Monofunctional Platinum Complexes Showing Potent Cytotoxicity against Human Liver Carcinoma Cell Line BEL-7402

Junyong Zhang,[†,#] Xiaoyong Wang,[†,#] Chao Tu,[†] Jun Lin,[†] Jian Ding,[‡] Liping Lin,[‡] Zheming Wang,[§] Cheng He,[§] Chunhua Yan,[§] Xiaozeng You,[†] and Zijian Guo*[,†]

State Key Laboratory of Coordination Chemistry, Coordination Chemistry Institute, Nanjing University, Nanjing 210093, P. R. China, Division of Anti-tumor Pharmacology, State Key Laboratory for New Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Shanghai 200031, P. R. China, and State Key Laboratory of Rare Earth Materials Chemistry and Applications, Peking University, Beijing 100871, P. R. China

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Three novel Pt(II) complexes [PtL$_1$′Cl] **I** (L$_1$′ = glycine-*N*′-8-quinolylamide), [PtL$_2$′Cl] **II** (L$_2$′ = L-alanine-*N*′-8-quinolylamide), and [PtL$_3$Cl] **III** [L$_3$ = *N*-(*tert*-butoxycarbonyl)-L-methionine-*N*′-8-quinolylamide] have been synthesized and characterized. The crystal structure of complexes **II** and **III** showed that the ligands are three-coordinated with only one Cl$^-$ as the leaving group. Complex **II** crystallized in the monoclinic system with space group *P*2(1), *a* = 9.502(2) Å, *b* = 4.724(1) Å, *c* = 14.800(3) Å, while complex **III** crystallized in the orthorhombic system with space group *P*2(1)2(1)2(1), *a* = 5.441(1) Å, *b* = 12.978(3) Å, *c* = 29.438(6) Å. These complexes have been tested against a wide range of tumor cell lines including BEL-7402, HCT-116, SPC-A4, MOLT-4, P388, HL-60, A-549, SGC-7901, MKN-28, and HO-8910. Complex **III** is highly cytotoxic against the HCT-116 (IC$_{50}$ = 0.38 μM), SPC-A4 (IC$_{50}$ = 0.43 μM), BEL-7402 (IC$_{50}$ = 0.43 μM), and MOLT-4 (IC$_{50}$ = 0.61 μM) cell lines. The cell line most sensitive to **III** is human liver carcinoma cell line BEL-7402, which has a response rate of 75.1% at 6.6 × 10^{-7} M, nearly 6 times higher than that of cisplatin.

Introduction

Severe toxicity such as nephrotoxicity and neurotoxicity, coupled with drug resistance developed after initial treatment, has limited the wider clinical application of cisplatin.[1,2] Although several new generations of platinum-based drugs with reduced toxicity have been introduced into the clinic, cross-resistance appeared due to their structural similarity to cisplatin.[3] Therefore, compounds with novel structural features and different biological properties are required to overcome these drawbacks.

Classical structure−activity rules for platinum-based drugs[4] have been questioned during the past decades. Many platinum(II) complexes that are trans in geometry[5,6] or charged[7] have been found to be active. According to the existing rules, monofunctional platinum complexes such as [Pt(dien)Cl]$^+$ are antitumor-inactive because of the lack of ability in formation of bifunctional DNA adducts.[8] However, several recent examples have shown that monofunctional platinum(II) complexes of nitrogen-containing heterocyclic amines such as pyridine, pyrimidine, purine, piperidine, picoline, and their derivatives can be cytotoxic.[9−13] The trinuclear platinum complex BBR3464, composed of two monofunctional Pt moieties, is currently in clinical trial.[14,15] The antitumor-active trans imino ether platinum complex preferentially forms monofunctional adducts at guanine residues in double helical DNA even after long incubation.[16] All these complexes appear to have different mechanisms of action compared to cisplatin and demonstrated distinct cytotoxicity profiles,[17,18] which suggest that the monofunctional adducts alone may be sufficient to exhibit the cytotoxicity.[19] Inspired by previous findings, we synthesized and characterized a series of novel monofunctional platinum(II) complexes to explore their potential antitumor activity and mechanism of action.

Chemistry

Three novel ligands, *N*-(*tert*-butoxycarbonyl)glycine-*N*′-8-quinolylamide (L$_1$), *N*-(*tert*-butoxycarbonyl)-L-alanine-*N*′-8-quinolylamide (L$_2$), and *N*-(*tert*-butoxycarbonyl)-L-methionine-*N*′-8-quinolylamide (L$_3$) and their *tert*-butoxycarbonyl (Boc) deprotected counterparts (L$_1$′−L$_3$′) were synthesized (Chart 1). The related complexes **I**−**III** (Chart 1) were also prepared and fully characterized by IR and NMR. The structures of complexes **II** and **III** were further determined by X-ray crystallography. In these ligands, the aminoquinoline moiety is condensed with amino acids glycine, L-alanine, and L-methionine, respectively, and all of them are able to coordinate with platinum(II) to give the monofunctional complexes. The crystal structures and numbering schemes of complexes **II** and **III** are shown in Figures 1 and 2, with the selected bond distances and angles listed in the figure caption. In complex **II**, platinum(II) is coordinated in a square planar geometry composed of three nitrogen atoms and a chloride anion. The approximately square-planar coordination of the platinum(II) atom generates two five-membered rings, and the torsion angles of Pt(1)−N(1)−C(9)−C(8), Pt(1)−N(3)−C(8)−C(9), Pt(1)−N(2)−C(10)−C(12), and Pt(1)−N(3)−C(12)−C(10) are −2.3°, 2.3° , −21.5°, and −0.9°, respectively. As shown

* To whom correspondence should be addressed. Phone: (+86)-25 3594549. Fax: (+86)-25 3314502. E-mail: zguo@nju.edu.cn.
† Nanjing University.
# Both authors contributed equally to the work.
‡ Shanghai Institute of Materia Medica.
§ Peking University.

