), 1427 (s), 1381 (s), 1280 (m), 1237 (m), 1096 (m). ^1H NMR (DMSO, 500 MHz): 0.90 (d, 6H, $CH(CH_3)_2$), 1.09 (d, 6H, Ar–$CH(CH_3)_2$), 1.78 (m, 1H, $CH(CH_3)_2$), 2.35 (s, 3H, CH_3–

Ar), 3.09–3.24 (m, 5H, 2CH_2NH_2, overlapped with 1H of *CH*–Ar), 4.61–4.69 (m, 2H, O*CHCH*O), 4.86 (d, 1H, O*CH*O), 6.50 (d, 1H, Ar), 7.02 (d, 1H, Ar). FAB$^+$-MS: *m*/*z* $[M+H]^+$ = 562 (100%). Data for **6**. Yield: 32%. Found (calculated for $C_{17}H_{26}N_2O_3Pt$) C 40.55 (40.72), H 5.27 (5.23), N 5.91 (5.99), Pt 38.48 (38.90). IR (KBr, ν, cm^{-1}): 3424 (m), 3214 (m), 2957 (m), 2865 (m), 1595 (s), 1560 (s), 1449 (s), 1380 (s), 1279 (m), 1238 (m). ^{1}H NMR (DMSO, 500 MHz): 1.00–1.11 (m, 10H, $CH_2CH_2CH_2CH_2$ of DACH, overlapped with 6H of $CH(CH_3)_2$), 1.31–1.46 (m, 4H, $CH_2CH_2CH_2CH_2$ of DACH), 1.92–1.94 (m, 2H, 2*CH*NH_2), 2.39 (s, 3H, CH_3–Ar), 3.15 (m, 1H, *CH*$(CH_3)_2$), 6.52 (d, 1H, Ar), 7.03 (d, 1H, Ar). FAB$^+$-MS: *m*/*z* $[M+H]^+$ = 502 (100%).

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