Journal of Medicinal Chemistry

Article

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Tuning the Activity of Platinum(IV) Anticancer Complexes through Asymmetric Acylation

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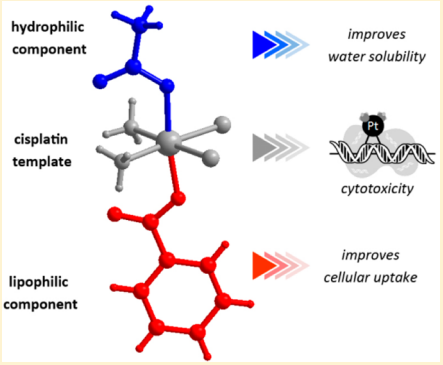
Supporting Information

ABSTRACT: Platinum(II) anticancer drug cisplatin is one of the most important chemotherapeutic agents in clinical use but is limited by its high toxicity and severe side effects. Platinum(IV) anticancer prodrugs can overcome these limitations by resisting premature aquation and binding to essential plasma proteins. Structure–activity relationship studies revealed a link between the efficacy of platinum(IV) complexes with the nature of their axial ligands, which can be modified to enhance the properties of the prodrug. The existing paradigm of employing platinum(IV) complexes with symmetrical axial carboxylate ligands does not fully exploit their vast potential. A new approach was conceived to control properties of platinum(IV) prodrugs using contrasting axial ligands via sequential acylation. We report a novel class of asymmetric platinum(IV) carboxylates based on the cisplatin template containing both hydrophilic and lipophilic ligands on the same scaffold designed to improve their aqueous properties and enhance their efficacy against cancer cells in vitro.



INTRODUCTION

Platinum(II) anticancer drug cisplatin is one of the most effective chemotherapeutic drugs used in the clinic (Figure 1).

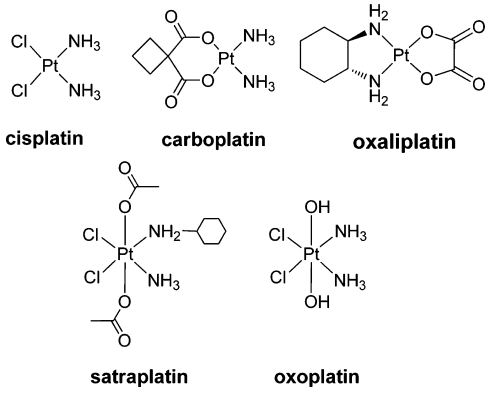


Figure 1. FDA-approved platinum(II) anticancer drugs, platinum(IV) prodrug in clinical trials, satraplatin, and platinum(IV) compound commonly used as precursor for acylation reactions, oxoplatin.

Together with carboplatin and oxaliplatin, they form a family of FDA-approved platinum-based therapeutic agents used in the first-line treatment of testicular, ovarian, lung, and colorectal cancer (Figure 1).[1,2] Their mechanism of action involves cell entry, where they undergo aquation before binding to purine bases in DNA to form platinated DNA cross-links and triggering apoptosis.[3,4] However, the efficacies of platinum drugs are limited by their high toxicity and severe side effects arising from premature aquation and nonselective binding toward essential biomolecules. Incidences of platinum-associated drug resistance, either inherent or acquired, also reduce the effectiveness of platinum-based chemotherapy.[5] Hence, there is renewed interest in developing stable platinum(IV) carboxylate prodrugs that are kinetically inert and would mitigate some of these limitations.[6] One prominent example is satraplatin, a platinum(IV) carboxylate currently undergoing phase III clinical trials against hormone-refractory prostate cancer as an orally available prodrug candidate (Figure 1).[7−10] The mechanism of action of platinum(IV) carboxylates involves chemical reduction to cytotoxic platinum(II) species, with concomitant loss of axial carboxylate ligands, followed by DNA binding.[11,12] The design of these platinum(IV) prodrugs is generally based on cytotoxic platinum(II) templates such as cisplatin and other FDA-approved platinum(II) drugs.[6] Platinum(IV) complexes which yields poorly cytotoxic platinum(II) species upon reduction, e.g., *mer*-[$PtCl_3$(dien)], are themselves inactive.[13]

