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Novel cisplatin-type platinum complexes and their cytotoxic activity

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Abstract—A series of novel cisplatin-type platinum complexes, formulated as $[PtA_2(OCOCH_2OR)_2]$ (A_2 = two monoamines or one diamine, R is an alkyl group), were designed, characteristic of alkoxyacetate as carboxylato ligands. The pertinent compounds were prepared and characterized by elementary analyses, IR, ^1H NMR, and ESI-MS spectra. The cytotoxic activities of compound **1a** in vitro toward HL-60 human leukemia and BEL-7402 human hepatocellular carcinoma cell lines were pioneeringly studied. Then, compounds **1b**–**3d** were evaluated for their in vitro cytotoxicity against Ramos human lymphoma, 3AO human ovarian carcinoma, and A549 human non-small cell lung cancer cell lines. Most of them showed better cytotoxic activity than carboplatin against above selected cell lines.

Cisplatin is one of the most frequently used chemotherapeutics in the treatment of malignant tumors,[1–3] nevertheless, the clinical application of cisplatin is greatly limited by its toxicity,[4–6] narrow range of activity, both intrinsic and acquired resistance, and low aqueous solubility. So far, tremendous efforts have been devoted to developing cisplatin analogues with broader spectra of activity, improved clinical efficacy, and reduced toxicity. More recently, there have been efforts directed at the design of non-classical Pt complexes that violate the original SAR, such as orally active platinum(IV) complexes,[7–16] sterically hindered platinum(II) complexes,[17–22] *trans*-platinum complexes,[17,23–27] multinuclear platinum complexes,[17,28–34] sulfur-containing platinum complexes,[35–40] etc. Although thousands of platinum compounds have been synthesized and biologically evaluated, only a small number of them have been advanced for human clinical trials. This situation is due to the shortcoming of cisplatin mentioned above. To overcome this impediment, a generally accepted strategy is to modulate both the aqueous solubility and liposolubility of the potential platinum compounds, since well-balanced solubility both in water and liposome is greatly helpful to transport drugs into target cells and reduce drug-related toxicities. One major approach to achieve this goal is to alternate the chloride anions of cisplatin to appropriate leaving groups. In this way, the previous literature work usually emphasizes on one of the following techniques. One is to replace the chloride anion with a short chain aliphatic carboxylato group or a cyclocarboxylato group to promote the aqueous solubility of the related Pt(II) complexes.[41–44] A successful example is carboplatin that has been widely used in clinic at present. The other is to change the chloride anion into a highly branched or long chain aliphatic carboxylato group to improve the liposolubility of the resulting Pt(II) complexes. One example in this way is the liposomal formulation of the platinum compound NDDP (L-NDDP/AR-726), developed by Aronex using neocaprate as leaving groups, which is currently in phase II clinical trials.[45] Another example, lipophilic SM-11355 developed by Sumitomo, is in phase II clinical trials against liver cancer, where $C_{13}H_{27}COO^-$ serves as leaving groups.[46] Considering that carboxylic acids containing alkoxy moieties have reasonable aqueous solubility and liposolubility, we selected alkoxyacetate as the candidate to take the place of chloride anions in cisplatin for developing its analogues with expectation of higher antitumor activities and lower toxicity. Based on this idea, we designed and synthesized a series of novel cisplatin analogues of the type $[PtA_2(OCOCH_2OR)_2]$ (Fig. 1, where R is an alkyl group). All complexes were evaluated for their in vitro cytotoxicity against a panel of human tumor cell lines.

Two general methods have been applied to prepare the platinum complexes containing alkoxy carboxylic anions.

Keywords: Platinum(II) complexes; Antitumor activity; Alkoxyacetate.
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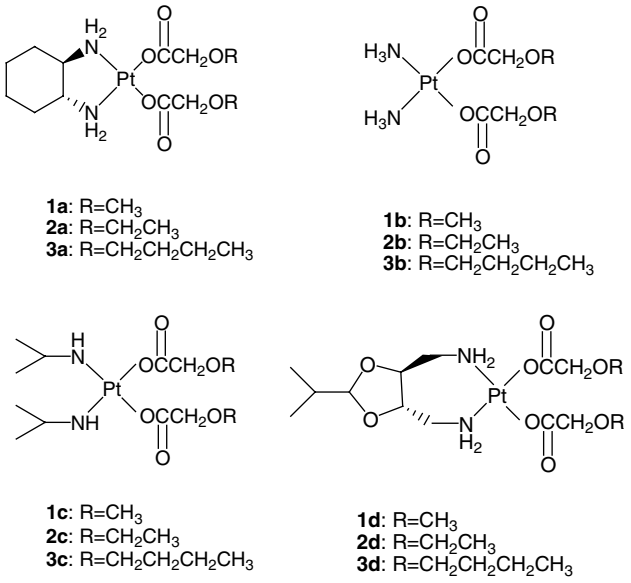

