Synthesis and in Vitro Antitumor Activity of Oligonucleotide-Tethered and Related Platinum Complexes

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Three classes of hydroxy-tethered platinum(II) complexes have been synthesized from K_2PtCl_4 and appropriate amino alcohols. A sequence of selective oxidation and hydrolysis has been developed to prepare hydroxy-tethered platinum(IV) complexes. A novel procedure for the synthesis of amminetrichloroplatinate(II) anion has been generated and used to synthesize a number of monohydroxy-tethered nonchelating platinum complexes. These tethered platinum complexes, including hydroxy-tethered, phosphoramidite-tethered, and monodeoxyribonucleotide-tethered platinum(II) and -(IV) complexes, have been examined in vitro for antitumor activity in both leukemia and ovarian cancer cell lines. Activity of some of these complexes was similar to *cis*-platin, and most of them showed much better potency than carboplatin. We observed an interesting structure−activity correlation for platinum(II) complexes for both PA-1 and SK-OV-3 ovarian cancer cell lines. However, platinum(IV) complexes showed much more diversified response among cancer cell lines studied. We observed enhanced selectivity among different cancer cell lines for some agents. The most promising is the monodeoxyribonucleotide-tethered platinum(IV) complex, which is the first analogue of the conjugates between a platinum fragment and monodeoxyribonucleotides, showing antitumor activity and selectivity among the cell lines. Finally, the p53 status of the cells appears to contribute to the effectiveness of these agents in that cells harboring wild-type p53 appear to be more sensitive to these agents.

Introduction

A current approach to the design of novel platinum (Pt) drugs is to target the Pt coordination moiety to DNA by attaching it to a suitable carrier ligand. A variety of ligands have been attached to Pt, including DNA intercalators, doxorubicin, estrogen analogues, amino acids, sugars, and antitrypanosomal drugs.[1] Studies of Pt compounds with biologically active carrier groups have yielded interesting results, such as improved activity in cisplatin resistant cell lines[2] and interesting structure−activity relationships (SAR).[3] Despite these different structure−activity relationships, still lacking are platinum compounds suitable as synthons in the synthesis of the conjugates. Although conjugates between platinum and oligodeoxynucleotides have been reported before,[4] there have been no prior reports of a physiologically active platinum center in the conjugates as we will report in the present study. Here we synthesized a variety of tethered platinum complexes and examined them for biological activity in vitro. These include hydroxy-tethered, phosphoramidite-tethered, and monodeoxyribonucleotide-tethered platinum complexes, and we examined their actions in leukemia and ovarian cancer cell lines.

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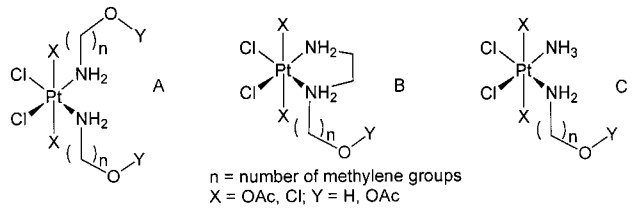
Scheme 1. Three General Classes of Platinum Compounds



Materials and Methods

The sketch of the compounds reported in this study is illustrated in Scheme 1. We will refer to these new agents as complexes A−C, each of which varies in the amine ligand.

Chemicals and Supplies for Synthesis. Potassium tetrachloroplatinate(II) (K_2PtCl_4) and potassium hexachloroplatinate(IV) (K_2PtCl_6) were purchased from Sausville Chemical Company (Garfield, NJ); phosphoric acid (H_3PO_4, 85%), hydrogen peroxide (30%), acetic acid HOAc), dimethylformamide (DMF), acetonitrile, methylene chloride, and ammonium chloride from Fisher Scientific (Pittsburgh, PA); acetic anhydride from Mallinckrodt Chemical (Paris, KY); hydrochloric acid (HCl) from EM Science (Gibbstown, NJ); deuterated dimethyl sulfoxide, water, methanol, and acetonitrile from Cambridge Isotope Company (Cambridge, MA); *N*,*N*-dimethylacetamide, 2-hydroxyethylamine, 3-hydroxypropylamine, 4-hydroxybutylamine, and tetraethylammonium chloride from Aldrich (Milwaukee, WI); and crystal violet and MTT from Sigma (St. Louis, MO). Compounds **8**−**10** and **20**−**22** were synthesized according to published procedures.[5] Elemental analyses were performed by Midwest Microlab (Indianapolis, IN).

NMR Measurements. All platinum(II) complexes undergo solvolysis at various rates in DMSO solutions. A minimum time exposure is required when conducting NMR studies in this solvent. Because of solubility limitations of some platinum(II) complexes, use of this solvent is sometimes unavoidable.

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The ^1H and $^{13}C\{^1H\}$ NMR spectra were obtained on either a Bruker Advance DRX/DPX 400 or 500 NMR spectrometer, and the chemical shifts were referenced to the residual solvent peaks. Standard parameters were used. The ^{195}Pt NMR spectra were obtained on a Varian 300 spectrometer, and the chemical shifts were referenced to *cis*-DDP in DMSO-d_6 at δ −2124 ppm, which was calibrated by using K_2PtCl_4 in water (δ −1623 ppm relative to Na_2PtCl_6) as an external standard. Typical parameters were the following: frequency at 64.324 MHz for platinum(II) and 64.494 MHz for platinum(IV) complexes, spectral width of 100 000 Hz, acqusition time of 6 ms, relaxation delay of 0 s, pulse width of 14 μs, temperature of 23 °C, decoupler off, line broadening of 150 Hz, and FT size of 1024.

