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Antitumor Platinum(II) Complexes Containing Platinum-Based Moieties of Present Platinum Drugs and Furoxan Groups as Nitric Oxide Donors: Synthesis, DNA Interaction, and Cytotoxicity

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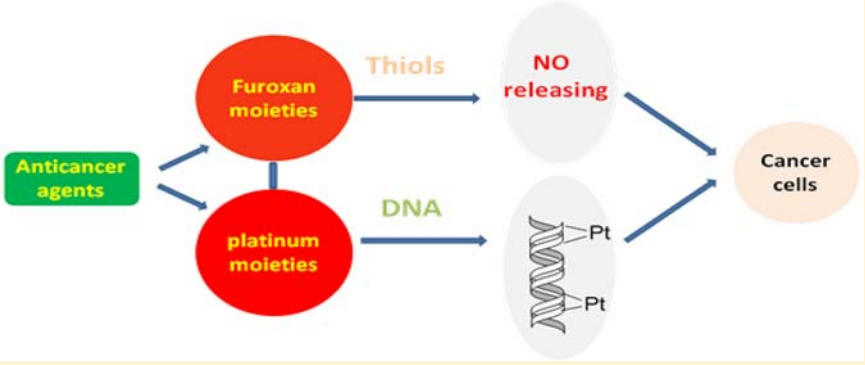

ABSTRACT: Six novel platinum(II) complexes **1**−**6** bearing different furoxan moieties as nitric oxide (NO) donors have been designed, synthesized, and characterized by elemental analysis and ^{1}H NMR, IR, and ESI-MS spectroscopy. The furoxan groups were introduced to the platinum complexes to release NO, which may take synergic action with the platinum-based moieties on the tumor cells. It was found that all compounds exhibited considerable cytotoxicity against human HCT-116 and SGC-7901 cell lines via DNA binding together with NO-releasing features, especially for compound **3**. This finding is in accordance with the previous reports that NO hybrids show higher cytotoxicity against colon cancer cell lines compared with their parent compounds.

INTRODUCTION

The successful application of cisplatin in clinical for cancer therapies has triggered considerable interest to search for more effective cisplatin analogues, and as a consequence carboplatin and oxaliplatin have been successively developed and used in clinic.[1,2] On the basis of the great success of platinum-based drugs, cisplatin has reached medicinal chemistry textbooks worldwide.[3] However, the cross resistance and severe side effects of platinum drugs have limited their clinical application to a great extent. In order to overcome these drawbacks, a number of drug-design strategies have been employed in the past years,[4−11] and one of the most successful approaches is to combine platinum-based drugs with some other active pharmacophore so that a synergic action can be achieved toward the tumor cells.[12−15]

Nitric oxide (NO) is one of the most important signaling molecules involved in many processes of vasodilation, neurotransmission, immune system, and apoptosis in human bodies.[16,17] Recently, it has been found that NO has cytotoxicity against cancer cells, as evidenced by the positive results of the study concerning the beneficial effects of NO in oncology.[18] The antitumor mechanisms of NO are various, including (a) inhibiting the proliferation of tumor cells, (b) causing apoptosis and necrosis of tumor cells, and (c) inducing the attenuation of angiogenesis.[19] However, NO is such a reactive molecule that it has a very short half-life in the body and is impossibly administered in aqueous solution. Besides, NO is found to be a two-sided sword in the treatment of cancer because many researches showed that NO plays a dual role as a proneoplastic and an antineoplastic agent simultaneously. Although the exact role of NO in oncology remains controversial, there is a consensus to date that the function of NO in the treatment of cancer depends on its action concentration. Basically, the high concentration of NO leads to inhibition of cell growth and induces apoptotic cell death, whereas the low concentration promotes cell growth.[18,19] Considering such character of NO, researchers are increasingly interested in NO donors that are stable in vitro and are able to release a relatively high level of NO in vivo.[16]

Furoxans (1,2,5-oxadiazole 2-oxides) represent an important class of NO donors. The biological activities of furoxan derivatives have been widely studied, including anti-infective properties, anticancer features, mutagenicity, immunosuppression, and central muscle relaxant properties.[20] Since the anticancer properties of some benzofuroxans were first reported by Ghosh and Whitehouse,[21−24] many furoxan and benzofuroxan derivatives have been tested against human cancer cell

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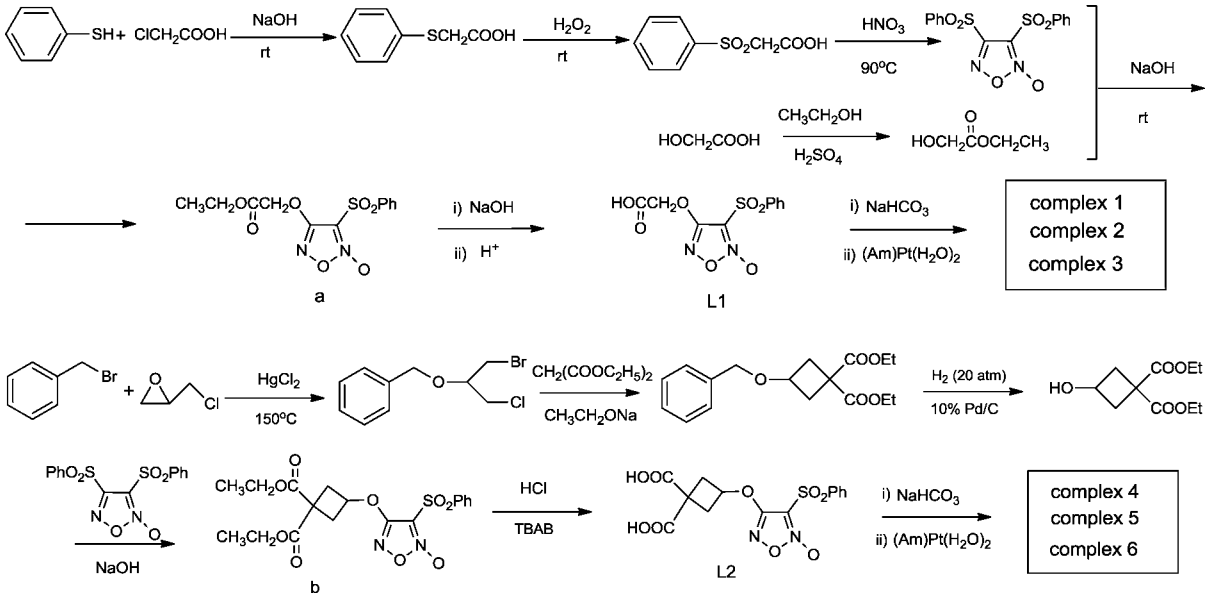
Scheme 1. Synthetic Route of Complexes 1−6