From a design perspective, the platinum(IV) scaffold provides an excellent platform upon which functionalities can be attached while maintaining the integrity of the platinum(II) pharmacophore [*cis*-$Pt(NH_3)_2Cl_2$] associated with cisplatin.[11] Enzyme inhibitors and moieties that can potentiate cisplatin activity have been added as ligands to platinum(IV) scaffolds to

Received: April 25, 2012
Published: August 9, 2012

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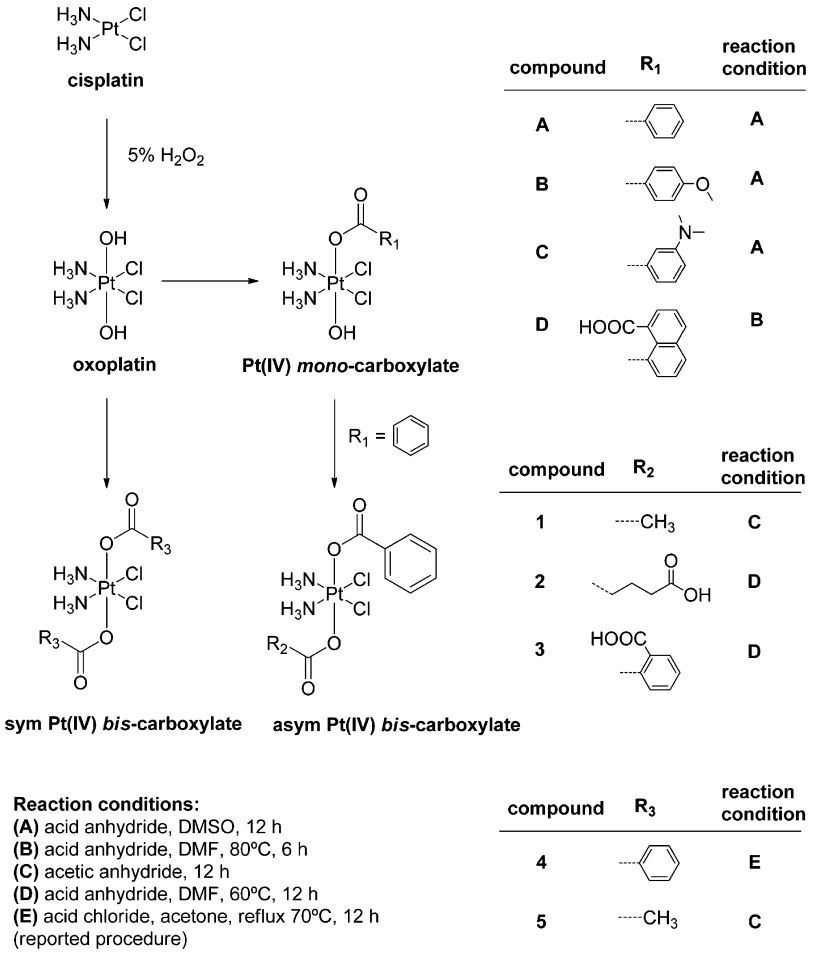
Scheme 1. Synthetic Route to Prepare Platinum(IV) Carboxylates



induce synergistic effects.[14,15] Photoactive platinum(IV) complexes that decompose to generate cytotoxic platinum(II) products have also been developed.[16] Structure−activity relationship studies have shown that the redox potential, rate of reduction, lipophilicity, and cytotoxicity of platinum(IV) prodrugs can be altered by varying the axial ligands.[17−20] For example, platinum(IV) prodrugs bearing hydrophobic aromatic carboxylate ligands exhibit more than 10-fold increase in cytotoxicity against a panel of lung, colon, and breast carcinoma cell lines compared to cisplatin due to improved lipophilicity, while those containing alkyl carboxylate ligands are generally less efficacious.[21]