Syntheses. Dichlorodi(3-hydroxypropylamine)platinum(II), 2. K_2PtCl_4 (0.500 g, 1.20 mmol) was dissolved in 4 mL of H_2O. 3-Hydroxypropylamine (221 mg, 2.2 eq) was added in one portion. The solution was stirred at room temperature (RT) for 4 h in the dark. The solvent was removed, and the solid was washed with acetic acid to get a yellow oily solid. The solid was extracted by CH_3OH to get rid of KCl. The CH_3OH solution was passed through a column of Dowex-X8 H^+ form 20−50 mesh cation-exchange resin by using CH_3OH as eluate. Removal of the solvent yielded a yellow powder of 261 mg; yield 52%. ^1H NMR in CD_3OD: δ 4.780 (br s, 4H, NH_2), 3.721 (t, $^3J_{HH}$ = 6.0 Hz, 2H, CH_2O), 2.931 (pent, $^3J_{HH}$ = 6.7 Hz, 2H, CH_2N), 1.958 (pent, $^3J_{HH}$ = 6.5 Hz, 2H, CH_2-CH_2O). $^{13}C\{^1H\}$ NMR in CD_3OD: δ 61.2 (CH_2-O), 46.6 (CH_2-N), 34.3 (CH_2-CH_2O). ^{195}Pt NMR in CD_3OD: δ −2255. Elemental analysis, found: C, 17.31; H, 4.27; N, 6.60. Calcd for $C_6H_{18}N_2OCl_2Pt$: C, 17.31; H, 4.36; N, 6.73.

Dichlorodi(4-hydroxybutylamine)platinum(II), 3. K_2PtCl_4 (0.500 g, 1.20 mmol) was dissolved in 4 mL of H_2O. 4-Hydroxybutylamine (236 mg, 2.2 equiv) was added in one portion. The solution was stirred at RT for 4 h in the dark. The solvent was removed, and the solid was extracted by CH_3OH to get rid of KCl, yielding a yellow waxy solid, which was washed with acetic acid and diethyl ether to obtain a yellow powder of 310 mg; yield 58%. ^1H NMR in CD_3OD: δ 4.780 (brs, 4H, NH_2), 3.606 (t, $^3J_{HH}$ = 6.3 Hz, 2H, CH_2O), 2.806 (pent, $^3J_{HH}$ = 6.5 Hz, 2H, CH_2N), 1.780 (pent, $^3J_{HH}$ = 7.0 Hz, 2H, CH_2-CH_2O), 1.609 (pent, $^3J_{HH}$ = 6.6 Hz, 2H, CH_2-CH_2N). $^{13}C\{^1H\}$ NMR in CD_3OD: δ 62.6 (CH_2-O), 48.4 (CH_2-N), 30.8 (CH_2-CH_2O), 28.9 (CH_2-CH_2N). ^{195}Pt in CD_3OD, δ: −2224. Elemental analysis, found: C, 21.69; H, 4.85; N, 5.98. Calcd for $C_8H_{22}N_2OCl_2Pt$: C, 21.63; H, 4.99; N, 6.30.

Tetrachlorodi(3-hydroxypropylamine)platinum(IV), 6. Complex **2** (260 mg, 0.625 mmol) was suspended in 3.0 mL of acetic acid. Acetic anhydride (500 mg, 4.90 mmol) and 30% H_2O_2 (100 mg, 1.5 equiv) were added sequentially. After being stirred for about 30 min at RT, the mixture changed to a clear-yellow solution. CH_3OH (0.5 mL) was added to quench the reaction. Upon removal of the solvent, the solid was dissolved in 3 mL of 15% HCl solution. After the mixture was stirred at RT for 6 h, the solvent was removed, and the solid was washed with diethyl ether to get a yellow powder of 185 mg; yield 61%. ^1H NMR in DMSO: δ 6.423 (br s, 4H, NH_2), 3.394 (m, 2H, CH_2O), 2.874 (m, 2H, CH_2N), 1.838 (m, 2H, CH_2-CH_2O). $^{13}C\{^1H\}$ NMR in DMSO-d_6: δ 60.3 (CH_2-O), 45.6 (CH_2-N), 32.4 (CH_2-CH_2O). ^{195}Pt NMR in DMSO: δ −146. Elemental analysis, found: C, 15.77; H, 3.80; N, 5.45; Pt, 39.2. Calcd for $C_6H_{18}N_2OCl_4Pt$: C, 14.79; H, 3.72; N, 5.75; Pt, 40.05.

Tetrachlorodi(4-hydroxybutylamine)platinum(IV), 7. The procedure was the same as that used for **6**; yield 69%. ^1H NMR in DMSO-d_6: δ 6.490 (brs, 4H, NH_2), 3.380 (m, 2H, CH_2O), 2.740 (m, 2H, CH_2N), 1.711 (m, 2H, CH_2-CH_2O), 1.419 (m, 2H, CH_2-CH_2N). $^{13}C\{^1H\}$ NMR in DMSO-d_6: δ 61.2 (CH_2-O), 47.1 (CH_2-N), 30.9 (CH_2-CH_2O), 26.5 (CH_2-CH_2N). ^{195}Pt in DMSO δ: −152. Elemental analysis, found: C, 18.41; H, 4.28; N, 5.26. Calcd for $C_8H_{22}N_2OCl_4Pt$: C, 18.65; H, 4.30; N, 5.44.