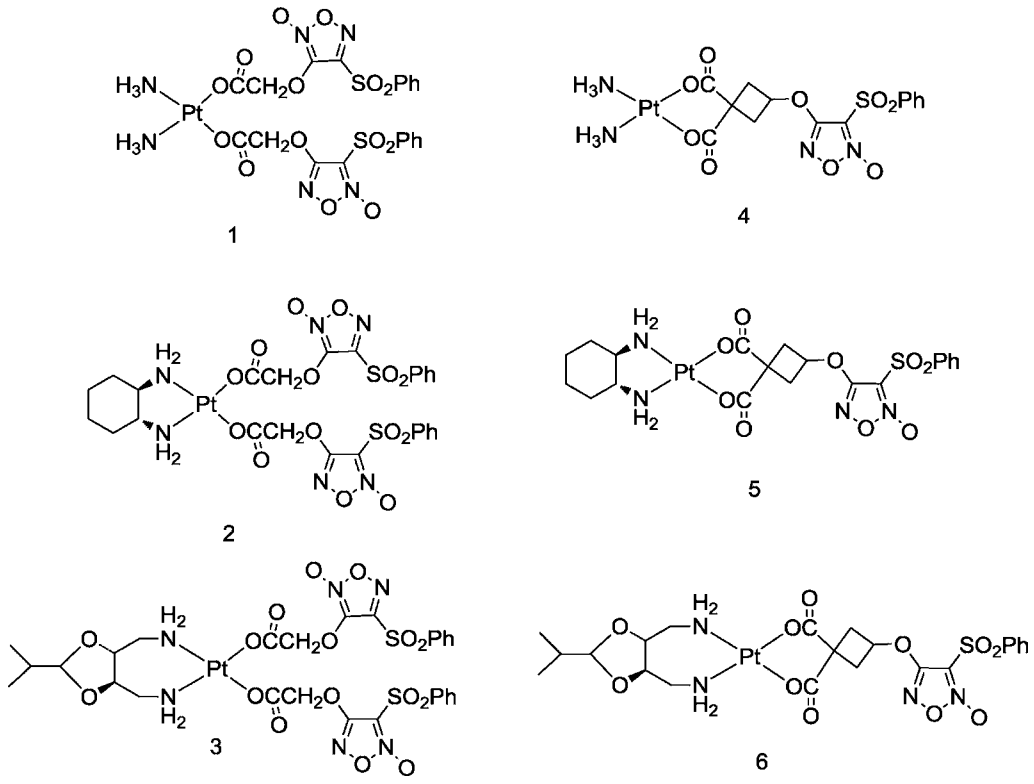


Figure 1. Schematic representation of the complexes studied in this work.

lines as cytotoxic compounds.[25,26] Recently, a few researchers have demonstrated that NO-donor compounds can enhance the cytotoxicity of platinum drugs against human cancer cells[27,28] and sensitize drug-resistant human cancer cells to cisplatin,[29] indicating that there is a positive synergy between NO and platinum-based compounds in the aspect of anticancer. It is noted that all previous attempts were performed by administrating NO-donor compounds and platinum drugs as two individual agents, but there has been no attempt to combine them into a molecule so far. From this point of view, it will be interesting and significant to study the hybrid compounds of platinum-based moieties with NO donors as anticancer agents.

In this work, we have designed and synthesized a series of hybrid compounds consisting of both platinum-based moieties of the present effective platinum drugs and furoxan species for the first time. It is anticipated that the newly synthesized agents could bind DNA in the same way as cisplatin and its analogues and exhibit good cytotoxicity against tumor cells owing to the anticancer property of NO and its possible synergic action with platinum-based complexes. At first, we intended to add furoxan species to the classical carrier ligands [e.g., ammine, (1*R*,2*R*)-