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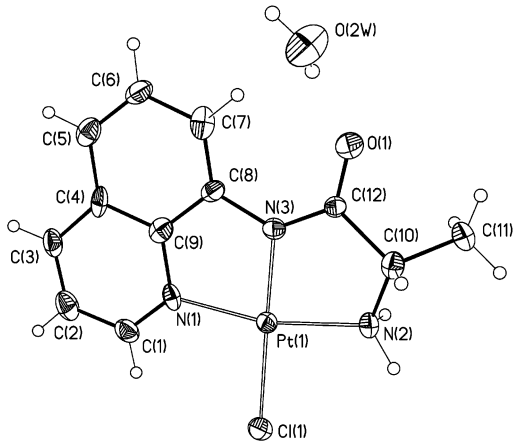

Figure 1. X-ray structure of complex **II** with atom-numbering scheme. Thermal ellipsoids are drawn at the 30% probability level. Selected bond distances (Å) and angles (deg) are the following: Pt(1)−N(1) 2.009(10), Pt(1)−N(2) 2.023(10), Pt(1)−N(3) 1.954(10), Pt(1)−Cl(1) 2.318(3); N(1)−Pt(1)−N(3) 83.7(4), N(1)−Pt(1)−Cl(1) 97.6(3), N(2)−Pt(1)−N(3) 83.5(4), N(2)−Pt(1)−Cl(1) 95.4(3), N(3)−Pt(1)−Cl(1) 178.0(3).

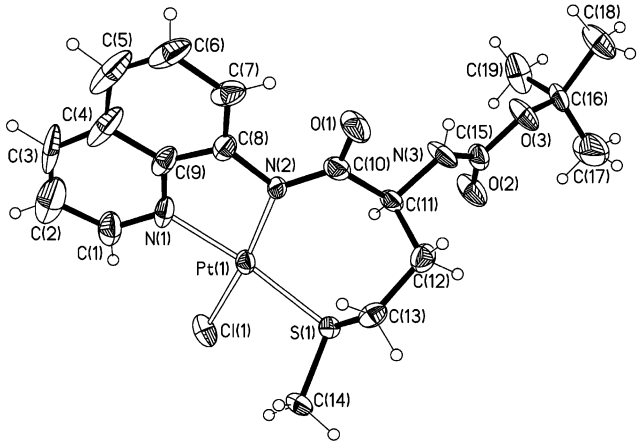

Figure 2. X-ray structure of complex **III** with atom-numbering scheme. Thermal ellipsoids are drawn at the 30% probability level. Selected bond distances (Å) and angles (deg) are the following: Pt(1)−N(1) 2.032(5), Pt(1)−N(2) 2.053(5), Pt(1)−S(1) 2.278(2), Pt(1)−Cl(1) 2.313(2); N(1)−Pt(1)−N(2) 80.5(2), N(1)−Pt(1)−Cl(1) 93.2(2), N(2)−Pt(1)−S(1) 100.9(2), N(2)−Pt(1)−Cl(1) 172.6(2), S(1)−Pt(1)−Cl(1) 85.9(7).

in Figure 2, L_3 in complex **III** acts as a tridentate ligand coordinating to the platinum(II) center through the quinolyl nitrogen N(1), the amide nitrogen N(2), and the sulfur atom S(1) with the chloride anion Cl(1) occupying the fourth coordinating site. This coordination mode gives five-membered and a seven-membered rings. The coordination of thioether sulfur of L_3 generates a chiral center at the sulfur; therefore, two potential diastereoisomers (*R* and *S*) are expected. The structural data show that in the solid-state complex **III** adopts an *R* configuration at the chiral sulfur atom. However, two sets of ^{1}H NMR resonances were observed, suggesting that the two isomers are in equilibrium with a roughly 1:1 ratio in $CDCl_3$ solution. Crystal data for complexes **II** and **III** are listed in Table 1.

Table 1. Crystal Data and Structure Refinement for Complexes **II** and **III**

	complex **II**	complex **III**
empirical formula	$C_{12}H_{14}ClN_3O_2Pt$	$C_{19}H_{24}ClN_3O_3PtS$
formula weight	462.8	605.01
temp, K	293(2)	293(2)
crystal size, mm	0.30 × 0.10 × 0.10	0.35 × 0.05 × 0.02
crystal system	monoclinic	orthorhombic
space group	*P*2(1)	*P*2(1)2(1)2(1)
a, Å	9.502(2)	5.441(1)
b, Å	4.724(1)	12.978(3)
c, Å	14.800(3)	29.438(6)
α, deg	90.00	90
β, deg	95.17(3)	90
γ, deg	90.00	90
V, Å^3	661.7(2)	2078.9(7)
Z	2	4
D_{calcd}, g·cm^{-3}	2.323	1.933
absorption coeff, mm^{-1}	10.805	7.004
F(000)	436	1176
θ range, deg	1.38−28.27	3.76−27.48
limiting indices	$-10 \le h \le 12$, $-6 \le k \le 6$, $-19 \le l \le 16$	$-7 \le h \le 7$, $-16 \le k \le 16$, $-38 \le l \le 38$
reflns collected	4092	27622
independent reflns	2697 (R_{int} = 0.0904)	4719 (R_{int} = 0.0873)
max and min transm	0.345 and 0.085	0.873 and 0.693
data/restraints/params	2697/1/188	4719/10/253
goodness-of-fit on F^2	1.011	1.013
final *R* indices	R1 = 0.0448	R1 = 0.0388
[$I > 2\sigma(I)$]	wR2 = 0.1070	wR2 = 0.0577