Figure 1. Structures of platinum(II) complexes **1a**–**3d**.

One is involved in the reaction of $[PtA_2(H_2O)_2](NO_3)_2$ with sodium carboxylate,[47] the other is involved in the reaction of $[PtA_2I_2]$[47,48] with silver carboxylate.[49,50] The result shows all complexes have both good aqueous solubility and liposolubility. Compounds **1a**, **2a**, **3a**, and **3d** with lower water solubility have been prepared by the former method,[58] compounds **1b**, **2b**, **3b**, **1c**, **2c**, **3c**, **1d**, and **2d** with higher water solubility have been obtained by the latter method.[59]

All compounds were characterized by microanalyses, IR, ^{1}H NMR, and ESI-MS spectra. The elemental analysis for each compound was in good agreement with the empirical formula proposed. In the IR spectra of all complexes, the amino group participation in binding with Pt(II) was confirmed by the examination of νNH_2 and δNH_2 frequencies, which were shifted to lower frequencies comparing with a free amino group, due to Pt(II)–NH_2 coordination. The presence of a band near 1662–1614 cm^{-1} and the absence of the C=O absorption of free carboxylic acids near 1700 cm^{-1} demonstrated that the carboxylate anion was coordinated to the metal atom in each case.[51] Most of the compounds showed a peak of $[M-ROCH_2COO^-+H_2O]^+$ in their positive ESI mass spectra, however, several compounds presented $[M+Na]^+$ peaks, which are corresponding to their molecular weight. It is noted that all the mass spectra of the platinum complexes have three protonated ion peaks because of the isotopes ^{194}Pt (33%), ^{195}Pt (34%), and ^{196}Pt (25%). ^{1}H NMR spectral peaks of all compounds were compatible to the related molecular structures given in Figure 1.

The in vitro cytotoxicities of the platinum compounds against a panel of tumor cell lines were screened by the National Center for Drug Screening of Chinese Academy of Sciences. For the tentative test, the cytotoxic activities of compound **1a** in vitro toward HL-60 human leukemia and BEL-7402 human hepatocellular carcinoma cell lines were pioneeringly studied. It was tested by trypan blue dye exclusion test and sulforhodamine B (SRB) colorimetric assay as described in the literature, respectively.[52–57,60] Cells were continuously exposed to **1a**, cisplatin, and carboplatin for 72 h, and the results are summarized in Table 1. As shown in Table 1, **1a** was more active than cisplatin and carboplatin against these two selected cell lines. **1a** displayed as near 9-fold and 68-fold high cytotoxicity toward HL-60 and BEL-7402 as carboplatin, respectively.

Next, cytotoxicities of complexes **1b**–**3d** toward Ramos human lymphoma, 3AO human ovarian carcinoma, and A549 human non-small cell lung cancer cell lines were determined by sulforhodamine B (SRB) assay. The cytotoxicities of these compounds were compared with those of cisplatin, carboplatin, and oxaliplatin. In this study, cells were continuously exposed to test compounds **1b**–**3d** for 72 h. The results are given in Table 2.

From the above biological results, it is concluded that Ramos human lymphoma cell was most sensitive to all the platinum analogue treatment. The cytotoxicity of compounds **1b**–**3d** against Ramos was comparable to that of cisplatin except for **3c** which was much less cytotoxic. Complex **2a** displayed the highest cytotoxicity against all these three cell lines, with the lower IC_{50} value by factors of 1.3- to 6.3-fold compared to those of cisplatin, 1.6- to 8.9-fold compared to those of oxaliplatin, and 12.8- to 98.3-fold compared to those of carboplatin. Complexes **3a**, **2d**, and **1d** are the next potent agents. It has been seen in Table 2 that the cytotoxicity of **3a** is very close to that of cisplatin against A549 and 2-fold better than cisplatin against 3AO. Complexes **2d** and **1d** are slightly better than cisplatin against Ramos and 3AO.