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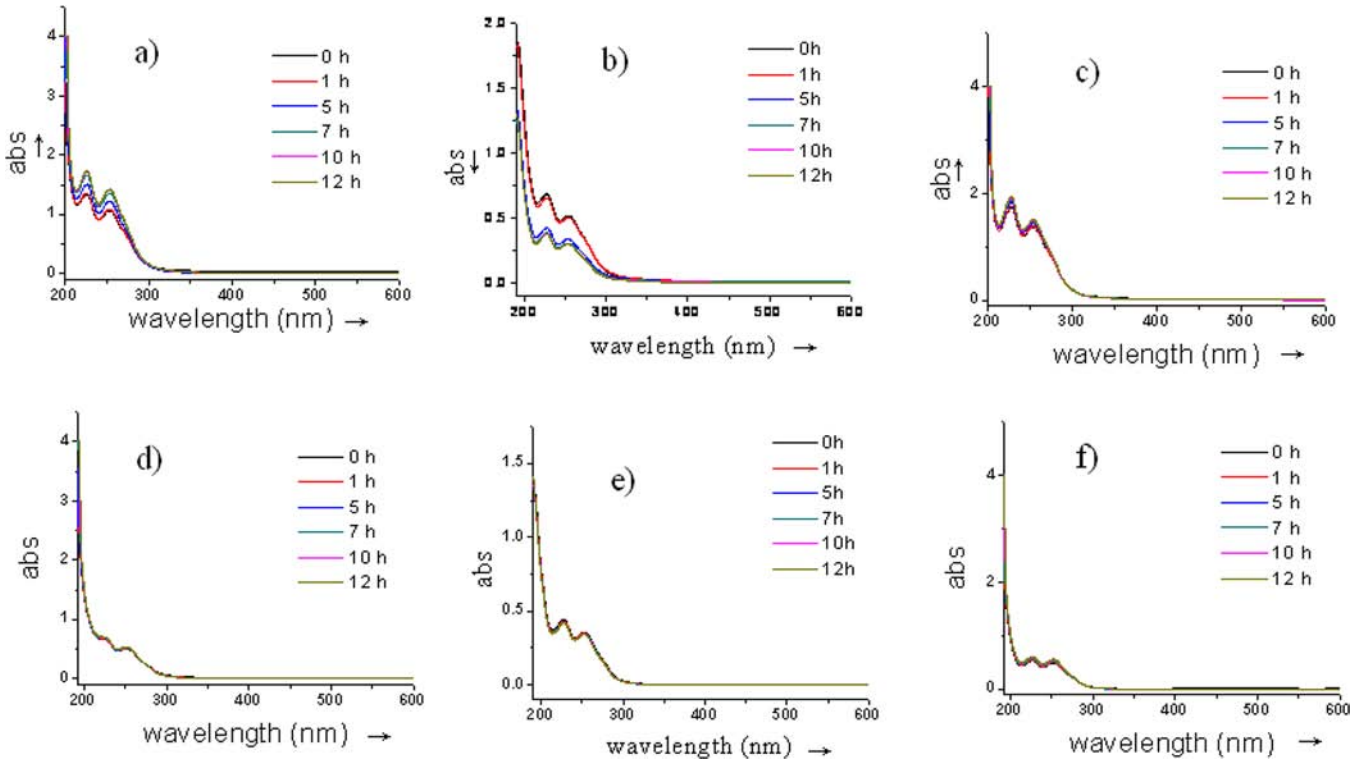
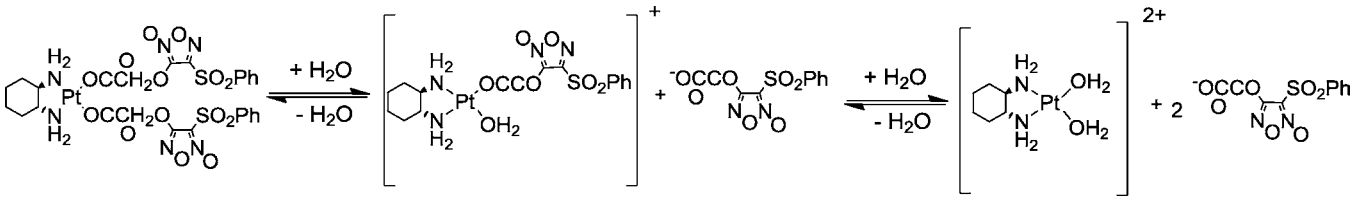


Figure 2. UV−vis spectra of complexes **1**−**6** in 5% CH_3CH_2OH/95% H_2O recorded at different times over 12 h of incubation at 298 K: (a) complex **1**; (b) complex **2**; (c) complex **3**; (d) complex **4**; (e) complex **5**; (f) complex **6**.

Scheme 2. Hydrolysis Equation for Complex 2



1,2-diaminocyclohexane, (4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane]; however, the lack of hydroxyl groups in these carrier ligands limits the introduction of furoxan species. Thus, we had to tether the furoxan moieties to the leaving group ligands containing a hydroxyl group like glycolic acid and 3-hydroxy-cyclobutane-1,1-dicarboxylic acid. Herein reported are the synthesis (Scheme 1), characterization, and in vitro biological properties of a series of novel anticancer candidates (Figure 1) that combine the platinum moieties of the present platinum drugs like carboplatin, oxaliplatin, or heptaplatin with furoxan groups.

RESULTS AND DISCUSSION

Synthesis of Complexes 1−6. Ligands L1 and L2 as well as platinum(II) complexes **1**−**6** were prepared following the procedure shown in Scheme 1. We failed to get L1 by a direct reaction of glycolic acid with 3,4-bis(phenylsulfonyl)-1,2,5-oxadizazole oxide, so the carboxylic acid was protected by esterification and then hydrolyzed under a basic condition with pH < 10. L2 was obtained in a manner analogous to that of L1. Because furoxans are stable under acidic conditions and have high resistance against heat,[17] hydrolysis took place under acidic conditions with tetrabutylammonium bromide as a phase-transfer catalyst at 100 °C.

The synthesized compounds were spectrally characterized by elemental analysis and ^{1}H NMR, IR, and electrospray ionization mass (ESI-MS) spectroscopy. In the ESI-MS spectra, all of the platinum complexes showed 100% of $[M + H]^+$ or $[M + Na]^+$ peaks, except for **1**, which showed 100% of the $[M - L1]^+$ peak. It is noted that complexes **1**−**6** have three protonated ion peaks, respectively, because of the existence of the isotopes ^{194}Pt (33%), ^{195}Pt (34%), and ^{196}Pt (25%). ^{1}H NMR spectra confirmed the existence of furoxan groups, which are compatible to the chemical structures of the corresponding complexes.

Stability of Complexes 1−6. Because the stability of platinum-based compounds is one of the key criteria for being an anticancer candidate, complexes **1**−**6** were evaluated by UV spectral analysis at different times in 5% CH_3CH_2OH/95% H_2O (Figure 2). No detectable changes of the absorption bands of complexes **4**−**6** have been observed within 12 h, which demonstrates the good stability of these compounds with bidentate carboxylate under the test conditions. However, the absorption bands of complex **2** decreased gradually at around 250 nm within the first 7 h and then maintained stability in the later 5 h, while the absorption bands of complexes **1** and **3** increased gradually at the beginning and then became unchanged in the later hours, indicating that complexes **1**−**3**

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with two monodentate carboxylates were easily hydrolyzed until was equilibrium reached. It is observed that the UV spectral changes of the complexes with different carrier ligands were not the same. A representative equilibrium equation for complex **2** is shown in Scheme 2.