The reaction of complexes **I**−**III** toward a DNA model compound guanosine 5′-monophosphate (5′-GMP) was investigated using electrospray mass spectrometry (ESMS). Two days after complex **I** reacted with 5′-GMP in DMF/water (v/v = 1:1), a peak at 791.7 was observed in the ESMS spectrum that could be attributed to one negatively charged species $\{[Pt(L_1')(5'\text{-GMP})] + 2H_2O\}^-$ ($C_{21}H_{26}N_8O_{11}PPt$, calcd 792.5). In the case of complex **II**, a peak at 794.0 appeared in the ESMS spectrum that

Chart 1. Structures of Ligands L_{1-3}, L'_{1-3}, and Complexes **I**−**III**

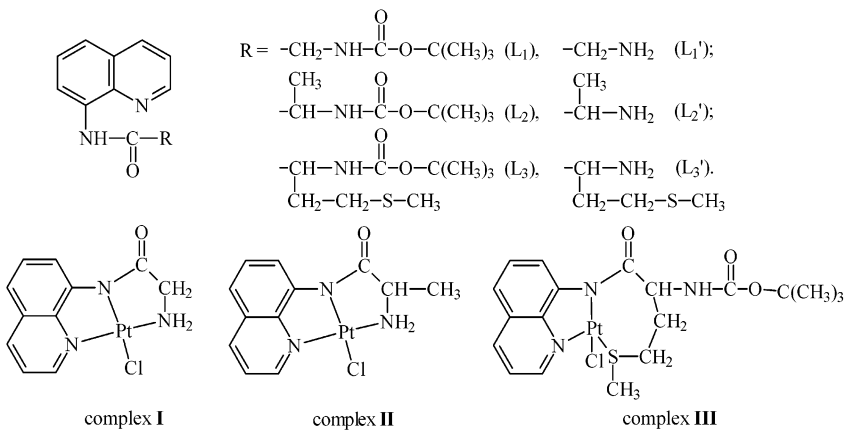

Table 2. Cytotoxic Activities of Complexes **I**−**III** against Selected Tumor Cell Lines at Different Concentrations (μg/mL), Expressed as Inhibition Rate (%)

tumor cell lines	for concentrations of complex **I**					for concentrations of complex **II**					for concentrations of complex **III**				
	10	3.3	1.1	0.4	0.1	10	3.3	1.1	0.4	0.1	10	3.3	1.1	0.4	0.1
BEL-7402[a]	84.5	92.6	18.3	12.6	4.1	83.5	94.4	45.4	0.4	0	89.3	93.2	90.9	75.1	13.9
HCT-116[a]	95.5	88.9	21.3	5.7	28.3	97.0	84.6	0	0	0	97.5	96.3	92.4	30.4	45.5
P-388[b]	86.2	64.2	25.7	15.6	9.6	73.7	58.1	0	0	0	85.9	52.3	45.2	15.4	13.2
HL-60[b]	100	61.5	0	0	0	100	57.4	0	0	0	98.7	89.6	21.0	4.0	0.6
A-549[a]	95.3	58.7	7.6	0	0	92.2	51.7	2.5	0	0	94.2	75.9	8.1	0	0

[a] Tested by the SRB assay. Cells were exposed to drugs for 72 h. [b] Tested by the MTT assay. Cells were incubated with drugs for 48 h.

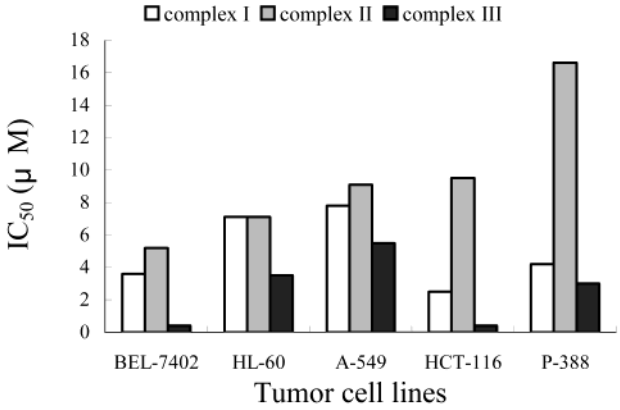

Figure 3. IC_{50} values of complexes **I**−**III** toward different cell lines.