Based on the comparison of IC_{50} values of these compounds, 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 **1d**, **2a**, **2d**, **3a**, and **3d**, are generally more active than those with the monoamine (ammine) carrier ligand when the leaving group is

Table 1. In vitro cytotoxicity against selected human tumor cell lines of **1a**[a]

Complex	Carrier ligand A	Leaving group R=	IC_{50} (μM)	
			HL-60[b,d]	BEL-7402[c,e]
1a	DACH[f]	$-CH_3$	0.31	0.53
Cisplatin			0.33	0.77
Carboplatin			2.86	36.12

[a] All IC_{50} values (platinum complex concentration at which cell growth was inhibited by 50%) calculated based on the Pt-content are means ± SD (SD < 12% of the mean value) from at least three separate experiments.
[b] Tested by trypan blue dye exclusion test.
[c] Tested by sulforhodamine B colorimetric assay.
[d] Leukemia.
[e] Hepatocellular carcinoma.
[f] DACH: *trans*-1*R*,2*R*-diaminocyclohexane.

Table 2. In vitro cytotoxicity against selected human tumor cell lines of **1b**–**3d**[a]

Complex	Carrier ligand	Leaving group	IC_{50} (μM)		
			Ramos[b,c]	3-AO[b,d]	A549[b,e]
1b	$2NH_3$	$–CH_3$	0.25	2.95	1.97
1c	2(Isopropylamine)	$–CH_3$	0.41	8.76	11.81
1d	BAMID[f]	$–CH_3$	0.13	0.91	2.01
2a	DACH[g]	$–CH_2CH_3$	0.04	0.17	0.78
2b	NH_3	$–CH_2CH_3$	0.21	1.38	2.07
2c	2(Isopropylamine)	$–CH_2CH_3$	0.39	7.13	8.67
2d	BAMID	$–CH_2CH_3$	0.10	0.87	2.09
3a	DACH	$–CH_2CH_2CH_2CH_3$	0.35	0.53	1.05
3b	NH_3	$–CH_2CH_2CH_2CH_3$	0.41	3.87	2.24
3c	2(Isopropylamine)	$–CH_2CH_2CH_2CH_3$	1.57	7.13	18.43
3d	BAMID	$–CH_2CH_2CH_2CH_3$	0.08	10.46	4.91
Oxaliplatin			0.20	1.51	1.26
Carboplatin			0.54	16.71	9.97
Cisplatin			0.17	1.07	1.00

[a] All IC_{50} values (platinum complex concentration at which cell growth was inhibited by 50%) calculated based on the Pt-content are means ± SD (SD < 12% of the mean value) from at least three separate experiments.
[b] Tested by sulforhodamine B colorimetric assay.
[c] Lymphoma.
[d] Ovarian carcinoma.
[e] Non-small cell lung cancer carcinoma.
[f] BAMID: (4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane.
[g] DACH: *trans*-1*R*,2*R*-diaminocyclohexane.

the same. However, among these compounds, there are some reversing cases, such as **3d** < **3c** < **3b** in 3AO cell, and **1d** < **1b**, **2d** < **2b**, **3d** < **3b** in A549 cell. The activities of the complexes with ammine seem to be higher than those of the compounds with isopropylamine in all three selected cell lines.

As seen in Table 2, the order of cytotoxicities in Ramos is **2a** > **3d** > **2d** > **1d** > cisplatin > oxaliplatin > **2b** > **1b** > **3a** > **2c** > **1c** = **3b** > carboplatin > **3c**, in 3AO is **2a** > **3a** > **2d** > **1d** > cisplatin > **2b** > oxaliplatin > **1b** > **3b** > **2c** = **3c** > **1c** > **3d** > carboplatin, and in A549 is **2a** > cisplatin > **3a** > oxaliplatin > **1b** > **1d** > **2b** > **2d** > **3b** > **3d** > **2c** > carboplatin > **1c** > **3c**.