The existing paradigm of employing platinum(IV) complexes with symmetrical axial bis-carboxylate ligands is too limited to fully exploit the vast potential of this prodrug strategy. Because platinum(IV) prodrugs take on the properties of their coordinating ligands, it should be practical to influence their properties by selectively "mixing-and-matching" the ligands. We surmised that it may be feasible to prepare stable platinum(IV) carboxylates that are lipophilic yet water-soluble, and hence more compatible with pharmaceutical applications, by combining carboxylate ligands with different characteristics into the same scaffold. This class of asymmetric platinum(IV) carboxylate complexes would contain two different ligands at the axial sites, which would serve as handles to fine-tune the lipophilic properties of the complex and, ultimately, to enhance their efficacy against cancer cells. Hence we devised a practical method to carry out acylation onto a platinum(IV) scaffold in sequential steps, a departure from existing procedures designed to generate symmetric bis-carboxylates, thereby paving the way for a rational development of asymmetric platinum(IV) carboxylates containing the [*cis*-$Pt(NH_3)_2Cl_2$] pharmacophore with engineered properties. In this report, we applied this synthetic approach in the preparation of new mono- and asymmetric bis-platinum(IV) carboxylates based on the cisplatin template in the context of tuning their aqueous solubilities and lipophilicities with the ultimate aim of enhancing their efficacies against cancer cells.

RESULTS

Synthetic Strategy. Our approach of accessing lipophilic asymmetric platinum(IV) complexes was to limit the acylation reaction to only one hydroxyl ligand of oxoplatin leaving the remaining site for further systematic manipulations (Scheme 1). Reported acylation methods to introduce aryl ligands required harsh reaction conditions due to the poorly reactive oxoplatin. For example, acylation using acid chlorides promoted the exclusive formation of platinum(IV) bis-carboxylates and required extensive washing with water to quench the reaction and remove side products.[21,22] Such a method would be detrimental to the isolation of platinum(IV) mono-carboxylates, which can be expected to exhibit greater aqueous solubility. To access the mono-carboxylate product, several reaction schemes were attempted, including microwave-assisted heating and refluxing under different solvent conditions. In most instances, both mono- and bis-carboxylates were obtained

dx.doi.org/10.1021/jm300580y | *J. Med. Chem.* 2012, 55, 7571−7582

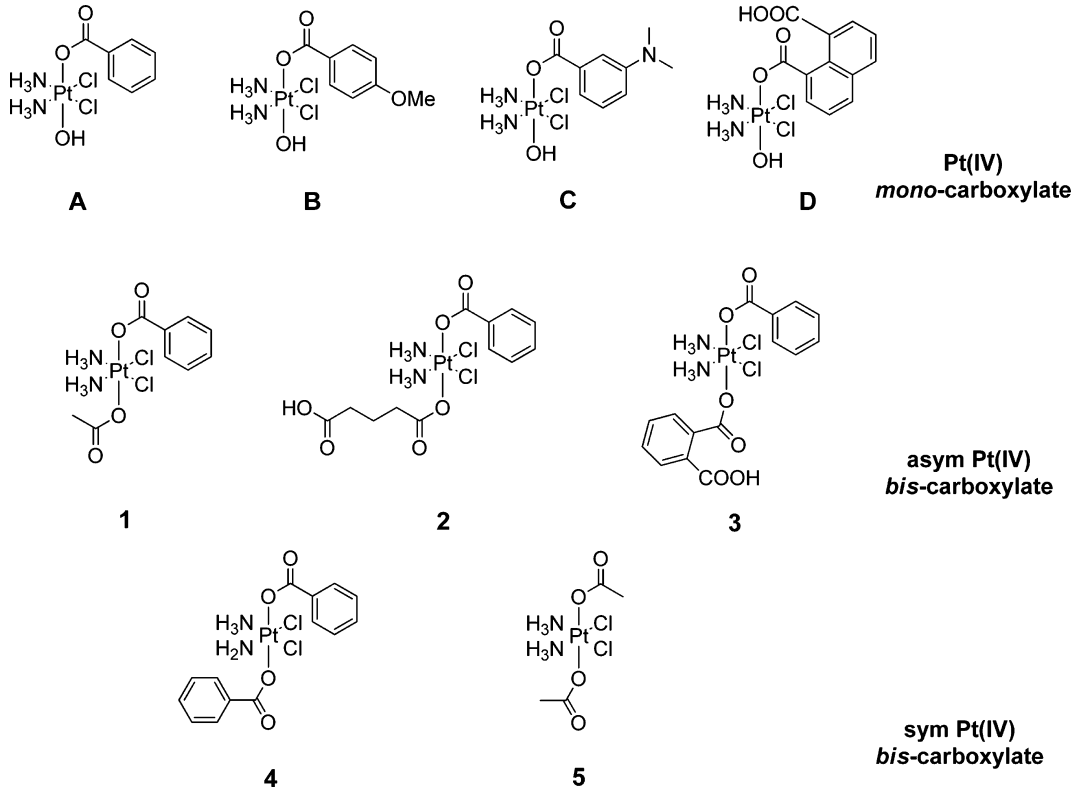


Figure 2. Different classes of platinum(IV) mono- and bis-carboxylates investigated in this report.