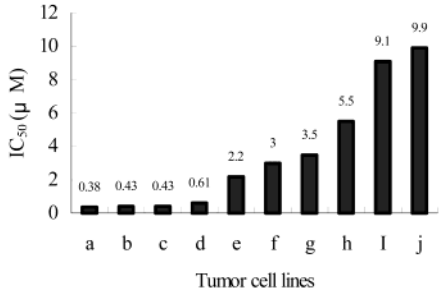

Figure 4. IC_{50} values of complex **III** toward different cell lines: (a) HCT-116; (b) SPC-A4; (c) BEL-7402; (d) MOLT-4; (e) HO-8910; (f) P-388; (g) HL-60; (h) A-549; (i) SGC-7901; (j) MKN-28. The IC_{50} values (μM) of cisplatin toward P-388, A-549, and HL-60 are 0.38, 0.54, and 0.87, respectively.

could be ascribed to one positively charged species $\{[Pt(L_2')(5'\text{-GMP})] + Na^+\}^+$ ($C_{22}H_{25}N_8O_9NaPPt$, calcd 794.5). The ESMS spectrum of the reaction of complex **III** with 5′-GMP showed the presence of a peak at 946.1 that can be tentatively assigned to a negatively charged species $\{[Pt(L_3)(5'\text{-GMP})] + H_2O\}^-$ ($C_{29}H_{38}N_8O_{12}$ PSPt, calcd 948.7). However, under the same reaction conditions, the intensity of this signal is lower compared to those of complexes **I** and **II**. The calculated molecular masses noted above and the isotopic distribution of the peaks perfectly matched the results from corresponding formulas according to the ISOPRO 3.0 program. These results showed that all three platinum(II) complexes could react with 5′-GMP to give monofunctional adducts and have the potential for DNA binding.

Biological Activity

Cytotoxicity experiments of platinum complexes are performed on a diverse panel of tumor cell lines including the human liver carcinoma cell line (BEL-7402), the human colon carcinoma cell line (HCT-116), the murine leukemia cell line (P-388), the human acute promyelocytic leukemia cell line (HL-60), and the human non-small-cell lung cancer cell line (A-549). The results of the cytotoxicity assay demonstrated that all three complexes were effective at 3.3 μg mL^{-1} against the cell lines examined (Table 2). At lower concentrations, complexes **I** and **II** did not exhibit significant activities but complex **III** still showed strong activities against BEL-7402 and HCT-116 cell lines and maintained a relatively high activity at 0.4 μg mL^{-1} for BEL-7402 cell line. The IC_{50} values of these complexes toward the same tumor cells further confirmed that complex **III** is the most active one under the experimental concentrations (Figure 3). Accordingly, complex **III** was selected to test its cytotoxic activities toward a broader range of tumor cell lines including the human lung adenocarcinoma cell line (SPC-A4), the T-cell leukemia cell line (MOLT-4), the human epithelial ovarian cancer cell line (HO-8910), the human stomach cancer cell line (SGC-7901), the human gastric cancer cell line (MKN-28), and the above five cell lines. From the IC_{50} values shown in Figure 4, it is found that complex **III** demonstrated more potent activities against HCT-116, SPC-A4, BEL-7402, and MOLT-4 cells than against other tumor cell lines. The activity of complex **III** against BEL-7402 is especially prominent, which is much more potent than cisplatin at whole concentration ranges (10^{-5}−10^{-7} M).

Discussion

In the process of preparing platinum(II) complexes from L_1−L_3, the −*tert*-Boc protecting groups in L_1 and L_2 were deprotected while that in L_3 remained intact under similar conditions. The leaving of −*tert*-Boc groups in L_1 and L_2 may be caused by the platinum(II) ion, which could act as a Lewis acid and facilitate the cleavage of the −*tert*-Boc group. Sulfur is generally thought to have a much higher affinity than nitrogen toward platinum(II),[20] and binding of the sulfur atom in L_3 to platinum(II) weakens the Lewis acidity of platinum(II) and makes it difficult for the −*tert*-Boc group to leave in complex **III**. The coordination of the thioether sulfur to platinum(II) generates a rare seven-membered S,N-chelate ring, and a chiral center at sulfur arose simultaneously because of the different configurations of the *S*-methyl group and the lone pair of electrons on the sulfur. Therefore, two potential diastereomers (*R* and *S*) are expected. Complex **III** indeed has two sets of ^{1}H NMR resonances in $CDCl_3$ solution, and the two diastereomers are in equilibrium with a roughly 1:1 ratio. Although the two diastereomers interconvert slowly enough to allow separate sets of signals to be observed in solution, the crystal structural data of complex **III** indicated that it only adopts an *R* configuration in the solid state. As shown in Figure 1,

a water molecule was crystallized with complex **II**, which may be stabilized by hydrogen bonding between O(1) of complex **II** and hydrogen atom of water.

It has been reported that monofunctional platinum(II) complexes can significantly destabilize DNA and affect the conformation of DNA duplex.[11,21] In our case, model reactions showed that although complexes **I**−**III** form only monofunctional adducts with 5′-GMP, they still exhibit considerable antitumor activity. These facts further suggest that the ability to form bifunctional DNA adducts may not be a precondition for platinum(II) complexes to exhibit cytotoxicity against tumor cells.