The cytotoxicities of the resulting platinum complexes are also related to the nature of the leaving group. It has been seen from Table 2 that nearly all platinum complexes with diethoxyacetate have higher cytotoxicity than those with dimethoxyacetate or dibutoxyacetate when the amine carrier ligand is the same, that is **2a** > **3a**; **2b** > **1b** and **3b**; **2c** > **1c** and **3c**; **2d** > **1d** and **3d**. However, there are some exceptions, such as **2d** < **3d** in Ramos; **2c** = **3c** in 3AO; **2b** < **1b** and **2d** < **1d** in A549.

In conclusion, most compounds exhibit better cytotoxic activity than carboplatin against tested cell lines. Complex **2a**, *cis*-(*trans*-1*R*,2*R*-diaminocyclohexane)bis(ethoxyacetate)platinum(II), displays the highest cytotoxicity against all three tested cell lines. Complexes **1a**, **2a**, and **3a** not only exhibit higher in vitro cytotoxicity, but also possess desirable physico-chemical properties such as excellent solubility in both water and organic solvents. Complex **1a** exhibits better aqueous solubility than carboplatin, oxaliplatin, and cisplatin, the pertinent values being 34, 17, 8, and 1 mg/mL at room temperature, respectively. Complexes **2a** and **3a** present lower aqueous solubility and higher liposolubility than **1a**.[61]

Acknowledgments

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58. *Synthesis of complexes* **1a**, **2a**, **3a**, and **3d**. Corresponding diiododiamineplatinum(II) or dichlorodiamineplatinum(II) complex (2 mmol) was first suspended in 20 mL water, and next an aqueous solution (10 mL) of silver nitrate (4 mmol) was added. After stirring under a nitrogen atmosphere for 6 h at 60 °C in the dark, the mixture was cooled down and filtered off, and the resulting deposits were washed several times with water. The filtrate was blended with sodium alkoxyacetate (4 mmol) in 10 mL water, and then stirred at 60 °C for 12 h. The solution was concentrated to 5 mL by rotavapor and then cooled to 0 °C. Light yellow or white crystals were collected, washed with a small amount of chilled water and ethanol, and dried at 60 °C in vacuum. All of such resulting complexes are stable in air. Data for **1a**. Yield: 63%, light yellow crystals. IR (ν, cm^{-1}): 3449vs (br), 3218s, 3111m, 2937m, 2861w, 2821w, 2362w, 2342w, 1633vs, 1449w, 1385s, 1338m, 1305m, 1120s, 1064w, 1035w. ^1H NMR (D_2O/TMS): δ 1.08 (m, 4H, 4H of $CH_2CH_2CH_2CH_2$ of DACH), 1.82 (m, 2H, 2H of $CH_2CH_2CH_2CH_2$ of DACH), 2.16 (m, 2H, 2H of $CH_2CH_2CH_2CH_2$ of DACH), 3.12 (m, 6H, 6H of $2OCH_3$ of methoxyacetate), 3.42 (m, 2H, 2H of *CHCH* of DACH), 3.66–3.78 (m, 4H, 4H of $2OCH_2COO$ of methoxyacetate). ESI-MS: m/z $[M+Na]^+ = 510$ (100%). Anal. ($C_{12}H_{24}N_2O_6Pt$) C, H, N. Data for **2a**. Yield: 29%, light yellow solids. IR (ν, cm^{-1}): 3442m, 3199m, 3112m, 2936m, 2817w, 1620vs, 1385s, 1342m, 1306m, 1200m, 1123s, 1064w. ^1H NMR (DMSO-d_6/TMS): δ 1.06 (m, 6H, 6H of $2CH_3$ of