in a mixture, and in other cases, unreacted oxoplatin would be recovered abundantly. Because the bis-carboxylate can be readily separated from the mono-carboxylate using acetone, the main challenge was to ascertain the conditions which would promote the consumption of oxoplatin yet minimize the formation of the bis-carboxylate. We found that agitating oxoplatin with benzoic anhydride in DMSO under high dilution conditions generated mono-carboxylate **A** in good yields (Scheme 1). The use of the polar DMSO solvent improved the consumption of the insoluble oxoplatin, while a highly diluted reaction system reduced the likelihood of the second acylation step. Complex **A** was purified by recrystallization from DMF and diethyl ether. Complexes **B**–**D** were prepared in a similar manner but with lower yields (Scheme 1). This strategy was also reported by Dabrowiak to be successful in the mono-acylation of oxoplatin with succinic anhydride.[23] Reaction yields constituted the major challenge to this synthetic approach, and repeated optimizations were required to achieve moderate recovery.

To generate a series of structurally analogous complexes for structure–activity relationship studies, **A** was further acylated at the remaining hydroxyl site to yield asymmetric platinum(IV) bis-carboxylates (Scheme 1). The reactions were generally facile, and conversion was quantitative. Complex **A** was converted to **1** in neat acetic anhydride at rt for 12 h, and reaction completion was indicated by complete dissolution of the reactants. Reaction workup was straightforward, involving lyophilizing the reaction mixture and triturating the residue with diethyl ether to remove the excess and unreacted acetic anhydride. Complexes **2** and **3** were obtained using a similar procedure using acetone as a solvent and excess of glutaric and phthalic anhydride, respectively, in good yields.

Characterization. The synthesized platinum(IV) complexes were characterized using ESI-MS, ^{1}H and ^{195}Pt{^{1}H} NMR spectroscopy, and their purity were determined by RP-HPLC (Figure 2). Elemental analyses were performed on **A** and **1**. Complex **1** was further characterized by single crystal X-ray diffraction. The $[M - H]^-$ parent ions of the platinum(IV) complexes were readily observed using ESI-MS. Fragmentation analysis resulted in the loss of NH_3, HCl, and carboxylate ligands, consistent with the proposed structures of the complexes. Characteristic resonances could be observed at ca. 6.1 ppm in ^{1}H NMR, assigned to the ammine ligands, and 1015–1025 ppm in the ^{195}Pt{^{1}H}-NMR. After acylation, these resonances were shifted downfield to 6.50–6.70 and 1100–1200 ppm, respectively, for asymmetric platinum(IV) bis-carboxylates **1**–**3**. Other than complex **D**, spin-to-spin coupling for ammine protons to quadrupolar ^{14}N nuclei ($^{1}J_{HN}$ = 51–52 Hz) and ^{195}Pt nucleus ($^{2}J_{HPt}$ = 52 Hz) in ^{1}H NMR spectra were well-resolved. These chemical shifts were consistent with other reported symmetric bis-carboxylates, e.g., **4** and **5** obtained directly from their corresponding anhydrides, as well as other reported platinum(IV) alkyl and aryl carboxylates.[21,24,25] One possible explanation of the downfield shift in their ^{195}Pt resonances compared to platinum(IV) mono-carboxylate would be due to the presence of an additional electron-withdrawing carboxylate ligand, resulting in greater deshielding effect on the platinum(IV) metal center.