The binding of thioether sulfur to platinum(II) is reported to be reversible under physiological conditions. Both inter- and intramolecular displacement of the S-bound thioethers on platinum(II) by N^7-bound 5′-GMP[22] or nucleopeptide Met-d(TpG) (5′-*O*-methioninate-*N*-ylcarbonylthymidine 2′-deoxyguanosine monophosphate)[23] can readily occur. However, the bound *S*-Me group in complex **III** was not replaced by the presence of 5′-GMP both in DMF/water and in DMSO/water medium. Since the S,N-chelate ring in complex **III** is seven-membered and might be expected to be less stable thermodynamically and kinetically than the five-membered ring in complexes **I** and **II**, the possibility of ring opening could not be excluded in vivo. If so, it may facilitate the Pt migration from S to the N of DNA bases and hence provide a possible account for the greater cytotoxicity of complex **III** compared with the other two complexes. However, as far as our experimental conditions are concerned, none of these complexes undergo ring-opening reactions. Novel routes to DNA platination for these complexes may exist in vivo. To elucidate the potential mechanism and to evaluate these effective antitumor agents, the action mode with DNA and other pharmacological data are needed.

Conclusion

This work illustrates that monofunctional platinum(II) complexes can be activated by the use of planar ligands, which may therefore provide a new avenue of approach in the design of antitumor agents. The complexes exhibited broad antitumor spectra. The activity of complex **III** against BEL-7402 is especially prominent, with **III** being much more potent than cisplatin at whole concentration ranges (10^{-5}−10^{-7} M). The present report provides further examples of monofunctional platinum(II) complexes with high antitumor activity; however, the exact mechanism of action of these complexes remains to be elucidated.

Experimental Section

Materials and Methods. Solvents such as ethyl acetate, tetrahydrofuran (THF), acetone, anhydrous ether, and $CHCl_3$ and common reagents such as triethylamine, trifluoroacetic acid (TFA), and NaOH are all analytical grade and used as received. Glycine, L-alanine, L-methionine, and the disodium salt of 5′-GMP were purchased from Sigma, and 8-aminoquinoline and di-*tert*-butyl dicarbonate were purchased from Acros Organics. $K_2[PtCl_4]$ was synthesized by the reduction of $K_2[PtCl_6]$ using hydrazine dihydrochloride, and cisplatin was prepared from $K_2[PtCl_4]$ according to the method described previously.[24] Streptomycin was obtained from GIBCO (Grand Island, NY).

The ^{1}H NMR spectra were recorded at 298 K on a Bruker DRX-500 spectrometer using standard pulse sequences. Electrospray mass spectra were recorded using an LCQ electron spray mass spectrometer (ESMS, Finnigan), and the predicted isotope distribution patterns for each of the complexes were calculated using the ISOPRO 3.0 program. The raw crystallographic data were collected on a Siemens SMART CCD diffractometer. Empirical absorption correction was carried out using the SADABS program,[25] and the crystal structures were solved by the SHELXTL software package.[26]

The growth inhibitory effect of platinum complexes on the leukemia cell lines (P388, HL-60, and MOLT-4) was measured by the MTT assay, while that on human solid tumor cell lines (BEL-7402, SPC-A4, SGC-7901, MKN-28, HO-8910, and HCT-116) was tested by the SRB assay. The OD (optical density) value was read on a multiwell spectrophotometer (VERSA Max, Molecular Devices). The cytotoxicity results of the complexes were expressed as IC_{50} (the drug concentration that reduces by 50% the absorbance in treated cells with respect to untreated cells), which was calculated by the Logit method. The mean IC_{50} value was calculated using the data from three replicate tests.

Preparation of the Ligands. L_1 was obtained by the following procedure. To a cooled solution of glycine (1.5 g, 20 mmol) in a mixture of dioxane (16 mL), water (8 mL), and 1 M NaOH (42 mL), di-*tert*-butyl dicarbonate (4.8 g, 22 mmol) was added with stirring. The reaction was continued at 0 °C for 1 h and then at room temperature overnight. The resulting mixture was titrated with a saturated citric acid solution to adjust the pH to about 5 and then extracted with ethyl acetate (2 × 70 mL). The ethyl acetate layer was dried with Na_2SO_4 and concentrated to give a pale-yellow syrup of *N*-(*tert*-Boc)-glycine (3.33 g). This product (1.23 g, 7 mmol) was dissolved in THF (35 mL), and the mixture was cooled to about −15 °C to which triethylamine (7 mmol) and $ClCO_2Et$ (7 mmol) were added slowly by syringe. After the mixture was stirred at −15 °C for 1 h, a cold solution of 1 mol equiv of 8-aminoquinoline in THF was added. The reaction was continued at −15 °C for 1 h and then at room temperature overnight. The resulting mixture was filtered, and the filtrate was concentrated to give L_1: yield, 92%. L_1 was deprotected by treating it with TFA in $CHCl_3$ for 24 h and adding anhydrous ether as the precipitating agents to give L_1': yield, 95%. Elemental anal. found (calculated) for L_1 (%): C, 63.5 (63.8); H, 6.29 (6.31); N, 14.7 (14.0). For L_1' (%): C, 65.3 (65.7); H, 5.44 (5.47); N, 21.3 (20.9). ESMS *m*/*z* for L_1: 324.1 [$L_1 + Na^+$]. For L_1': 202.2 [$L_1' + H^+$]. ^{1}H NMR for L_1 (DMSO-d_6): δ 8.88 (d, 1H), 8.64 (d, 1H), 8.42 (d, 1H), 7.66 (d, 1H), 7.64 (q, 1H), 7.59 (t, 1H), 3.83 (s, 29H), 1.44 (s, 9H). For L_1' (D_2O): δ 8.56 (d, 1H), 8.11 (d, 1H), 7.84 (d, 1H), 7.56 (d, 1H), 7.33 (m. 2H), 3.90 (d, 2H).