ethoxyacetate), 1.25 (m, 2H, 2H of CH_2 $CH_2CH_2CH_2$ of DACH), 1.60 (m, 2H, 2H of $CH_2CH_2CH_2CH_2$ of DACH), 1.96 (m, 2H, 2H of $CH_2CH_2CH_2CH_2$ of DACH), 2.35 (m, 2H, 2H of $CH_2CH_2CH_2CH_2$ of DACH), 3.40–3.90 (m, 10H, 4H of $2OCH_2CH_3$of ethoxyacetate, 4H of $2OCH_2$-COO of ethoxyacetate, overlapped with 2H of *CHCH* of DACH). ESI-MS: *m/z* $[M-CH_3CH_2OCH_2COO^-+H_2O]^+$ = 430 (100%). Anal. ($C_{14}H_{28}N_2O_6Pt$) C, H, N. Data for **3a**. Yield: 32%, yellow solids. IR (ν, cm^{-1}): 3449w(br), 3199s, 3104m, 2957s, 2933s, 2867s, 1614vs, 1454w, 1383s, 1336m, 1297m, 1205w, 1122s, 1064w, 1030w. ^1H NMR (D_2O/TMS): δ 0.84 (m, 6H, 6H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 1.11 (m, 2H, 2H of $CH_2CH_2CH_2CH_2$ of DACH), 1.29 (m, 6H, 2H of $CH_2CH_2CH_2CH_2$ of DACH, overlapped with 4H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 1.49 (m, 6H, 2H of $CH_2CH_2CH_2CH_2$ of DACH, overlapped with 4H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 1.99 (m, 2H, 2H of $CH_2CH_2CH_2CH_2$ of DACH), 2.33 (m, 2H, 2H of *CHCH* of DACH), 3.45 (m, 4H, 4H of 2 $OCH_2CH_2CH_2CH_3$ of butoxyacetate), 3.84–3.97 (m, 4H, 4H of $2OCH_2COO$ of butoxyacetate). ESI-MS: *m/z* $[M-C_4H_9OCH_2COO^-+H_2O]^+$ = 458 (100%). Anal. ($C_{18}H_{36}N_2O_6Pt$) C, H, N. Data for **3d**. Yield: 35%, white solids. IR(ν, cm^{-1}): 3448br, 3233s, 3124m, 2960s, 2934m, 2871m, 1660vs, 1640vs, 1581m, 1458w, 1421m, 1371m, 1335m, 1279m, 1129s. ^1H NMR (DMSO-d_6/TMS): δ 0.87 (m, 12H, 6H of $2CH_3$ of BAMID, overlapped with 6H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 1.31 (m, 4H, 4H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 1.46 (m, 4H, 4H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 1.77 (m, 1H, 1H of $CH(CH_3)_2$ of BAMID), 2.81 (m, 4H, 4H of $2CH_2NH_2$ of BAMID), 3.10–3.40 (m, 6H, 4H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate, overlapped with 2H of *OCHCHO* of BAMID), 3.60–3.88 (m, 4H, 4H of $2OCH_2COO$ of butoxyacetate), 4.80 (d, 1H, 1H of *OCHO* of BAMID), 4.54–4.78 (br, NH_2); ESI-MS *m/z* $[M+Na]^+$ = 654 (100%), $[M+Na+H_2O]^+$ = 672 (85%), $[M+H]^+$ = 632 (80%), $[M-C_4H_9OCH_2COO^-+H_2O]^+$ = 518 (60%). Anal. ($C_{20}H_{40}N_2O_8Pt$) C, H, N. After the light yellow crystals were collected, the filtrate for complex **1a**was further concentrated and cooled, the new and more crystals were collected after the process was repeated three times. All the crystals were united, so the product yield for complex **1a** is higher than those for complexes **2a**, **3a**, and **3d** in spite of the same preparation method employed for them.