Single crystals of **1** suitable for X-ray diffraction studies were grown through vapor diffusion of diethyl ether into acetone solution of **1**. To the best of our knowledge, this was the first reported example of *cis*,*cis*,*trans*-diamminedichlorobiscarboxylatoplatinum(IV) structure containing two different carboxylate ligands at the axial positions. There were two molecules of **1** and one molecule of acetone in a monoclinic unit cell with

dx.doi.org/10.1021/jm300580y | J. Med. Chem. 2012, 55, 7571–7582

$P2_1/c$ space group. A molecular representation of **1** was shown in Figure 3, and selected crystallographic and structural data

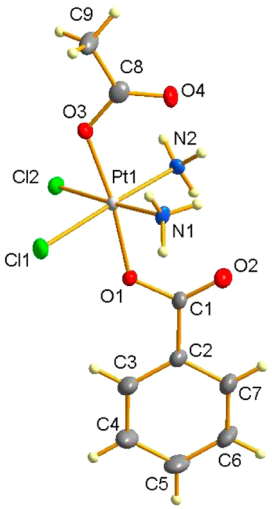


Figure 3. Molecular representation of **1**; thermal ellipsoids are 50% equiprobability envelopes. There are two molecules of complex and one molecule of acetone solvent per unit cell.

were given in Tables 1 and 2, respectively. The coordination geometry about the platinum(IV) metal center was octahedral. The average Pt−Cl and Pt−N bond lengths of 2.315(3) Å and 2.035(5) Å, respectively, were of typical values; they were held

Table 1. Selected X-ray Crystallographic Data for 1[a]

complex	2 × (compound **1**)·acetone
formula	$[Pt(NH_3)_2Cl_2(CO_2C_6H_5)(CO_2CH_3)]_2{\cdot}CH_3COCH_3$
formula weight	1018.50
temperature [K]	293(2)
wavelength [Å]	0.71073
crystal size [mm^3]	0.50 × 0.14 × 0.08
crystal system	monoclinic
space group	$P2_1/c$
a [Å]	13.660(3)
b [Å]	9.867(2)
c [Å]	23.937(6)
α [deg]	90
β [deg]	98.520(5)
γ [deg]	90
V [$Å^3$]	3190.6(13)
Z	8
D_c [mg/m^3]	2.120
μ [mm^{-1}]	9.145
θ range [deg]	2.11−27.50
no. of unique data	22207
max, min transmn	0.5282 and 0.0918
final R indices	$R1$ = 0.0427
[$I > 2\sigma(I)$]	$wR2$ = 0.0953
R indices (all data)	$R1$ = 0.0514
	$wR2$ = 0.0988
goodness-of-fit on F^2	1.100
peak/hole [e $Å^{-3}$]	4.522 and −1.310

[a] $R = \sum||F_o| - |F_c||/\sum|F_o|$, $wR2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$. Goodness-of-fit (GOF) = $\{\sum[w(F_o^2 - F_c^2)^2]/(n-p)\}^{1/2}$, where n is the number of data and p is the number of parameters refined.

Table 2. Comparison of Bond Distances (Å) and Angles (deg) of 1, 4, and 5

complex	1[a]	4[b]	5[b]
Pt−$O_{acetate}$	2.007(5)	2.01(1)	
Pt−$O_{benzoate}$	2.021(5)		2.030(6)
Pt−N	2.035(5)	2.05(1)	2.049(6)
Pt−Cl	2.316(2)	2.30(1)	2.318(2)
C−O(Pt)	1.301(8)	1.30(2)	1.303(11)
O=C(O)	1.230(8)	1.22(2)	1.213(11)
C−C(O)	1.496(10)	1.50(2)	1.502(14)
O−Pt−O	172.6(2)	170.9(2)	176.7(3)
N−Pt−Cl	178.0(2)	179.2(4)	177.1(2)

[a]The bond parameters are average values between the two separate platinum(IV) molecules in the unit cell. [b]The bond parameters listed are average values given the symmetrical nature of the complexes. The data was obtained from literature reports.[21,26]

in a square−planar conformation similar to cisplatin. The axial O−Pt−O bond across the acetate and benzoate ligands was slightly bent due to the intramolecular H-bonding between the ammine H-atoms and the carboxylate O atoms.[21] The Pt−$O_{acetate}$ bond length of was 2.021(5) Å, which was slightly longer than Pt−$O_{benzoate}$ at 2.007(5) Å but consistent with reported values.[21,26] In comparison, the average Pt−O bond length in symmetric bis-benzoate **4** and bis-acetate **5** complexes was reported to be 2.01(1) Å and 2.030(6) Å, respectively.