The L_2, L_3, and their Boc-deprotected counterparts L_2' and L_3' were synthesized following the same procedure as that for ligands L_1 and L_1' except that the starting materials were L-alanine and l-methionine, respectively. Elemental anal. found (calculated) for L_2 (%): C, 64.6 (64.8); H, 6.68 (6.67); N, 6.40 (6.35). For L_2' (%): C, 66.6 (67.0); H, 6.00 (6.05); N, 19.8 (19.5). ESMS *m*/*z* for L_2: 338.1 [$L_2 + Na^+$]. For L_2': 216.3 [$L_2' + H^+$]. ^{1}H NMR for L_2 (DMSO-d_6): δ 8.87 (d, 1H), 8.64 (d, 1H), 8.41 (d, 1H), 7.67 (m, 1H), 7.64 (q, 1H), 7.59 (t, 1H), 4.18 (q, 1H), 1.36 (d, 3H), 1.43 (s, 9H). For L_2' (D_2O): δ 8.92 (s, 1H), 8.51 (d, 1H), 8.19 (d, 1H), 7.93 (d, 1H), 7.70 (m, 2H), 4.51 (q, 1H), 1.78 (d, 3H). Elemental anal. found (calculated) for L_3 (%): C, 60.4 (60.8); H, 6.63 (6.67); N, 11.5 (11.2). For L_3' (%): C, 61.0 (61.1); H, 6.15 (6.18); N, 15.7 (15.3). ESMS *m*/*z* for L_3: 398.2 [$L_3 + Na^+$]. For L_3': 276.4 [$L_3' + H^+$]. ^{1}H NMR for L_3 ($CDCl_3$): δ 10.35 (s, 1H), 8.85 (d, 1H), 8.75 (d, 1H), 8.26 (d, 1H), 7.58(m, 2H), 7.52 (m, 1H), 5.53 (m, 1H), 4.66 (m, 1H), 2.67 (m, 2H), 2.32 (m, 1H), 2.14 (s, 3H), 2.13 (m, 1H), 1.48 (s, 9H). For L_3' (D_2O): δ 8.92 (s, 1H), 8.46(d, 1H), 8.22 (d, 1H), 7.92 (d, 1H), 7.68 (m, 2H), 4.56 (t, 1H), 2.80 (m, 2H), 2.43 (m, 2H), 2.16 (s, 3H).

Preparation of the Complexes. Platinum complexes **I**−**III** were prepared by the following procedure. The aqueous solution of K_2PtCl_4 (0.2 mmol, 2 mL) was mixed with the acetone solution of the ligand (L_1', L_2', or L_3) (2 mL) at a 1:1 molar ratio. The mixture was then refluxed for 2 h. A yellow

precipitate was formed when the mixture was cooled to room temperature, which was then collected by filtration. Single crystals of complexes **II** and **III** were both obtained by slow evaporation of the solvent of acetone and water. It is interesting to note that **I** or **II** can also be synthesized by the reaction of ligand L_1 or L_2 with K_2PtCl_4 in a mixture of acetone and water, during which the Boc group is cleaved, as shown by ^{1}H NMR spectra and X-ray crystal analysis. However, L_3 does not undergo the same cleavage reaction during the preparation of complex **III**. Elemental anal. found (calculated) for complex **I** (%): C, 29.0 (29.4); H, 2.63 (2.68); N, 9.39 (9.36). ^{1}H NMR of complex **I** in DMSO-d_6 (ppm): δ 8.99 (d, 1H), 8.68 (d, 1H), 8.58 (d, 1H), 7.69 (m, 1H), 7.57 (t, 1H), 7.50 (d, 1H), 6.00 (s, 2H). Elemental anal. found (calculated) for complex **II** (%): C, 30.8 (31.1); H, 3.00 (3.03); N, 9.13 (9.08). ^{1}H NMR of complex **II** in DMSO-d_6 (ppm): δ 9.01 (d, 1H), 8.67 (d, 1H), 8.60 (d, 1H), 7.70 (m, 1H), 7.57 (t, 1H), 7.50 (d, 1H), 3.81 (q, 1H), 1.33 (d, 3H). Elemental anal. found (calculated) for complex **III** (%): C, 32.0 (32.2); H, 3.40 (3.44); N, 8.07 (8.04). Two sets of ^{1}H NMR resonances for complex **III** in $CDCl_3$ (ppm), set 1: δ 9.365 (d, 1H), 8.735 (d, 1H), 8.426 (t, 1H), 8.638 (m, 1H), 7.572 (m, 1H), 7.495 (m, 1H), 6.002 (m, 1H), 3.425 (m, 1H), 3.065 (m, 2H), 2.857 (s, 3H), 2.305 (m, 2H), 1.481 (s, 9H). For set 2: δ 9.300 (d, 1H), 8.645 (d, 1H), 8.426 (t, 1H), 8.638 (m, 1H), 7.572 (m, 1H), 7.495 (m, 1H), 6.656 (m, 1H), 3.114 (m, 1H), 2.620 (m, 2H), 2.761 (s, 3H), 2.171 (m, 2H), 1.481 (s, 9H).

Reaction of Complexes with 5′-GMP. Platinum(II) complex **I** (**II** or **III**) (0.05 mmol) was dissolved in a mixture of DMF and water, and an aqueous solution of 5′-GMP (0.05 mmol) was added with stirring. The reaction was continued for about 48 h at room temperature, and then the solvent was removed. ESMS spectra were recorded for the resulting product.