Potassium Amminetrichloroplatinate(II) [$KPtCl_3(NH_3)$], 11. *Cis*-DDP (4.0 g, 13.3 mmol), tetraethylammonium chloride (Et_4NCl, 2.82 g, 16.0 mmol), and ammonium chloride (NH_4Cl, 0.20 g, 3.7 mmol) were dissolved in *N*,*N*-dimethylacetamide (150 mL). The reaction mixture was heated at 100 °C, purging with a slow stream of N_2 for 8−10 h. The color of the solution changed from yellow to orange during this period. No black precipitate was observed. After the reaction, the solvent was partially removed down to 50 mL. Hexane/ethyl acetate (1:1, 300 mL) was then added, and the mixture was kept overnight at −20 °C. The supernatant was then decanted, and the solid was extracted with 60 mL of CH_3CN. The remaining solid (probably the mixture of NH_4Cl and *cis*-DDP) was washed with water to recover unreacted *cis*-DDP, 1.3 g. Water (5 mL) was added to the CH_3CN solution, and CH_3CN was removed by a mechanical pump, as judged by the volume of the solution. Dowex 50 W-X8 H^+ exchange resin (50 mL) was added, and the mixture was kept stirring for 1 h at RT. The resin was removed by filtration, and the solution was concentrated to 2 mL. Saturated KCl solution (5 mL) was added with stirring, and the mixture was kept at 0 °C for 2 h. The precipitate was collected and dried by air to get 2.8 g of $K[PtCl_3(NH_3)]\cdot(^1/_2)H_2O$. The yield was 58% based on *cis*-DDP added and 87% based on the *cis*-DDP consumed. The recovered *cis*-DDP is reusable. ^{195}Pt NMR in H_2O: δ −1888. Elemental analysis, found: Cl, 28.90; H, 1.55; N, 4.82. Calcd for $K[PtCl_3(NH_3)]\cdot(^1/_2)H_2O$: Cl, 29.01; H, 1.10; N, 3.82.

Amminechloroiodo(2-hydroxyethylamine)platinum(II), 12. Complex **11** (300 mg, 0.818 mmol) was dissolved in 2.0 mL of H_2O. NaI (240 mg, 1.9 eq) and 2-hydroxyethylamine (1.1 equiv) were added sequentially. The color of the solution changed from orange to red. Yellow precipitate began to form after about 5 min. The mixture was kept at 0 °C for 1 h. The solid was filtered and washed with precooled water and a small amount of diethyl ether to obtain 210 mg of **13**; yield 59%. ^1H NMR in CD_3OD: δ 3.888 (t, $^3J_{HH}$ = 5.0 Hz, 2H, CH_2O), 2.882 (pent, $^3J_{HH}$ = 5.3 Hz, 2H, CH_2N). $^{13}C\{^1H\}$ NMR in DMSO-d_6: δ 60.7 (CH_2-O), 47.9 (CH_2-N). $^{13}C\{^1H\}$ NMR in CD_3OD: δ 62.3 (CH_2-O), 50.4 (CH_2-N). ^{195}Pt NMR in DMF: δ −2685. Elemental analysis, found: C, 5.54; H, 2.26; N, 6.21. Calcd for $C_2H_{10}N_2OClIPt$: C, 5.52; H, 2.31; N, 6.43.

Amminechloroiodo(4-hydroxybutylamine)platinum(II), 13. Complex **11** (300 mg, 0.818 mmol) was dissolved in 1.5 mL of H_2O. NaI (240 mg, 1.9 eq) was added, and the color of the solution changed from orange to red. 4-Hydroxybutylamine (1.1 equiv) was added. Yellow precipitate began to form immediately. The mixture was kept at 0 °C for 1 h. The solid was filtered and washed with precooled water and a small amount of acetone (0.5 mL) to obtain 270 mg of **13**; yield 71%. ^1H NMR in CD_3OD: δ 4.629 (brs, 3H, NH_3), 3.607 (t, $^3J_{HH}$ = 6.4 Hz, 2H, CH_2O), 2.810 (pent, $^3J_{HH}$ = 7.5 Hz, 2H, CH_2N), 1.802 (pent, $^3J_{HH}$ = 7.6 Hz, 2H, CH_2-CH_2O), 1.608 (pent, $^3J_{HH}$ = 7.5 Hz, 2H, CH_2-CH_2N). $^{13}C\{^1H\}$ NMR in DMSO-d_6: δ 61.2 (CH_2-O), 46.0 (CH_2-N), 30.2 (CH_2-CH_2O), 27.8 (CH_2-CH_2N). $^{13}C\{^1H\}$ NMR in CD_3OD: δ: 62.4 (CH_2-O), 46.9 (CH_2-N), 30.7 (CH_2-CH_2O), 28.9 (CH_2-CH_2N). ^{195}Pt NMR in CD_3OD: δ −2718. Elemental analysis, found: C, 10.12; H, 2.95; N, 5.85. Calcd for $C_4H_{14}N_2OClIPt$: C, 10.36; H, 3.04; N, 6.04.

Amminedichloro(2-hydroxyethylamine)platinum(II), 14. Complex **12** (160 mg, 0.367 mmol) was suspended in 2.0 mL of H_2O. $AgNO_3$ (1.6 equiv) was added, and a light-yellow precipitate formed immediately. The mixture was stirred at RT for 4 h in the dark or until the solution was found to be Ag^+-free. Activated carbon (0.1 g) was added, and the mixture was stirred for 30 min. Concentrated HCl (1.0 mL) was added, and the solution was stirred for 30 min. The volatile was removed, and the solid was washed with diethyl ether and dried to afford 81 mg of **14**; yield 64%. ^1H NMR in DMSO-d_6: δ 4.712 (brs, 3H, NH_3), 3.983 (brs, 2H, NH_2), 3.574 (t, $^3J_{HH}$ = 5.5 Hz, 2H, CH_2OH), 2.609 (pent, $^3J_{HH}$ = 6.1 Hz, 2H, CH_2N). ^1H NMR in CD_3OD: δ 3.812 (t, $^3J_{HH}$ = 5.2 Hz, 2H, CH_2O), 2.766 (pent, $^3J_{HH}$ = 5.5 Hz, 2H, CH_2N). $^{13}C\{^1H\}$ NMR in DMSO-d_6: δ 60.8 (CH_2-O), 49.2 (CH_2-N). ^{195}Pt NMR in DMSO: δ −2183. Elemental analysis, found: C, 7.11; H, 2.98; N, 7.90; Pt, 56.4. Calcd for $C_2H_{10}N_2OCl_2Pt$: C, 6.98; H, 2.93; N, 7.90; Pt, 56.4.

Amminedichloro(4-hydroxybutylamine)platinum(II), 15. The synthetic procedure is the same as that for **14**.