Lipophilicity and Aqueous Solubility. Log P_{ow} values of the synthesized platinum(IV) complexes were determined using the shake-flask method (Table 3). Besides shake-flask, Log P_{ow} values of other platinum(IV) complexes had also been previously determined using RP-HPLC and group-additive methods.[27] The compounds were partitioned between *n*-octanol and 0.9% w/v NaCl, both presaturated with 0.9% w/v NaCl and *n*-octanol respectively, and the aqueous fractions for before and after partitioning analyzed using ICP-OES. The synthesized complexes, each containing at least an axial aromatic carboxylate ligand, were significantly more hydrophilic that bis-benzoate **4**, as evidenced by their lower Log P_{ow} values. Mono-carboxylate **A**−**D** were evidently more hydrophilic than their bis-carboxylate congeners, presumably due to the uncarboxylated hydroxyl ligand. Functionalization of the benzoate ligands with H-bonding groups increased hydrophilicity, with **D** displaying the lowest Log P_{ow} among its class despite the bulkier naphthalate ligand. Within the series of bis-carboxylate **1**−**4**, replacement of one benzoate with a less lipophilic ligand, namely acetate, glutarate, or phthalate, improved hydrophilicity significantly. In particular, **3** differed structurally from **4** by the addition of an *ortho*-carboxylic group and exhibit a 0.75 unit reduction in Log P_{ow}. Notably, **A** and **1** exhibited a Log P_{ow} reduction of 1.60 and 1.20 unit compared to **4** and differing only in the nature of the one of the axial ligand. In comparison, cisplatin, oxoplatin, and bis-acetate **5** were highly hydrophilic, with Log P_{ow} values of −2.03, −2.12, and −2.00, respectively.

With the increased hydrophilicity, aqueous solubility of the newly synthesized platinum(IV) complexes was expected to improve. Indeed, at rt, the solubility of **A** and **1** in water were observed to be higher than 300 μg/mL, whereas **4** was too poorly soluble to be determined. In comparison, cisplatin and **5** both exceeded 1 mg/mL solubility. For a more quantitative measurement, saturated solutions of **A**, **1**, and **4** were prepared at 80 °C under agitation, filtered, and their platinum levels

dx.doi.org/10.1021/jm300580y | J. Med. Chem. 2012, 55, 7571−7582

Table 3. Properties of Platinum(IV) Carboxylates

complex	Log P_{ow}[a]	solubility (mg/mL)[b]	IC_{50} [μM][c] A2780	IC_{50} [μM][c] A2780/Cis	resistance factor[d]
A	−0.59 ± 0.12	1.22 ± 0.03	2.41 ± 0.27	7.30 ± 2.31	3.0
B	−0.50 ± 0.09		5.11 ± 0.61	16.97 ± 4.01	3.3
C	−0.87 ± 0.16		5.80 ± 0.73	20.21 ± 5.26	3.5
D	−1.14 ± 0.18		19.03 ± 0.91	>50	
1	−0.19 ± 0.04	0.62 ± 0.04	0.83 ± 0.14	6.24 ± 0.94	7.5
2	−0.46 ± 0.04		1.95 ± 0.30	26.41 ± 1.72	13.5
3	0.25 ± 0.02		4.95 ± 0.77	24.90 ± 1.81	5.0
4	1.01 ± 0.01	0.27 ± 0.01	0.048 ± 0.004	0.23 ± 0.07	4.8
5	−2.00 ± 0.18		>20	>20	
cisplatin	−2.03 ± 0.47	3.37 ± 0.05	1.63 ± 0.17	13.91 ± 2.04	8.5
oxoplatin	−2.12 ± 0.24		>50	>50	

[a]Log P_{ow} values determined via the shake-flask method against 1:1 *n*-octanol:0.9% w/v NaCl partition. [b]Compounds were dissolved in 1 mL of water to saturation at 80 °C and filtered, and [Pt] was determined using ICP-OES. [c]IC_{50} values is the concentration of platinum complexes required to inhibit 50% of the cell growth with respect to control groups, measured by MTT assay after 72 h exposure. Data obtained are based on the average of at least three independent trials, and the reported errors are the corresponding standard deviations. The IC_{50} were corrected using actual [Pt] determined using ICP-OES. [d]Based on the ratio of IC_{50}[A2780/Cis] to IC_{50}[A2780].

determined by ICP-OES. The results indicated that monocarboxylate **A** was twice as soluble as asymmetric bis-carboxylate **1** and four times more soluble than bis-benzoate **4**. This provided a validation on the proposed approach of controlling the lipophilicity and solubilities of platinum(IV) carboxylate complexes through asymmetric ligands with contrasting attributes.