Cytotoxicity Assays. Cytotoxicity assays of platinum complexes are performed on a diverse panel of human tumor cell lines. Tumor cell lines were grown in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in a highly humidified atmosphere of 95% air with 5% CO_2 at 37 °C.

Growth inhibitory effect of platinum complexes on the leukemia cell lines (P388, HL-60, and MOLT-4) was measured by the microculture tetrazolium [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT] assay.[27] Briefly, cells in 100 μL of culture medium were plated in each well of 96-well plates (Falcon, CA). The cells were treated in triplicate with grade concentrations of platinum complexes and the reference drug cisplatin at 37 °C for 48 h. A 20 μL aliquot of MTT solution (5 mg/mL) was added directly to all the appropriate wells. The culture was then incubated for 4 h. Then 100 μL of "triplex solution" (10% SDS/5% isobutanol/12 mM HCl) was added. After the plates were incubated at 37 °C overnight, they were measured by the absorbance at 570 nm using a multiwell spectrophotometer.

For human solid tumor cell lines (BEL-7402, A-549, SPC-A4, SGC-7901, MKN-28, HO-8910, and HCT-116), the growth inhibition was tested by the sulforhodamine B (SRB) assay.[28] Briefly, adherent cells in a 100 μL medium were seeded in 96-well plates and allowed to attach for 24 h before drug addition. The cell densities were selected on the basis of preliminary tests to maintain control cells in an exponential phase of growth during the period of the experiment and to obtain a linear relationship between the OD and the number of viable cells. Each cell line was exposed to grade concentrations of the three compounds at desired final concentrations for 72 h, and each concentration was tested in triplicate wells. After exposure, cells were fixed by gentle addition of 100 μL of cold (4 °C) 10% trichloroacetic acid to each well, followed by incubation at 4 °C for 1 h. Plates were washed with deionized water five times and allowed to air-dry. Cells were stained by addition of 100 μL of SRB solution (0.4% SRB (w/v) in 1% acetic acid (v/v) to wells for 15 min. Then the plates were quickly washed five times with 1% acetic acid to remove any unbound dye and allowed to air-dry. Bound dye was solubilized with 10 mmol·mL^{-1} Tris (pH 10.5) prior to reading plates. The OD value was read on a plate reader at a wavelength of 515 nm. Media and DMSO control wells, in which complexes were absent, were included in all the experiments. The percent growth inhibitory rate of treated cells was calculated by $(OD_{control} - OD_{test})/OD_{control} \times 100$. Results of complexes were expressed as IC_{50} (the drug concentration that reduces by 50% the absorbance in treated cells with respect to untreated cells) that was calculated by the Logit method. Finally, the mean IC_{50} was calculated using the data from three replicate tests.

Note. Crystallographic data of complexes **II** and **III** have been deposited at the Cambridge Crystallographic Data Centre, CCDC Nos. 192536 and 192537. Any queries relating to the data can be e-mailed to deposit@ccdc.cam.ac.uk.

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References

(1) Wong, E.; Giandomenico, C. M. Current status of platinum-based antitumor drugs. *Chem. Rev.* **1999**, *99*, 2451−2466.
(2) Lippert, B., Ed. *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Wiley-VCH: Weinheim, Germany, 1999.
(3) Hambley, T. W. Platinum binding to DNA: structural controls and consequences. *J. Chem. Soc., Dalton Trans.* **2001**, 2711−2718.
(4) Cleare, M. J.; Hoeschele, J. D. Antitumor activity of group VIII transition metal complexes. I. Platinum(II) complexes. *Bioinorg. Chem.* **1973**, *2*, 187−210.
(5) Coluccia, M.; Nassi, A.; Boccarelli, A.; Giordano, D.; Cardellicchio, N.; Locker, D.; Leng, M.; Sivo, M.; Intini, F. P.; Natile, G. In vitro and in vivo antitumour activity and cellular pharmacological properties of new platinum−iminoether complexes with different configuration at the iminoether ligands. *J. Inorg. Biochem.* **1999**, *77*, 31−35.
(6) Bierbach, U.; Qu, Y.; Hambley, T. W.; Peroutka, J.; Nguyen, H. L.; Doedee, M.; Farrell, N. Synthesis, structure, biological activity, and DNA binding of platinum(II) complexes of the type *trans*-[$PtCl_2(NH_3)$L] (L = planar nitrogen base). Effect of L and cis/trans isomerism on sequence specificity and unwinding properties observed in globally platinated DNA. *Inorg. Chem.* **1999**, *38*, 3535−3542.
(7) Pratesi, G.; Perego, P.; Polizzi, D.; Righetti, S. C.; Supino, R.; Caserini, C.; Manzotti, C.; Giuliani, F. C.; Pezzoni, G.; Tognella, S.; Spinelli, S.; Farrell, N.; Zunino, F. A novel charged trinuclear platinum complex effective against cisplatin-resistant tumors: hypersensitivity of p53-mutant human tumor xenografts. *Br. J. Cancer* **1999**, *80*, 1912−1919.
(8) Hollis, L. S.; Sundquist, W. I.; Burstyn, J. N.; Heiger-Bernays, W. J.; Bellon, S. F.; Ahmed, K. J.; Amundsen, A. R.; Stern, E. W.; Lippard, S. J. Mechanistic studies of a novel class of trisubstituted platinum(II) antitumor agents. *Cancer Res.* **1991**, *51*, 1866−1875.
(9) Hollis, L. S.; Amundsen, A. R.; Stern, E. W. Chemical and biological properties of a new series of *cis*-diammineplatinum-(II) antitumor agents containing three nitrogen donors: *cis*-[Pt-$(NH_3)_2$(N-donor)Cl]$^+$. *J. Med. Chem.* **1989**, *32*, 128−136.
(10) Lempers, E. L. M.; Bloemink, M. J.; Brouwer, J.; Kidani, Y.; Reedijk, J. The new antitumor compound, *cis*-[$Pt(NH_3)_2$(4-methylpyridine)Cl]Cl, does not form N7,N7-D(GpG) cheletes with DNA−an unexpected preference for platinum binding at the 5′G in D(GpG). *J. Inorg. Biochem.* **1990**, *40*, 23−35.
(11) Bauer, C.; Peleg-Shulman, T.; Gibson, D.; Wang, A. H. J. Monofunctional platinum amine complexes destabilize DNA significantly. *Eur. J. Biochem.* **1998**, *256*, 253−260.
(12) Okada, T.; El-Mehasseb, I. M.; Kodaka, M.; Tomohiro, T.; Okamoto, K.; Okuno, H. Mononuclear platinum(II) complex with 2-phenylpyridine ligands showing high cytotoxicity against mouse Sarcoma 180 cells acquiring high cisplatin resistance. *J. Med. Chem.* **2001**, *44*, 4661−4667.
(13) Zhang, J. Y.; Liu, Q.; Duan, C. Y.; Shao, Y.; Ding, J.; Miao, Z. H.; You, X. Z.; Guo, Z. J. Structural evidence for the facile chelate-ring opening reactions of novel platinum(II)−pyridine carboxamide complexes. *J. Chem. Soc., Dalton Trans.* **2002**, 591−597.