Briefly, complex **13** (160 mg, 0.345 mmol) was suspended in 2.0 mL of H_2O. $AgNO_3$ (1.6 equiv) was added, and the mixture was stirred at RT for 4 h in the dark or until the solution was found to be Ag^+-free. Activated carbon (0.1 g) was added, and the mixture was stirred for 0.5 h. Concentrated HCl (1.0 mL) was added, and the solution was stirred for 0.5 h. The volatile was removed, and the solid was washed with diethyl ether and dried to afford 101 mg of **15**. Yield 79%. ^{1}H NMR in DMSO-d_6: δ 4.711 (t, $^3J_{HH}$ = 5.0 Hz, 3H, NH_3), 3.975 (brs, 2H, NH_2), 3.385 (q, $^3J_{HH}$ = 5.3 Hz, 2H, CH_2OH), 2.513 (m, 2H, CH_2N), 1.566 (pent, $^3J_{HH}$ = 7.7 Hz, 2H, CH_2-CH_2O), 1.423 (pent, $^3J_{HH}$ = 7.1 Hz, 2H, CH_2-CH_2N). ^{1}H NMR in CD_3OD/acetone-d_6 (1:1): δ 4.612 (brs, 3H, NH_3), 3.503 (t, $^3J_{HH}$ = 6.3 Hz, 2H, CH_2O), 2.747 (pent, $^3J_{HH}$ = 6.9 Hz, 2H, CH_2N), 1.738 (pent, $^3J_{HH}$ = 7.4 Hz, 2H, CH_2-CH_2O), 1.515 (pent, $^3J_{HH}$ = 7.4 Hz, 2H, CH_2-CH_2N). ^{13}C{^{1}H} NMR in DMSO-d_6: δ 61.3 (CH_2-O), 47.3 (CH_2-N), 30.2 (CH_2-CH_2O), 27.7 (CH_2-CH_2N). ^{195}Pt NMR in H_2O: δ −2207. ^{195}Pt NMR in CD_3OD: δ −2189. Elemental analysis, found: C, 12.75; H, 3.72; N, 7.30. Calcd for $C_4H_{14}N_2OCl_2Pt$: C, 12.91; H, 3.79; N, 7.53.

Amminetetrachloro(2-hydroxyethylamine)platinum-(IV), 18. Compound **14** (85 mg, 0.247 mmol) was suspended in 1 mL of HOAc. Acetic anhydride (500 mg, 20 equiv) and H_2O_2 30% solution (121 mg, 4.5 equiv) were added sequentially. Stirring the mixture for 2 h at RT solubilized it. Methanol was added to quench the reaction, and the solvent was removed. The solid was dissolved in 3 mL of 15% HCl solution, and the solution was stirred at RT for 6 h. The solvent was removed, and the solid was washed with diethyl ether and dried, yielding 88 mg of the yellow product **18**; yield 86%. ^{1}H NMR in D_2O: δ 3.763 (t, $^3J_{HH}$ = 5.2 Hz, 2H, CH_2OH), 3.043 (pent, $^3J_{HH}$ = 5.2 Hz, 2H, CH_2N). ^{13}C{^{1}H} NMR in D_2O: δ 59.0 (CH_2-O), 47.7 (CH_2-N). ^{195}Pt NMR in D_2O: δ −334. Elemental analysis, found: C, 5.84; H, 2.43; N, 6.55; Pt, 47.00. Calcd for $C_2H_{10}N_2OCl_2Pt$: C, 5.79; H, 2.43; N, 6.75; Pt, 47.00.

Amminetetrachloro(4-hydroxybutylamine)platinum-(IV), 19. Compound **15** (170 mg, 0.457 mmol) was suspended in 5 mL of HOAc. Acetic anhydride (400 mg, 8.6 equiv) and H_2O_2 30% solution (80 mg, 4.5 equiv) were added sequentially. The mixture was stirred for 0.5 h at RT, solubilizing it. Methanol was added to quench the reaction, and the solvent was removed. The solid was dissolved in 3 mL of 15% HCl solution, and the solution was stirred at RT for 6 h. The solvent was removed, and the solid was washed with diethyl ether and dried to yield 141 mg of the yellow product **19**; yield 70%. ^{1}H NMR in D_2O: δ 3.617 (t, $^3J_{HH}$ = 6.3 Hz, 2H, CH_2OH), 2.929 (t, $^3J_{HH}$ = 7.4 Hz, 2H, CH_2N), 1.767 (pent, $^3J_{HH}$ = 7.6 Hz, 2H, CH_2-CH_2O), 1.616 (pent, $^3J_{HH}$ = 7.2 Hz, 2H, CH_2-CH_2N). ^{13}C-{^{1}H} NMR in D_2O: δ 60.9 (CH_2-O), 46.2 (CH_2-N), 28.6 (CH_2-CH_2O), 25.7 (CH_2-CH_2N). ^{195}Pt NMR in D_2O: δ −316. Elemental analysis, found: C, 10.80; H, 3.20; N, 6.18; Pt, 43.5. Calcd for $C_4H_{14}N_2OCl_4Pt$: C, 10.84; H, 3.18; N, 6.32; Pt, 44.03.