According to Figure 2, it is noted that the changes of the absorption bands of complex **3** were very tiny compared to those of complexes **1** and **2** but somewhat like those of complexes **4**−**6** with bidentate carboxylate ligands, demonstrating that complex **3** was relatively more stable than complexes **1** and **2**. The departure of the leaving group from the complex with two monodentate carboxylates was also detected by the ESI-MS technique. The typical peak appearing in the ESI-MS spectrum of complex **1** was attributed to $[M - L1]^+$ species. It is notable that the departure of the leaving group from the complex with two monodentate carboxylates was common, which similarly occurred in the case of cisplatin.[30] The absorption bands at around 228 and 254 nm were assigned to the furoxan derivatives, which were stable in aqueous solution. Overall, complexes **4**−**6** with a bidentate O−O leaving group were more stable than complexes **1**−**3**, and the synthesized furoxan derivatives were stable in aqueous solution as well.

DNA Binding Study. The interaction of platinum compounds with DNA to form platinum/DNA adducts is the main mechanism of platinum complexes leading to tumor cell death and anticancer effects.[3,31] With the purpose of assessing the binding of our synthesized complexes with DNA, agarose gel electrophoresis was applied to study the interaction of pET22b plasmid DNA with complexes **1**−**6** and cisplatin, respectively. The compounds tested were divided into three groups, and each group was composed of untreated pET22b plasmid DNA, cisplatin, and newly synthesized compounds bearing the same carrier ligands. Each compound was tested at four different concentrations (10, 50, 100, and 200 μM).

When pET22b plasmid DNA was incubated with complexes **1**−**6** and cisplatin increased from 10 to 200 μM, it was found that the intensity of the circular supercoiled DNA (form I) band decreased, while that of the nicked (form II) bands increased. This can be attributed to the unwinding of supercoiled DNA to open circular DNA, demonstrating the binding between DNA and the platinum complexes (Figure 3). For complexes **1** and **3**, the circular supercoiled DNA disappeared at a concentration of 200 μM and a small fraction of linear DNA (form III) appeared, which is indicative of the strong DNA binding activities of complexes **1** and **3**. It was noticed that the linear DNA was not observed in the case of complex **2** under the same conditions. As for complexes **4**−**6**, only a high concentration of complex **6** caused the disappearance of the circular supercoiled DNA. The DNA binding abilities of the tested platinum complexes follows the order **1**, **3** > **2**, **6**, cisplatin > **4**, **5**, as indicated by the disappearance of the circular supercoiled DNA at a concentration of 200 μM. Evidently, the ability of complexes **4**−**6** with bidentate carboxylate to interact with DNA was lower than that of complexes **1**−**3** with two monodentate carboxylates. This is probably due to the fact that the bidentate dicarboxylate anion, which forms a six-membered coordination ring with the metal atom, is more stable and harder to dissociate from the parent compound, while the corresponding monodentate anion, which binds the metal atom only with a single coordination bond, appears to be much easier to dissociate from the parent compounds. In general, it could be concluded that complexes **1**−**3** and **6** showed cleavage ability comparable to that of cisplatin, while the binding ability of complexes **4** and **5** was a little weak. Notably, the behaviors of plasmid DNA binding with our complexes and cisplatin are similar, which is in accordance with our expectation that the newly synthesized agents could bind DNA in the same manner as cisplatin.

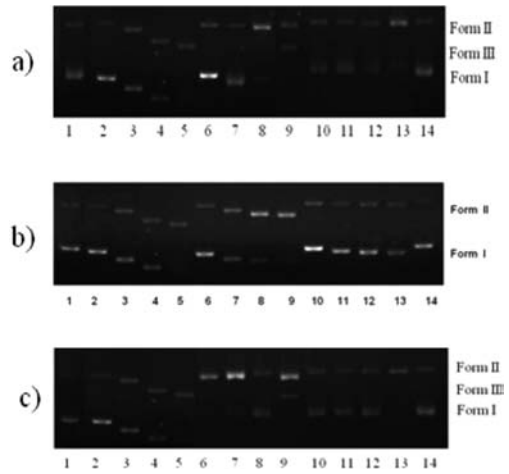

Figure 3. Gel electrophoretic mobility pattern of pET22b plasmid DNA when incubated with various concentrations of platinum complexes. (a) Lanes 1 and 14: control DNA. Lanes 2−5: cisplatin (10, 50, 100, and 200 μM) + DNA. Lanes 6−9: complex **1** (10, 50, 100, and 200 μM) + DNA. Lanes 10−13: complex **4** (10, 50, 100, and 200 μM) + DNA. (b) Lanes 1 and 14: control DNA. Lanes 2−5: cisplatin (10, 50, 100, and 200 μM) + DNA; Lanes 6−9: complex **2** (10, 50, 100, and 200 μM) + DNA. Lanes 10−13: complex **5** (10, 50, 100, and 200 μM) + DNA. (c) Lanes 1 and 14: control DNA. Lanes 2−5: cisplatin (10, 50, 100, and 200 μM) + DNA. Lanes 6−9: complex **3** (10, 50, 100, and 200 μM) + DNA. Lanes 10−13: complex **6** (10, 50, 100, and 200 μM) + DNA.