59. *Synthesis of the rest complexes.* A suspension of the corresponding diiododiamineplatinum(II) complex (2 mmol) and silver alkoxyacetate (4 mmol) in H_2O (100 mL) was stirred at 60 °C in the dark overnight, the resulting deposits filtered off and washed with water. The filtrate was evaporated to near dryness and refrigerated to give white solids, which were filtered off and dried under vacuum. All of such resulting complexes are liable to be hygroscopic when exposed in air. Data for **1b.** Yield: 35%, white solids. IR (ν, cm^{-1}): 3279s, 3222s, 3124s, 2989m, 2901m, 2827m, 1662vs, 1571vs, 1466m, 1423vs, 1393vs, 1335vs, 1284s, 1205s, 1117vs, 1015m, 989m. ^1H NMR (D_2O/TMS): δ 3.34 (m, 6H, 6H of $2OCH_3$ of methoxyacetate), 4.02 (m, 4H, 4H of $2OCH_2COO$ of methoxyacetate). ESI-MS: *m/z* $[M+Na]^+$ = 430 (100%). Anal. ($C_6H_{16}N_2O_6Pt$) C, H, N. Data for **2b**. Yield: 35%, white solids. IR (ν, cm^{-1}): 3497m, 3260sh, 2978w, 2885w, 1631vs, 1451w, 1420s, 1387s, 1330s, 1121vs, and 1036w. ^1H NMR (D_2O/TMS): δ 1.20 (m, 6H, 6H of 2 OCH_2CH_3 of ethoxyacetate), 3.60 (m, 4H, 4H of $2OCH_2CH_3$ of ethoxyacetate), 3.94–4.07 (m, 4H, 4H of $2OCH_2COO$ of ethoxyacetate). ESI-MS: *m/z* $[M-CH_3OCH_2COO^-+H_2O]^+$ = 350 (100%). Anal. ($C8H_{20}N_2O_6Pt$) C, H, N. Data for **3b.** Yield: 57%, white crystals. IR (ν, cm^{-1}): 3460m, 3381m, 3270s, 3111s, 2958m, 2932m, 2871m, 1651vs, 1601vs, 1577vs, 1461w, 1388vs, 1339m, 1292s, 1136s, 1116s. ^1H NMR (D_2O/TMS): δ 0.83 (m, 6H, 6H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 1.28 (m, 4H, 4H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 1.49 (m, 4H, 4H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 3.45 (m, 4H, 4H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 3.84–3.99 (m, 4H, 4H of $2OCH_2COO$ of butoxyacetate). ESI-MS: *m/z* $[M-C_4H_9OCH_2COO^-+H_2O]^+$ = 378 (100%). Anal. ($C_{12}H_{28}N_2O_6Pt$) C, H, N. Data for **1c**. Yield: 36%, white solids. IR (ν, cm^{-1}): 3451m, 3207s, 3106m, 1630s, 1449w, 1384vs, 1333m, 1298m, 1173m, 1119s, 1065m, 1031m;^1H NMR (DMSO-d_6/TMS): δ 1.25 (m, 12H, 12 H of $4CH_3$ of *i*-propylamine), 3.22 (m, 6H, 6H of $2OCH_3$ of methoxyacetate), 3.36–3.85 (m, 6H, 2H of $2CH$ of *i*-propylamine, overlapped with 4H of $2OCH_2COO$ of methoxyacetate); ESI-MS: *m/z* $[M+Na]^+$ = 514 (100%). Anal. ($C_{12}H_{28}N_2O_6Pt$) C, H, N. Data for **2c.** Yield: 27.0%, white solids. IR (ν, cm^{-1}): 3448m, 3196sh, 2973m, 2933w, 2876w, 1642vs, 1569m, 1384vs, 1339vs, 1172w, 1118s, 1016w. ^1H NMR (D_2O/TMS): δ 1.18 (m, 12H, 12H of $4CH_3$ of *i*-propylamine), 1.20 (m, 6H, 6H of $2OCH_2CH_3$ of ethoxyacetate), 3.59 (m, 2H, 2H of $2CH$ of *i*-propylamine), 3.64 (m, 4H, 4H of $2OCH_2CH_3$ of ethoxyacetate), 3.90–4.10 (m, 4H, 4H of $2OCH_2COO$ of ethoxyacetate). ESI-MS: *m/z* $[M-CH_3CH_2OCH_2COO^-+H_2O]^+$ = 434 (100%). Anal. ($C_{14}H_{32}N_2O_6Pt$) C, H, N. Data for **3c**. Yield: 40%, white solids. IR (ν, cm^{-1}): 3448w (br), 3223sh, 2964m, 2935m, 2875m, 1633vs, 1575w, 1465w, 1382vs, 1342vs, 1273w, 1159w, 1120s. ^1H NMR(D_2O/TMS): δ 0.89 (m, 6 H, 6H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 1.30 (m, 16H, 12H of $4CH_3$ of *i*-propylamine, overlapped with 4H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 1.55 (m, 4H, 4H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 2.81 (m, 2H, 2H of 2CH of *i*-propylamine), 3.50 (m, 4H, 4H of $2OCH_2CH_2CH_2CH_3$ of butoxyacetate), 3.89–4.03 (m, 4H, 4H of $2OCH_2COO$ of butoxyacetate). ESI-MS: *m/z* $[M+Na]^+$ = 598 (100%), $[M-C_4H_9OCH_2COO^- +H_2O]^+$ = 462 (85%). Anal. ($C_{18}H_{40}O_6Pt$) C, H, N. Data for **1d**. Yield: 28%, white solids. IR (ν, cm^{-1}): 3449s, 3206s, 2964s, 2825m, 1617vs, 1419s, 1385s, 1311m, 1261w, 1200s, 1114vs, 1014w, 988m. ^1H NMR (D_2O/TMS): δ 0.95 (d, 6H, 6H of $2CH3$ of BAMID), 1.91 (m, 1H, 1H of $CH(CH_3)_2$ of BAMID), 2.50–2.98 (m, 4H, 4H of $2CH_2NH_2$ of BAMID), 3.39 (m, 6H, 6H of $2OCH_3$ of methoxyacetate), 3.99 (m, 4H, 4H of$2OCH_2COO$ of methoxyacetate), 4.04 (m, 2H, 2H of *OCHCHO* of BAMID), 5.34 (d, 1H, 1H of *OCHO* of BAMID). ESI-MS: *m/z* $[M+Na]^+$ = 570 (100%), $[M-CH_3OCH_2COO^- +H_2O]^+$ = 475 (75%). Anal. ($C_{14}H_{28}N_2O_8Pt$) C, H, N. Data for **2d**. Yield: 19%, white solids. IR (ν, cm^{-1}): 3449m, 3212sh, 2974m, 2878m, 1637s, 1384vs, 1302w, 1116m, 1011w. ^1H NMR (D_2O/TMS): δ 0.86 (d, 6H, 6H of $2CH_3$ of BAMID), 1.12 (m, 6H, 6H of $2OCH_2CH_3$ of ethoxyacetate), 1.84 (m, 1H, 1H of $CH(CH_3)_2$ of BAMID), 2.73 (m, 2H, 2H of CH_2NH_2 of BAMID), 3.20 (m, 2H, 2H of CH_2NH_2 of BAMID), 3.50 (m, 4H, 4H of $2OCH_2CH_3$ of ethoxyacetate), 3.80–4.00 (m, 6H, 4H of $2OCH_2COO$ of ethoxyacetate, overlapped with 2H of *OCHCHO* of BAMID), 4.93 (d, 1H, 1H of *OCHO* of BAMID). ESI-MS: *m/z* $[M-CH_3CH_2OCH_2COO^- +H_2O]^+$ = 490 (100%). Anal. ($C_{16}H_{32}N_2O_8Pt$) C, H, N.