Cell Culture and Cytotoxicity for Leukemia Cancer Cell Lines. Teniposide (VM-26)-resistant leukemia cells, CEM/VM-1,[6] and the parental cell line, CEM, were cultured in minimum essential medium (BioWhittaker, Walkersville, MD) for suspension cells with 10% fetal bovine serum (FBS; Sigma Chemical Co., St. Louis, MO) and 2 mM L-glutamine (BioWhittaker). The cells were incubated at 37 °C with 5% CO_2. Exponentially growing CEM and CEM/VM-1 cells were seeded into 24-well plates in triplicate at a density of 3×10^5 cells/mL. All drug solutions were prepared in cell culture medium by first dissolving in DMSO or PBS, then using serial dilutions to twice the desired concentration, and adding to the plates at equal volume. The cells were incubated at 37 °C in an atmosphere of 5% CO_2 for 48 h. Cell growth inhibition was determined by counting on a Coulter Multisizer (Coulter Corporation, Miami, FL). The drug concentrations were plotted versus the percent of cell growth inhibition. The drug concentrations that inhibited cell growth by 50% were calculated by extrapolation and taken as the IC_{50} values.[7]

Colony Formation and Crystal Violet Assay for Ovarian Cancer Cell Lines. The ovarian cancer cell lines, PA-1 (wild-type [wt] p53), A2780 (wtp53), SW626 (mutant [m] p53), and SKOV-3 (p53-null) were obtained from American Type Culture Collection (ATCC) (Rockville, MD). These monolayer cells were cultured in RPMI 1640 (BioWhittaker) with 10% FBS (Sigma Chemical Co.) and 2 mM L-glutamine (BioWhittaker) and incubated at 37 °C in an atmosphere of 5% CO_2. Cell culture medium was aspirated from exponentially growing ovarian cells. The cells were washed with 1 × PBS, removed from the plate using a Trypsin−Versene mixture (BioWhittaker) and seeded in 6-well plates at a density of 300−500 cells/well. The cells were incubated 1 day in order to allow their proper attachment to the plates. Drug solutions were prepared in cell culture medium using serial dilutions and added to the cells in various concentrations as described above. All cells were incubated at 37 °C in an atmosphere of 5% CO_2. However, because of the varying growth rates of the ovarian lines, the PA-1 and A2780 cells were incubated with drug for 7 days and the SW626 and SKOV-3 cells were incubated with drug for 10 days. Following incubation, the cell culture medium was aspirated, the cells were washed with 1X PBS and fixed and stained using 0.1% crystal violet (Sigma) in methanol, and the colonies were counted using a colony counter (Artek Systems Corporation, Farmingdale, NY). The drug concentrations were plotted versus the percent inhibition of colony formation. Drug concentrations that inhibited colony formation by 50% were calculated by extrapolation and taken as IC_{50} values.

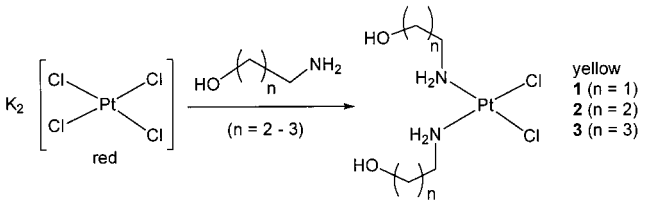
MTT Assay for Ovarian Cancer Cell Lines. The ovarian cancer cell lines, PA-1 (wild-type [wt] p53) and SKOV-3 (p53-null) were used for cytotoxic evaluation of some compounds. These monolayer cells were cultured in RPMI-1640 (BioWhittaker) with 10% FBS (Sigma Chemical Co.) and 2 mM L-glutamine (BioWhittaker) and incubated at 37 °C in an atmosphere of 5% CO_2. Cell culture medium was aspirated from exponentially growing ovarian cells. The cells were removed from the plate using a Trypsin−Versene mixture (BioWhittaker) and seeded in 96-well plates at a density of 1000 cells/well for PA-1 and 600 cells/well for SK-OV-3. The PA-1 cells were incubated for 1 day and SK-OV-3 cells were incubated for 2 days to allow for their proper attachment to the plates. Drug solutions were prepared in cell culture medium using serial dilutions and added to the cells in various concentrations as described above. All cells were incubated at 37 °C in an atmosphere of 5% CO_2. However, because of different growth rates of the ovarian cancer cell lines, the PA-1 cells were incubated with drugs for 5 days and the SKOV-3 cells were incubated with drugs for 7 days. At the end of the incubation, MTT (from Sigma, 25 μL, 4 mg/mL) in media without FBS and L-Gln were added to each well. The plate was incubated for 5 h at 37 °C and centrifuged in swinging bucket rotors at 1200 rpm for 15 min. After removal of the media, DMSO (200 μL) was added into each well. The mixture was then incubated for 30 min. The plates were read spectroscopically using a dual-wavelength filter (570 and 630 nm). The concentrations of complexes that decreased absorption by 50% were calculated by extrapolation and taken as the IC_{50} values.

Results and Discussion

Synthesis of Hydroxy-Tethered Platinum(II) and Platinum(IV) Complexes. Normally, the synthesis of the aminoplatinum(II) complexes starts with the interaction of K_2PtCl_4 with appropriate amines. This is indeed the procedure for the synthesis of *cis*-DDP.[8] We used this method successfully to prepare **2** and **3** (Scheme 2). However, the same procedure with ethanol amine gave **1** along with some other unidentified products in an oily form. Multiple efforts to purify this compound were unsuccessful probably because of the coordinative ability of the nearby hydroxy group to form a chelating five-membered ring.

Transformation of the above platinum(II) complexes into kinetically inert platinum(IV) carboxylate complexes was attempted following procedures developed

Scheme 2. Synthesis of Dihydroxy-Tethered Platinum(II) Complexes



for the synthesis of orally active platinum(IV) antitumor agents.[8] However, the tethered hydroxy groups were transformed into the ester groups, preventing direct coupling with other molecules such as oligodeoxynucleotides. Multiple attempts to selectively cleave the esters on the side chains were not successful except for hydrolysis in 20% HCl solution, which resulted in complexes **6** and **7** (Scheme 3). The same complexes were generated by the direct substitution of chlorides of K_2PtCl_6 by the corresponding amines but in very low yield.

Two types of monohydroxy-tethered platinum complexes have been developed. One involves a chelating diamino alcohol ligand.[5] Interaction of the ligand with K_2PtCl_4 generated platinum(II) complex **8**. Oxidation of **8** in the presence of acetic anhydride gave **9** with a free hydroxy group on the side chain. Hydrolysis in the presence of 20% HCl solution generated the corresponding tetrachloro amino platinum(IV) complex.[5b] The same compound, **10**, was also generated from the interaction of the chelating diamino alcohol with K_2PtCl_6 in low yield (Scheme 4).