In Vitro NO-Releasing Study. Considering that the principal oxidation product of NO is NO_2^- in aqueous solution, the Griess reaction[32] was applied to measure the quantity of NO released from complexes **1**−**6**. It has been reported that thiols can induce the release of NO from furoxan and its derivatives in vitro.[17] Therefore, complexes **1**−**6** were measured in the presence and absence of L-cysteine to assess their ability to produce NO under different conditions. The release of NO from complexes **1**−**6** was very fast at the beginning and then gradually tended to remain unchanged at last in the presence of L-cysteine (Figure 4). The NO-releasing abilities of complexes generally followed the order **6** > **4**, **3**, **5**, **2** > **1**. The final percentage of NO released from complexes **1**−**6** varied from 27.28% to 44.50%, which was different from some organic furoxan derivatives that varied widely (0.2−34.3%).[33] In contrast, no NO liberated from complexes **1**−**6** was observed in the absence of L-cysteine, suggesting that furoxan moieties are stable under the test conditions, which is in accordance with the UV spectral results. We know that thiol-containing molecules, such as GSH and L-cysteine,[34,35] could form stable bonds with platinum atoms and dramatically decrease the percentage of platinum-based drugs binding to DNA (only 5−10% of cisplatin binds to nuclear DNA),[36,37] which may lead to severe side effects and drug resistance.[38,39] Nevertheless, once the newly synthesized compounds were exposed to the thiol-containing molecules, some of the thiol-containing nucleophiles would react with the furoxan moieties

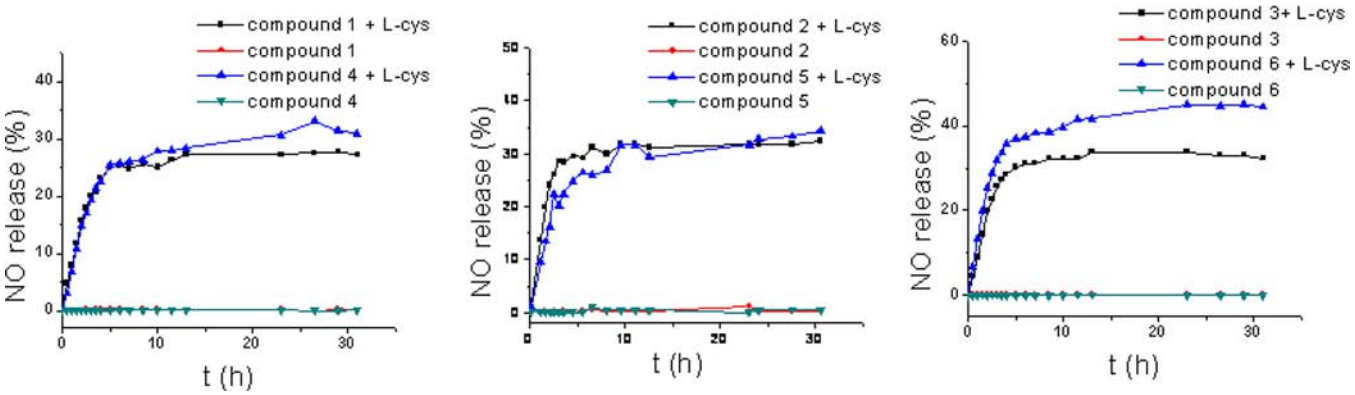

Figure 4. Percentage of NO release from complexes **1**–**6** in the presence or absence of L-cysteine in vitro.

to release NO, which may partly decrease the binding with platinum-based moieties. Overall, our compounds exhibited good NO-releasing properties in the presence of thiols and were stable under normal conditions in vitro.

In Vitro Cytotoxicity Assay. The in vitro cytotoxicity of complexes **1**–**6** and positive agents was evaluated by means of CCK-8 assay against HCT-116 (human colorectal cancer cell line) and SGC-7901 (human gastric cancer cell line). Carboplatin and oxaliplatin, whose structures are similar to those of our synthesized complexes, were used as positive controls. According to the IC_{50} values (Table 1) for HCT-116, complexes **1**–**6** showed cytotoxicity comparable to that of oxaliplatin, which is the first platinum-based complex effective in the treatment of colorectal cancer. As for SGC-7901, the synthesized compounds showed cytotoxicity comparable to that of carboplatin and less cytotoxicity than oxaliplatin. Obviously, complexes **1**–**6** were more sensitive to the colon cancer cell line than the gastric cancer cell line. It has been reported that NO hybrids, which seemed safer, were from 1.7- to 1083-fold more potent than their corresponding parent compounds against HT-29 and HCT-15 colon cancer cell lines in vitro.[19,40] Besides, NO aspirin promoted the anticancer activity compared with their parent compounds against the HT-29 colon cancer cell line by stimulating phophorylation of MAP kinases P38 and JNK in vitro.[41] Moreover, NO-donating aspirin showed a strong inhibitory effect against intestinal carcinogenesis in Min (APCMin/+) mice in vivo.[42] On the basis of the previous reports,[40−42] it is rational to conjecture that furoxan species may more or less play a role in the cytotoxic efficacy against the HCT-116 colon cancer cell line; i.e., compound **4** bearing a NO-releasing moiety showed 2-fold higher cytotocixity than its parent drug (carboplatin), suggesting that the furoxan species probably made a contribution to the cytotoxic activity on HCT-116.

Table 1. In Vitro Cytotoxicity (IC_{50}, μM) of Complexes 1−6, Carboplatin, and Oxaliplatin against Human Cancer Cell Lines[a]

	IC_{50} (μM)	
compound	HCT-116[b]	SGC-7901[c]
carboplatin	273.05 ± 12.3	58.11 ± 4.31
oxaliplatin	57.04 ± 5.61	17.35 ± 1.23
complex **1**	64.06 ± 7.81	217.93 ± 10.13
complex **2**	57.21 ± 4.32	94.23 ± 5.14
complex **3**	39.43 ± 2.34	34.64 ± 2.13
complex **4**	111.91 ± 7.89	248.07 ± 21.14
complex **5**	142.15 ± 12.12	59.10 ± 4.59
complex **6**	87.06 ± 8.07	70.83 ± 2.91

[a]Each value is the mean of three independent experiments. [b]Human colorectal cancer cell. [c]Human gastric cancer cell.