(14) Hegmans, A.; Qu, Y.; Kelland, L. R.; Roberts, J. D.; Farrell, N. Novel approaches to polynuclear platinum pro-drugs. Selective release of cytotoxic platinum−spermidine species through hydrolytic cleavage of carbamates. *Inorg. Chem.* **2001**, *40*, 6108−6114.

(15) Davies, M. S.; Thomas, D. S.; Hegmans, A.; Berners-Price, S. J.; Farrell, N. Kinetic and equilibria studies of the aquation of the trinuclear platinum phase II anticancer agent [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-*trans*-Pt(NH$_3$)$_2$(NH$_2$(CH$_2$)$_6$NH$_2$)$_2$}]$^{4+}$ (BBR3464). *Inorg. Chem.* **2002**, *41*, 1101−1109.

(16) Coluccia, M.; Nassii, A.; Loseto, F.; Boccarelli, A.; Mariggio, M. A.; Giordano, D.; Intini, F. P.; Caputo, P.; Natile, G. A *trans*-platinum complex showing higher antitumor activity than the *cis* congeners. *J. Med. Chem.* **1993**, *36*, 510−512.

(17) Brabec, V.; Vrána, O.; Nováková, O.; Kleinwächter, V.; Intini, F. P.; Coluccia, M.; Natile, G. DNA adducts of antitumor *trans*-[PtCl$_2$ (E-imino ether)$_2$]. *Nucleic Acids Res.* **1996**, *24*, 336−341.

(18) Farrell, N. DNA-binding and chemistry of dinuclear platinum comlexes. *Comments Inorg. Chem.* **1995**, *16*, 373−389.

(19) Natile, G.; Coluccia, M. Current status of *trans*-platinum compounds in cancer therapy. *Coord. Chem. Rev.* **2001**, *216*, 383−410.

(20) Farrell, N. *Transition Metal Complexes as Drugs and Chemotherapeautic Agents*; Kluwer Academic Press: Dordrecht, The Netherlands, 1989; p 51.

(21) Peleg-shulman, T.; Katzhendler, J.; Gibson, D. Effects of monofunctional platinum binding on the thermal stability and conformation of a self-complementary 22-mer. *J. Inorg. Biochem.* **2000**, *81*, 313−323.

(22) Barnham, K. J.; Djuran, M. I.; Murdoch, P. del S.; Sadler, P. J. Intermolecular displacement of S-bound L-methionine on platinum(II) by guanosine 5′-monophosphate: implications for the mechanism of action of anticancer drugs. *J. Chem. Soc., Chem. Commun.* **1994**, 721−722.

(23) Teuben, J.-M.; van Boom, S. S. G. E.; Reedijk, J. Intramolecular migration of co-ordinated platinum from a sulfur to N^7 in the nucleopeptide Met-d(TpG) (5′-*O*-methioninate-*N*-ylcarbonylthymidine 2′-deoxyguanosine monophosphate). *J. Chem. Soc., Dalton Trans.* **1997**, 3979−3980.

(24) Dhara, S. C. A rapid method for the synthesis of *cis*-[Pt(NH$_3$)$_2$Cl$_2$]. *Indian J. Chem.* **1970**, *8*, 193−194.

(25) Sheldrick, G. M. *SADABS, Program for empirical absorption correction of area detector data*; University of Gottingen: Gottingen, Germany, 1996.

(26) Sheldrick, G. M. *SHELXTL, Structure Determination Software Programs*, version 5.10; Bruker Analytical X-ray Systems Inc.: Madison, WI, 1997.

(27) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* **1988**, *48*, 589−601.

(28) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

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