However, the synthesis of monohydroxy-tethered platinum complexes without a chelating amino group was not straightforward (Scheme 5). Generation of the aminotrichloro platinum(II) anion, **11**, was first achieved by following a procedure in the literature.[5] However, our results by this method were low yield and lack of reproducibility. Multiple trials to improve the procedure resulted in our finding that a stabilizer, NH_4Cl, is critical for the consistent production of this starting material **11**. No decomposition was observed at prolonged heating (>18 h) at 110 °C or more than 1 h at 140 °C. It appears that the amount of NH_4Cl is not critical after a certain limit (from 0.25 to 2.0 molar equiv, no change in results was observed), probably maintaining a low concentration of NH_3 during the reaction. Without NH_4Cl as a stabilizer, a black precipitate began to form at less than 105 °C.

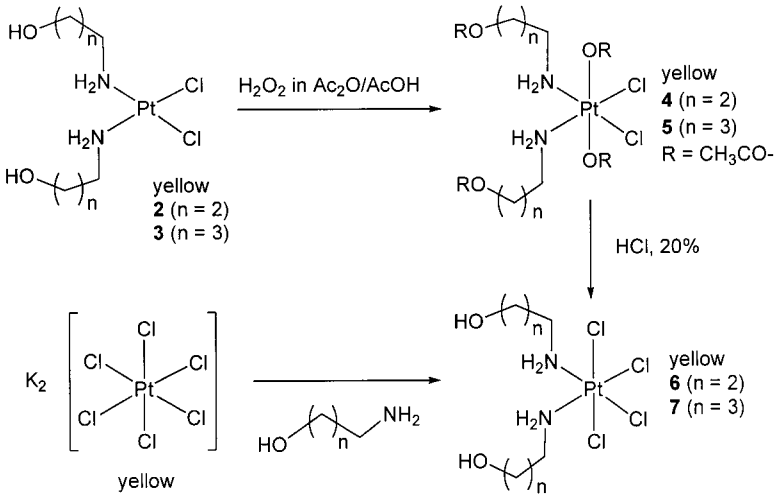
Replacement of the chloride by amines in the presence of NaI successfully gave **12** and **13**. Substitution of iodide with chloride in the presence of silver cations and subsequently quenched by HCl resulted in production of dichloro platinum(II) complexes **14** and **15**. At the last step, the reaction time is critical for its success. Prolonged reaction (overnight at RT, for example) tends to generate complicated mixtures. Monitoring of the reaction by ^{195}Pt NMR showed that the product formation is complete within 10 min. It appears that substitution of the iodide by chloride is critical for the subsequent oxidation; otherwise, a mixture was formed probably from partial iodo oxidation. H_2O_2 oxidation of **14** and **15** in the presence of acetic anhydride gave all protected complexes **16** and **17**. Subsequent hydrolysis in the presence of 20% HCl solution gave **18** and **19** with a free hydroxy group on the side chain ready for attachment of oligodeoxynucleotides.

Synthesis of Platinum Complexes Bearing Phosphoramidite Group and Monodeoxynucleotide-Tethered Platinum(II) and Platinum(IV) Complexes. These were synthesized according to methods previously reported from one of our laboratories (LC).[5] These are shown in Schemes 6 and 7.

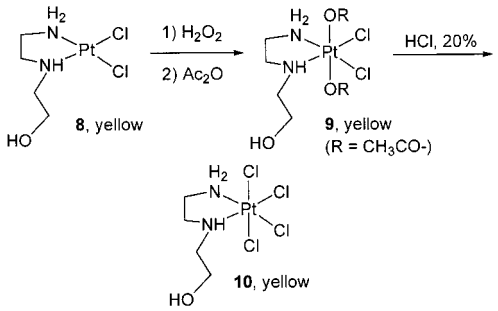
Cell Culture Studies of Tethered Platinum Complexes. The results of the growth inhibition and cytotoxicity studies of a series of our newly synthesized compounds are summarized in Tables 1 and 2, depending on the assay used to evaluate the compounds. The correlation of the IC_{50} values between those from PA-1 and SK-OV-3 is shown in Figure 1.

The dotted line represents the IC_{50} values from platinum(II) complexes and resembles an S-shaped curve. Except for *cis*-DDP, the curve follows an exponential function, with $IC_{50}(SKOV\text{-}3) = 17.5 + 28.4 \log[IC_{50}(PA\text{-}1)]$, $R = 0.999$. What is striking is the uniform change in potency for both cell lines, although a variety of differences exist for both cell lines, demonstrating that the major factors controlling the IC_{50} values were maintained for this group of platinum(II) complexes. The mechanism(s) that makes the SK-OV-3 more re-

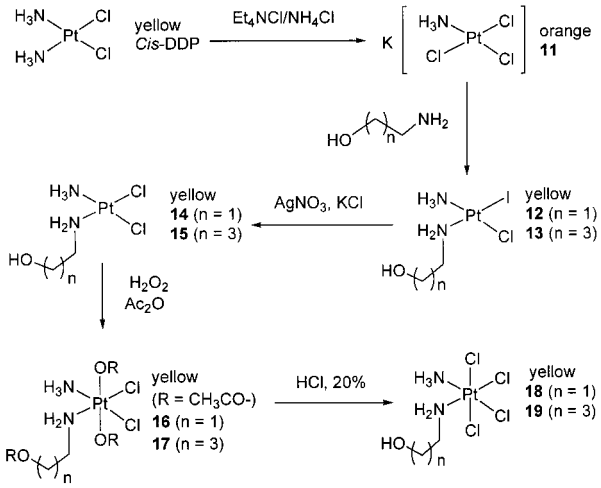
Scheme 3. Synthesis of Dihydroxy-Tethered Platinum(IV) Complexes