According to the IC_{50} values, the relative orders of the cytotoxicity of the newly synthesized compounds against HCT-116 and SGC-7901 cell lines are **3** > **2** > **1** > **6** > **4** > **5** and **3** > **5** > **6** > **2** > **1** > **4**, respectively. It is notable that complexes **1**–**3** with two monodentate carboxylates showed cytotoxicity superior to that of their corresponding compounds with bidentate dicarboxylates, which are in line with the gel electrophoresis results, demonstrating that the interaction of platinum-based moieties with DNA played the leading role in the anticancer effect. In a comparison of the same leaving ligands, the anticancer activities of different carrier ligands followed the order (4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane > (1*R*,2*R*)-1,2-diaminocyclohexane > ammine. Complex **3** is the most effective agent among the synthesized complexes, which has cytotoxicity similar to that of oxaliplatin against both HCT-116 and SGC-7901 cell lines. In general, complexes **1**–**6** showed considerable anticancer activity against the HCT-116 cell line, and most of them exhibited cytotoxic efficacy better than that of carboplatin and close to that of oxaliplatin.

■ CONCLUSION

Furoxan moieties were used for the first time as NO donors in the form of carboxylate to combine with platinum-based moieties of the present platinum drugs to prepare six novel platinum-based compounds. As expected, the DNA binding properties of the platinum-based compounds, analogous to those of cisplatin, were proven by agarose gel electrophoresis. In consideration of the complexity of the cellular system, the synergic action of NO-donor and platinum-based moieties was not studied in this paper. However, the newly synthesized compounds exhibited good stability and NO-releasing properties and showed considerable anticancer activity against HCT-116 and SGC-7901 cells in vitro. Besides, the interaction of complexes with DNA, which formed platinum/DNA adducts, played the leading role in the anticancer effects of complexes **1**–**6**, while the furoxan species were important for the cytotoxic activity on the HCT-116 colon cancer cell line. Thus, these compounds are promising anticancer agents and deliver a new view for the design of anticancer drugs beyond those currently in use.

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EXPERIMENTAL SECTION

Materials and Instrumentation. All reagents and solvents were of analytical purity and were used without any further purification. Potassium tetrachloroplatinate(II) was purchased from a local chemical company. ^{1}H NMR spectra were produced on a Bruker 500 MHz spectrometer. ESI-MS spectra were measured on a Bruker instrument. IR spectra were obtained on a Nicolet IR 200 spectrophotometer. All UV absorption spectra were recorded on a Shimadzu UV-1700 UV−vis spectrophotometer.

Synthesis and Characterization. *Synthesis of Ethyl Glycolate.* Glycolic acid (7.6 g, 0.1 mol) and concentrated sulfuric acid (5 mL) were added at room temperature to a mixture solution of absolute ethyl alcohol (17.4 mL, 0.3 mol) and benzene (50 mL) in a 250 mL flask equipped with an oil−water separator. The mixture was heated under reflux for 12 h and then evaporated under vacuum to remove the rest of ethanol and benzene; 50 mL of water was added and extracted with ethyl acetate (3 × 30 mL). The extract was washed with water (3 × 30 mL) and dried with anhydrous sodium sulfate. The solvent was evaporated off under vacuum. Ethyl glycolate was obtained, which was used without further purification for the synthesis of **a**. Yield: 7.8 g (75%). IR (KBr, cm^{-1}): 3398 (m), 2984 (m), 1741 (s), 1437 (w), 1223 (s), 1098 (s), 1021 (w).

Synthesis of L1. Ethyl glycolate (0.62 g, 6 mmol) and 3,4-bis(phenylsulfonyl)furoxan[43] (0.73 g, 2 mmol) were dissolved in 40 mL of tetrahydrofuran (THF) at room temperature. 25% NaOH (0.72 mL, 6 mmol) was added dropwise at 25 °C within 5 min to a stirred solution. The mixture was stirred for 2 h at 25 °C, and 40 mL of water was added. THF was removed under vacuum at 30 °C, and white deposits (**a**) were obtained by filtration. The intermediate **a** was dissolved in a mixture solution of 25 mL of water and 25 mL of THF. A pH of 10 was maintained by the addition of a 1 M NaOH aqueous solution, and the reaction was terminated when the pH was stable. The white solid was filtered off, and the pH of the filtrate was adjusted to 4 with a 1 M HCl solution. THF was removed under vacuum at 30 °C, affording white precipitate of L1. Yield: 0.37 g (62%), white solid. Elem anal. Calcd for $C_{10}H_8N_2O_7S$: C, 40.00; H, 2.69; N, 9.33; S, 10.68. Found: C, 39.68; H, 2.78; N, 9.22; S, 10.89. ^{1}H NMR (DMSO-d_6): δ 13.51 (m, 1H, OH), 8.05−8.03 (m, 2H, Ph), 7.92−7.89 (m, 1H, Ph), 7.77−7.74 (m, 2H, Ph), 5.07 (s, 2H, CH_2). ESI-MS:m/z 299 (100%; $[M - H]^-$).

Synthesis of L2. Diethyl 3-hydroxycyclobutane-1,1-dicarboxylate[44] (1.30 g, 6 mmol) and 3,4-bis(phenylsulfonyl)furoxan (0.73 g, 2 mmol) were dissolved in 40 mL of THF at room temperature. 25% NaOH (0.72 mL, 6 mmol) was added dropwise at 25 °C within 5 min to a stirred solution. The mixture was stirred for 2 h at 25 °C, and 40 mL of water was added. The solvent was removed under vacuum at 30 °C, and white deposits (**b**) were obtained by filtration. The intermediate **b** was suspended in 100 mL of water, a small amount of tetra-*n*-butylammonium bromide was added as a phase-transfer catalyst, and then 5 mL of concentrated hydrochloric acid was added to the solution. The mixture was heated under reflux for 12 h and extracted with ethyl acetate (3 × 50 mL). The extract was washed with water (3 × 30 mL) and separated by column chromatography (PE:EtOAc = 2:1). Yield: 0.25 g (32%), white solid. Elem anal. Calcd for $C_{14}H_{12}N_2O_9S$: C, 43.75; H, 3.15; N, 7.29; S, 8.34. Found: C, 43.43; H, 3.23; N, 7.46; S, 8.71. IR (KBr, cm^{-1}): 2923 (m), 1712 (s), 1618 (s), 1552 (m), 1450 (m), 1416 (m), 1357 (w), 1292 (w), 1163 (s), 999 (w), 736 (w), 599 (s), 552 (m). ^{1}H NMR (DMSO-d_6): δ 13.12 (m, 2H, OH), 8.04−8.02 (m, 2H, Ph), 7.91−7.88 (m, 1H, Ph), 7.76−7.73 (m, 2H, Ph), 5.14−5.11 (m, 1H, CH of cyclobutyl), 2.94−2.88 (m, 2H, CH_2 of cyclobutyl), 2.66−2.62 (m, 2H, CH_2 of cyclobutyl). ESI-MS: m/z 407 (100%; $[M + Na]^+$).