60. These cell lines were grown in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 U/mL), and streptomycin (100 μg/mL) in a highly humidified atmosphere of 5% CO_2 at 37 °C and passaged every 3 days to maintain normal growth (monolayer on

the flask). The exponentially grown cells were cultured for 24 h at a density of 5.0×10^5 cells/mL in 96-well culture plates and subsequently exposed to various concentrations of platinum complexes (0.01, 0.1, 1, 10, and 100 μM) for 72 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. The control cells were treated with the same amount of vehicle alone. For each of the variants tested, eight wells were used. For HL-60, at the end of the exposure, cell culture medium was removed by vacuum aspiration and the suspended cells were removed from the medium by centrifugation. A total of 100 μL suspensions of the cells were treated with equal amount of a 1:10 (v/v) mixture of 0.4% filtered trypan blue solution stain in Hanks' balanced salt solution (HBSS). Cells were observed under an inverted phase contrast microscope and counted as stained (dead) and non-stained (viable) cells on hemocytometer separately. For BEL-7402, Ramos, 3AO, and A549, after platinum complex treatment, the cultures were fixed with cold trichloroacetic acid and stained with 0.4% SRB dissolved in 1% acetic acid. Subsequently, unbound dye was removed by washing with acetic acid, and protein-bound dye was solubilized with Tris base. Then the absorbance of treated cells was read at 492 nm. Cytotoxicity was evaluated from the cell-growth inhibition in the treated cultures versus untreated controls. IC_{50}, the micromolar concentration of compound at which cell proliferation was 50% of that observed in control cultures, was determined by linear regression analysis.

61. The solubility of compounds **1a**–**3a** in several normal solutions such as water, ethanol, and chloroform has been tested. The solubility of complex **1a** is approximately 34.48, 0.17, and 0.1 mg/mL in water, ethanol, and chloroform, respectively. The solubility of complex **2a** is approximately 0.33, 1.00, and 1.25 mg/mL in water, ethanol, and chloroform, respectively. The solubility of complex **3a** is approximately 0.12, 10.00, and 1.00 mg/mL in water, ethanol, and chloroform, respectively.