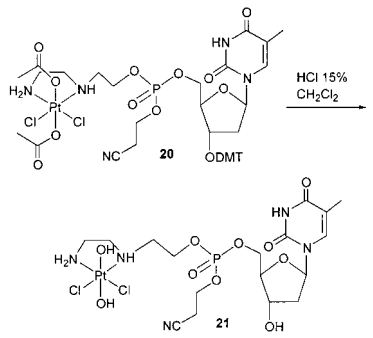
Scheme 4. Synthesis of Chelate Monohydroxy-Tethered Platinum(II) and Platinum(IV) Complexes



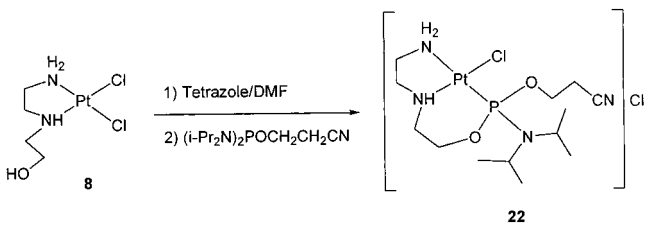
Scheme 5. Synthesis of Monohydroxy-Tethered Platinum(II) and Platinum(IV) Complexes



Scheme 6. Synthesis of Monodeoxynucleotide-Tethered Platinum(IV) Complexes



Scheme 7. Synthesis of a Phosphoramidite-Tethered Platinum(II) Complex



sistant toward platinum(II) complexes also appears to be the same for this group of compounds. Also of interest, the p53 status of the cells may be important for the action of these platinum(II) complexes. Thus, cells with wild-type p53 (PA-1 and A2780) are more sensitive to these complexes than those with mutant p53 (SW626 and SK-OV-3). Among the platinum(II) complexes examined, the following structural features appear to contribute to their potency. The chelated amine complex **8** had the lowest potency. The complexes with two amino alcohol ligands, **2** and **3**, performed better. It appears that some flexibility in the chain of the amino ligand is quite important. Monoamine complexes **14** and

Table 1. Cell Growth and Colony Formation Inhibition of Leukemia and Ovarian Cancer Cell Lines[a]

Pt complex	cell line					
	CEM[b]	CEM/VM-1	PA-1	A2780	SW626	SKOV-3
cis-DDP	<1	<1	0.3 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
6	8 ± 1	5 ± 0.8	0.6 ± 0.2	1.6 ± 0.3	3.0 ± 0.5	10.0 ± 1
7	10 ± 1	10 ± 1	1.0 ± 0.2	2.2 ± 0.3	7 ± 1	4 ± 0.5
9	7 ± 1	7 ± 1	1.5 ± 0.3	2.8 ± 0.2	28 ± 4	24 ± 3
20	35 ± 3	35 ± 3	3 ± 0.5	4 ± 0.5	30 ± 3	24 ± 2
21	100 ± 10	100 ± 10	13 ± 1	16 ± 2	40 ± 4	75 ± 8
22	none	none	165 ± 20	125 ± 20	>200	>200

[a] IC_{50} values of growth inhibition and colony formation were determined as described in Materials and Methods. Concentrations are in μM. Values are $X \pm$ SD of three separate experiments. [b] CEM and VM-1 are leukemia cancer cell lines (with MDR), and PA-1, A2780, SW626, and SKOV-3 are ovarian cancer cell lines.

Table 2. Cell Growth Inhibition of Ovarian Cancer Cell Lines[a]

Pt compounds	cell lines	
	PA-1	SK-OV-3
cis-DDP	0.08 ± 0.03	2.6 ± 0.5
15	0.4 ± 0.1	6.0 ± 1
14	0.7 ± 0.5	13 ± 3
2	1.2 ± 0.4	20 ± 4
3	1.5 ± 0.5	23 ± 5
carboplatin	2.4 ± 0.8	28.5 ± 6
8	2.9 ± 1.2	30 ± 6
19	0.2 ± 0.1	9 ± 2
17	0.8 ± 0.3	22 ± 5
18	0.9 ± 0.3	10 ± 2
10	1.7 ± 0.6	26 ± 5
16	2.8 ± 0.9	10 ± 2

[a] IC_{50} values of growth inhibition were determined by MTT assay as described in Materials and Methods. Concentrations are in μM. Values are $X \pm$ SD of four separate experiments.

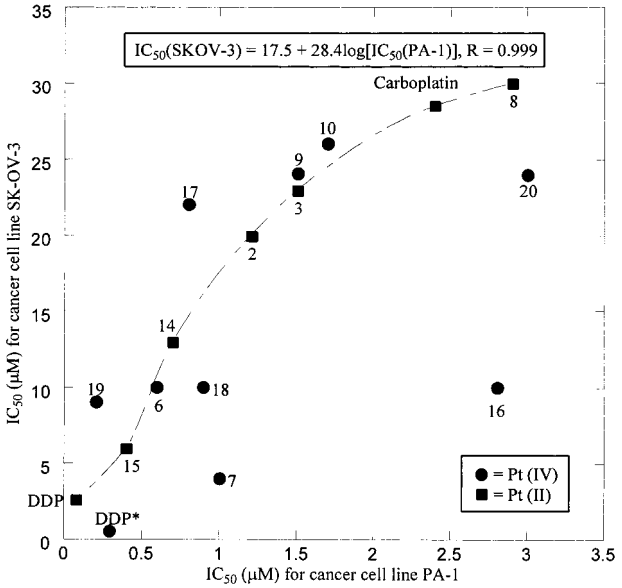


Figure 1. Correlation of IC_{50} values of the platinum complexes for cancer cell lines PA-1 and SK-OV-3. DDP represent the IC_{50} value from Table 1, and DDP* represents the IC_{50} value from Table 2. Squares represent platinum(II) complexes, and circles represent platinum(IV) complexes.

15 performed better than diamine complexes **2** and **3**. This result suggests again that the flexibility of the ligand around the platinum(II) center plays a central role in the activity of the drugs. Another interesting result is that within each class of amino ligands, longer alkyl chains were more potent, and for two adjacent alkyl groups, those with odd numbers of methylene groups performed better.