Standard Procedure for the Preparation of Complexes 1−6. A mixture of *cis*-[Pt(Am)I_2] or *cis*-[Pt(Am)Cl_2] (1 mmol) [Am = 2-ammine, (1*R*,2*R*)-1,2-diaminocyclohexane, and (4*R*,5*R*)-4,5-bis-(aminomethyl)-2-isopropyl-1,3-dioxolane] and silver nitrate (2 mmol) in distilled water was stirred in the dark for 24 h at 40 °C. The reaction mixture was filtered under vacuum, and then L1 (2 mmol) or L2 (1 mmol) mixed with NaOH (2 mmol) in 20 mL of water and 30 mL of THF was added to the filtrate and stirred for 24 h at room temperature. After that, the mixture was cooled to room temperature and concentrated to 5 mL, affording the precipitate. The precipitate was filtered and washed with cold distilled water.

Complex 1. *cis*-[Pt$(NH_3)_2I_2$] was used as the starting material. Yield: 0.50 g (60.9%), pale-yellow solid. Elem anal. Calcd for $C_{20}H_{20}N_6O_{14}S_2Pt$: C, 29.02; H, 2.44; N, 10.15; S, 7.75. Found C, 28.88; H, 2.29; N, 10.40; S, 7.89. IR (KBr, cm^{-1}): 3444 (m), 1708 (w), 1623 (s), 1458 (w), 1350 (w), 1164 (m), 834 (w), 730 (w), 687 (w), 597 (s), 553 (w). ^{1}H NMR (DMSO-d_6 + D_2O): δ 8.08−8.05 (m, 2H, Ph), 7.97−7.88 (m, 1H, Ph), 7.83−7.74 (m, 2H, Ph), 4.99 (s, 1H, CH_2), 4.94 (s, 1H, CH_2), 4.58 (s, 2H, CH_2). ESI-MS: m/z 528 (100%; $[M - L1]^+$).

Complex 2. *cis*-{Pt[(1*R*,2*R*)-1,2-diaminocyclohexane]Cl_2} was used as the starting material.[45] Yield: 0.61 g (67.1%), white crystalline solid. Elem anal. Calcd for $C_{26}H_{28}N_6O_{14}S_2Pt$: C, 34.40; H, 3.11; N, 9.26; S, 7.06. Found: C, 34.12; H, 3.34; N, 9.45; S, 6.95. IR (KBr, cm^{-1}): 3443 (w), 3216 (w), 3101 (w), 2941 (w), 1616 (s), 1550 (s), 1453 (m), 1295 (w), 1164 (m), 1021 (w), 730 (w), 597 (s), 555 (w). ^{1}H NMR (DMSO-d_6): δ 8.07−8.02 (m, 2H, Ph), 7.95−7.85 (m, 1H, Ph), 7.80−7.70 (m, 2H, Ph), 6.28−5.81 (m, 4H, NH_2), 4.93 (s, 2H, CH_2), 4.51 (s, 2H, CH_2), 2.49−1.01 (m, 10H, CH_2 of DACH). ESI-MS: m/z 930 (100%; $[M + Na]^+$).

Complex 3. *cis*-{Pt[(4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane] I_2} was used as starting material.[46] Yield: 0.65 g (66.9%). Pale yellow solid. Elemental analysis calculated for $C_{28}H_{32}N_6O_{16}S_2Pt$ (%): C, 34.75; H, 3.33; N, 8.68; S, 6.63. Found C, 34.49; H, 3.56; N, 8.95; S, 6.34. [1] IR (KBr)/cm^{-1}: 3442 (m), 1710 (m), 1624 (s), 1551 (s), 1459 (m), 1354 (w), 1252 (w), 1163 (s), 1086 (w), 731 (m), 599 (s); H NMR (d_6-DMSO+D_2O): δ 8.06−8.02 (m, 2H, Ph), 7.95−7.91 (m, 1H, Ph), 7.78−7.73 (m, 2H, Ph), 4.94 (s, 2H, CH_2), 4.84−4.83 (d, J(H,H)=4.5 Hz, 1H, CH), 4.43 (s, 2H, CH_2), 4.25 (s, 1H, CH), 4.22 (s, 1H, CH), 3.15 (m, 1H, CH_2NH_2), 2.99 (d, 1H, CH_2NH_2), 2.65 (m, 2H, CH_2NH_2), 1.86−1.79 (m, 1H, $CHMe_2$), 0.92 ppm (d, J(H,H) =7 Hz, 6H, $C(CH_3)_2$); ESI-MS: m/z $[M+Na]^+$ = 990 (100%).

Complex 4. *cis*-[Pt$(NH_3)_2I_2$] was used as the starting material. Yield: 0.36 g (58.9%), pale-yellow solid. Elem anal. Calcd for $C_{14}H_{16}N_4O_9SPt$: C, 27.50; H, 2.64; N, 9.16; S, 5.24. Found: C, 27.89; H, 2.76; N, 8.89; S, 5.10. IR (KBr, cm^{-1}): 3445 (w), 1615 (s), 1542 (s), 1451 (w), 1359 (s), 1167 (m), 742 (w), 684 (w), 599 (s), 556 (w). ^{1}H NMR (DMSO-d_6 + D_2O): δ 8.00−7.99 (d, J(H,H) = 7.5 Hz, 2H, Ph), 7.89−7.86 (t, J(H,H) = 7.5 Hz, 1H, Ph), 7.75−7.72 (t, J(H,H) = 7.5 Hz, 2H, Ph), 4.93−4.90 (t, J(H,H) = 7.0 Hz, 1H, CH of cyclobutyl), 3.38−3.34 (m, 2H, CH_2 of cyclobutyl), 2.79−2.71 (m, 2H, CH_2 of cyclobutyl). ESI-MS: m/z 612 (10%; $[M + H]^+$), 634 (100%; $[M + Na]^+$).

Complex 5. *cis*-{Pt[(1*R*,2*R*)-1,2-diaminocyclohexane]Cl_2} was used as the starting material. Yield: 0.44 g (64.2%), white crystalline solid. Elem anal. Calcd for $C_{20}H_{24}N_4O_9SPt$: C, 34.73; H, 3.50; N, 8.10; S, 4.64. Found: C, 34.56; H, 3.37; N, 8.41; S, 4.54. IR (KBr, cm^{-1}): 3448 (m), 3200 (w), 3095 (w), 2946 (w), 1620 (s), 1544 (m), 1450 (w), 1358 (m), 1168 (m), 900 (w), 732 (w), 596 (s), 554 (w). ^{1}H NMR (DMSO-d_6): δ 8.04−8.01 (m, 2H, Ph), 7.93−7.88 (m, 1H, Ph), 7.78−7.73 (m, 2H, Ph), 5.92 (m, 2H, NH_2), 5.23−5.20 (m, 2H, NH_2), 4.95−4.89 (m, 1H, CH of cyclobutyl), 3.40−3.38 (m, 2H, CH_2 of cyclobutyl), 2.83−2.73 (m, 2H, CH_2 of cyclobutyl), 2.04−0.85 (m, 10H, CH_2 of DACH). ESI-MS: m/z 692 (30%; $[M + H]^+$), 714 (100%; $[M + Na]^+$).

Complex 6. *cis*-{Pt[(4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane]I_2} was used as the starting material. Yield: 0.49 g (65.9%), yellow solid. Elem anal. Calcd for $C_{22}H_{28}N_4O_{11}SPt$: C, 35.16; H, 3.75; N, 7.45; S, 4.27. Found: C, 34.87; H, 3.89; N, 7.68; S, 4.04. [1] IR (KBr, cm^{-1}): 3443 (m), 2971 (w), 1721 (w), 1619 (s), 1545 (m), 1451 (w), 1358 (m), 1165 (m), 1091 (w), 734 (w), 597 (s), 556 (w). ^{1}H NMR (DMSO-d_6 + D_2O): δ 8.03−8.02 (d, J(H,H) = 7.5 Hz, 2H, Ph), 7.92−7.89 (t, J(H,H) = 7.5 Hz, 1H, Ph), 7.77−7.74 (t, J(H,H) = 7.5 Hz, 2H, Ph), 4.94−4.90 (m, 1H, CH of cyclobutyl), 4.85 (m, 1H, CH), 4.39 (s, 1H, CH), 4.26 (s, 1H, CH), 3.22−3.19 (m, 2H, CH_2 of cyclobutyl), 3.12 (m, 1H, CH_2NH_2), 3.00 (d, 1H, CH_2NH_2), 2.80 (m, 2H, CH_2 of cyclobutyl), 2.67 (m, 2H, CH_2NH_2), 2.19−2.17 (m, 1H, $CHMe_2$),

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0.87−0.86 (d, 6H, $C(CH_3)_2$). ESI-MS: m/z 752 (30%, $[M + H]^+$), 774 (100%; $[M + Na]^+$).

Stability Studies. The absorption spectra of complexes **1**−**6** in the UV−vis region were recorded on a Shimadzu UV-1700 UV−vis spectrophotometer. The electronic spectra of complexes **1**−**6** dissolved in 5% CH_3CH_2OH/95% H_2O were obtained at different times over 12 h at room temperature.

Interaction with Plasmid DNA. The pET22b plasmid DNA was used as the target to treat with complexes **1**−**6** and cisplatin to investigate the cleavage of DNA by agarose gel electrophoresis. The complexes were dissolved and diluted to different desired concentrations with distilled water, then 5 μL of pET22b plasmid DNA (0.5 μg/mL) was added to the solution, and the resulting solution was incubated in a water bath at 37 °C in the dark for 24 h for DNA unwinding. After that, the mixtures with a loading buffer (1 μL) were loaded onto the 1.0% agarose gel stained with ethidium bromide, and electrophoresis was carried out under a TA buffer (50 mM Tris-acetate, pH 7.5) for 60 min at 100 V. The gels were photographed by a Molecular Imager (Bio-Rad, USA) under UV light.

NO Release in Vitro. The standard curve of nitrite concentration against absorbance was measured as described before.[47] Complexes **1**−**6** were diluted to 12.5 μM with either (i) a phosphate-buffered solution (PBS) at pH 7.4 or (ii) PBS containing 5 mM L-cysteine at pH 7.4, and then Griess reagent was added to the solution to react with the nitrite at different times. The data were acquired by measuring the absorbance at 540 nm with a UV spectrophotometer.

Cytotoxicity Studies. The cytotoxicity of all of the complexes against HCT-116 and SGC-7901 cells was determined by means of the colorimetric assay CCK-8 (Beyotime, Beijing, China). The cells were plated at 5000 cells per well in 96-well culture plates with a culture medium and incubated for 24 h at 37 °C in a water atmosphere (5% CO_2). The compounds with the desired concentrations were obtained by dissolution in DMSO and dilution with a culture medium (DMSO final concentration < 0.4%). Then the diluted solution of complexes was treated with the cells for 48 h at 37 °C in a 5% CO_2 incubator. After that, 10 μL of a freshly diluted CCK-8 solution (5 mg/mL in PBS) was added to each well and the plate was incubated for 4 h. The cell survival was evaluated by measuring the absorbance at 540 nm with an automatic microplate ELISA reader. IC_{50} values were calculated from the chart of the cell viability (%) against the compound concentration (μM). All experiments were carried out in triplicate.

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Notes

The authors declare no competing financial interest.

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