Compared with platinum(II) complexes, the platinum(IV) complexes show much more varied responses in the two cell lines. No general correlation between IC_{50} values from the two cancer cell lines exists, as shown in Figure 1. In fact, platinum(IV) complexes **7**, **16**, **18**, and **20** show remarkable selectivity to the SK-OV-3 cancer cell line. The most striking examples are complexes **7** and **16**. If we accept that all platinum(IV) complexes are reduced to platinum(II) complexes before binding with DNA, more factors are involved for platinum(IV) complexes, such as the rate of reduction and transport. Recently, the direct interaction between platinum(IV) complexes and nucleotides has been demonstrated in solution chemistry.[9] These extra factors may render different selectivity for platinum(IV) complexes.

Although none of the new compounds are as potent as *cis*-DDP, several facts are evident. First, all platinum(IV) complexes examined are active cytotoxic agents. Second, compounds **6** and **7** (both are Pt(IV)) are the most active in all cell lines, with activities approaching that of *cis*-DDP. Third, the conjugates of platinum(IV) complexes and monodeoxynucleotides are active and show enhanced selectivity among different cancer cell lines. Fourth, and perhaps the most important, the compounds show activity comparable to that of *cis*-DDP in CEM/VM-1 cells that express multidrug resistance due in part to mutation in and decreased activity of topoisomerase II.[10]

This increased selectivity of the platinum(IV) complexes among these different cancer cell lines is an interesting result and suggests that the lower potency of our platinum complexes compared to *cis*-DDP may not be so important. The potency of an individual compound also appears to be affected by chemical groups far away from the platinum center, lending further support to the notion that our new agents are potential candidates as precursors of selective cytotoxic drugs. For example, for **9** vs **20**, the platinum center is the same. While **20** is less potent than **9** in PA-1, both are about the same for SK-OV-3.

The reduced potency of the mononucleotide−Pt conjugates relative to *cis*-DDP is not surprising. The mononucleotides alone show negligible affinity toward either mRNA or DNA. This likely comes from the observation that the minimum length of an ODN that is necessary for targeting a unique sequence in mRNA is approximately 15−20 bases.[11] The tether connecting the mononucleotide and Pt fragment consists of only two methylene groups. This may not be an optimized length for the simultaneous binding of a mononucleotide and Pt to the biological targets.

Our result does not support the general notion that Pt(IV) complexes are less potent than their Pt(II) counterparts;[12] instead, from the numerous examples we have shown here, the opposite is true, such as in **2** vs **6**, **3** vs **7**, **8** vs **9**, and **8** vs **10**. Only **15** vs **17** and **14** vs **18** show the normal pattern. Others such as **14** vs **16** and **14** vs **18** show different responses among different cancer cell lines.

The kinetically inert platinum(IV) complexes can serve as protecting elements to prevent the premature liberation of the platinum(II) center. Under physiological conditions, we expect that reduction of the platinum(IV) center by ascorbic acid or glutathione and a reductase enzyme should result in the liberation of the modified *cis*-DDP, and this should interact with mRNA or dsDNA. This type of reduction has been achieved with the orally administered drug *cis*-(ammine)(cyclohexylamine)dichloro-*trans*-(diacetato)platinum(IV)[13] and with our own in vitro study of the reaction of the platinum(IV) complexes with ascorbic acid or thiols.[14] With ascorbic acid (0.1 M), all platinum(IV) complexes were reduced to platinum(II) complexes within 1−2 days, and with thiols, they were reduced within 1−3 h, demonstrating the possibility of activation by the physiological reductants. Of importance in the present study, all of the platinum(IV) complexes we tested on cancer cell lines showed inhibition of cell growth or colony formation at low concentrations. This result suggests that platinum(IV) complexes can be activated under physiological conditions. However, we have not yet demonstrated this nor have we demonstrated that our compounds remain conjugated after they enter the cells.

Conclusions

We have prepared in the present study a series of hydroxy-tethered Pt(II) and Pt(IV) complexes that can serve as synthons to construct other conjugates of biological interest. These tethered platinum complexes and the conjugate between a platinum fragment with a monodeoxyribonucleotide have been evaluated in in vitro growth inhibition and cytotoxicity assays. Except for compound, **22**, which has a coordinated phosphoramidite group and was inactive, all other compounds were active and some revealed slightly less potency when compared to *cis*-DDP. For platinum(II) complexes, the response between PA-1 and SK-OV-3 is remarkably correlated, with PA-1 the more sensitive to the platinum complexes. Among the amine ligands examined, the potency of the platinum complexes increases from those of chelate diamine, two amines, to those of the monoamine ligands. All of the amine ligands benefits from long aliphatic chains, with slight favor for odd-numbered ones. Overall, the p53 status may be important in the actions of these agents. However, multidrug resistance associated with altered DNA topoisomerase II is not a factor in the actions of these compounds because they appear to have equivalent potency in these cells and their drug sensitive parents. For the platinum(IV) complexes, the response is more diversified. More factors are involved for the cytotoxicity for platinum(IV) complexes, including transport, reduction, and possibly direct interaction with DNA. Groups far away from a platinum center also have a substantial effect on the IC_{50} values. These tethered complexes demonstrate substantial selectivity toward the SK-OV-3 cancer cell line. In this regard, the p53 status may be a critical factor for the enhanced selectivity among different cancer cell lines, at least the ovarian carcinoma lines.

Thus, cells expressing the wild-type protein appear to be more sensitive to the changes of the tether than those with mutant p53. Finally, these conjugates between a platinum fragment and ODNs have the potential characteristics necessary for sequence-specific inhibition of oncogenes such as erbB-2, which is overexpressed in 25−30% of primary breast and ovarian cancer patients.[15] These compounds described in this study represent the first steps in the design of such sequence-specific inhibitions of oncogene expression.

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