Reduction of Platinum(IV) Carboxylates and Interactions with 5′-dGMP. The reactivity of platinum(IV) carboxylates **A**, **1**, and **4** as well as oxoplatin towards 5′-dGMP was investigated, using 5′-dGMP as a model for DNA, since DNA would be the putative biological target for platinum drugs. The formation of reaction products was analysed using analytical RP-HPLC, and unknown peak fractions were isolated and identified using ESI-MS analysis. Upon treatment with 3 mM ascorbic acid as a reductant, the platinum(IV) complexes reacted with 5′-dGMP to form monofunctional [$Pt(NH_3)_2Cl$-(5′-dGMP)] and bifunctional [$Pt(NH_3)_2(5'\text{-dGMP})_2$] adducts (Figure 4 and Supporting Information Figures S9−12). These adducts were similar to those formed by cisplatin under ambient conditions.[28,29] In the absence of a reductant, however, no visible reaction was observed. Taken together, these results suggested that these inert and stable platinum(IV) complexes functioned as prodrugs that were activated upon chemical reduction.

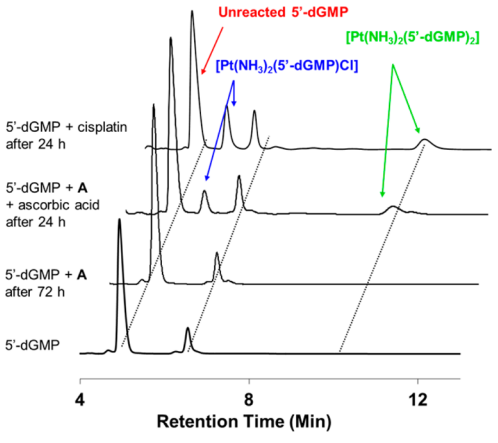


Figure 4. HPLC chromatograms showing 5′-dGMP, reaction of **A** with 5′-dGMP, reaction of **A** with 5′-dGMP in the presence of 3 mM ascorbic acid, and the reaction of cisplatin with 5′-dGMP.

Inhibition of Cell Viability of Cisplatin-Sensitive/Cisplatin-Resistant Cell Lines and Possible Involvement of p53 Transcription Factor. The efficacy of the newly synthesized platinum(IV) complexes on the growth inhibition of A2780 and A2780/Cis human ovarian carcinoma cells was investigated and compared to cisplatin and oxoplatin. Briefly, after the cells were adhered to the multiwall plate surface, they were exposed to varying concentrations of the platinum complexes in serum-free medium for 6 h. The media was replaced with complete media and left to incubate for a further 66 h before the viability of the remaining cells was determined. The data was presented as % survival against nontreated controls, and IC_{50} was interpolated from the graph as concentration required to inhibit cell viability by 50% (Figure 5). All experiments were carried out in triplicates and repeated. The purity of the compounds was established using RP-HPLC and found to exceed 95%. To further mitigate the effects of contamination, platinum concentration of the stock solutions were determined using ICP-OES and the IC_{50} values were adjusted to actual platinum concentration values.

The archetypal complex **4** was 30-fold more cytotoxic than cisplatin in both cell lines. The enhanced activity was earlier attributed to its high lipophilicity and increased drug uptake.[19,21] On the other hand, the highly lipophilic **5**, despite sharing the same [*cis*-$Pt(NH_3)_2$]-pharmacophore as **4**, was poorly cytotoxic. With the exception of **D**, the newly synthesized platinum(IV) carboxylates exhibited the same order of magnitude of cytotoxicity as cisplatin (Table 3). Complex **A** was the most efficacious among the monocarboxylates tested, while **1** was the most effective within the panel of asymmetric bis-carboxylates. Their IC_{50} values of these complexes against both cell lines were in the same order of magnitude range comparable to cisplatin. Notably, all platinum(IV) prodrug complexes exhibited cross-resistance to the cisplatin-resistant A2780 variant (A2780/Cis) with resistance factors between 3.3 and 13.5.

To investigate the possible involvement of p53 in the mechanism of action of platinum(IV) carboxylate complexes, A2780 and A2780/Cis as well as BJ and BJ/shp53 were treated

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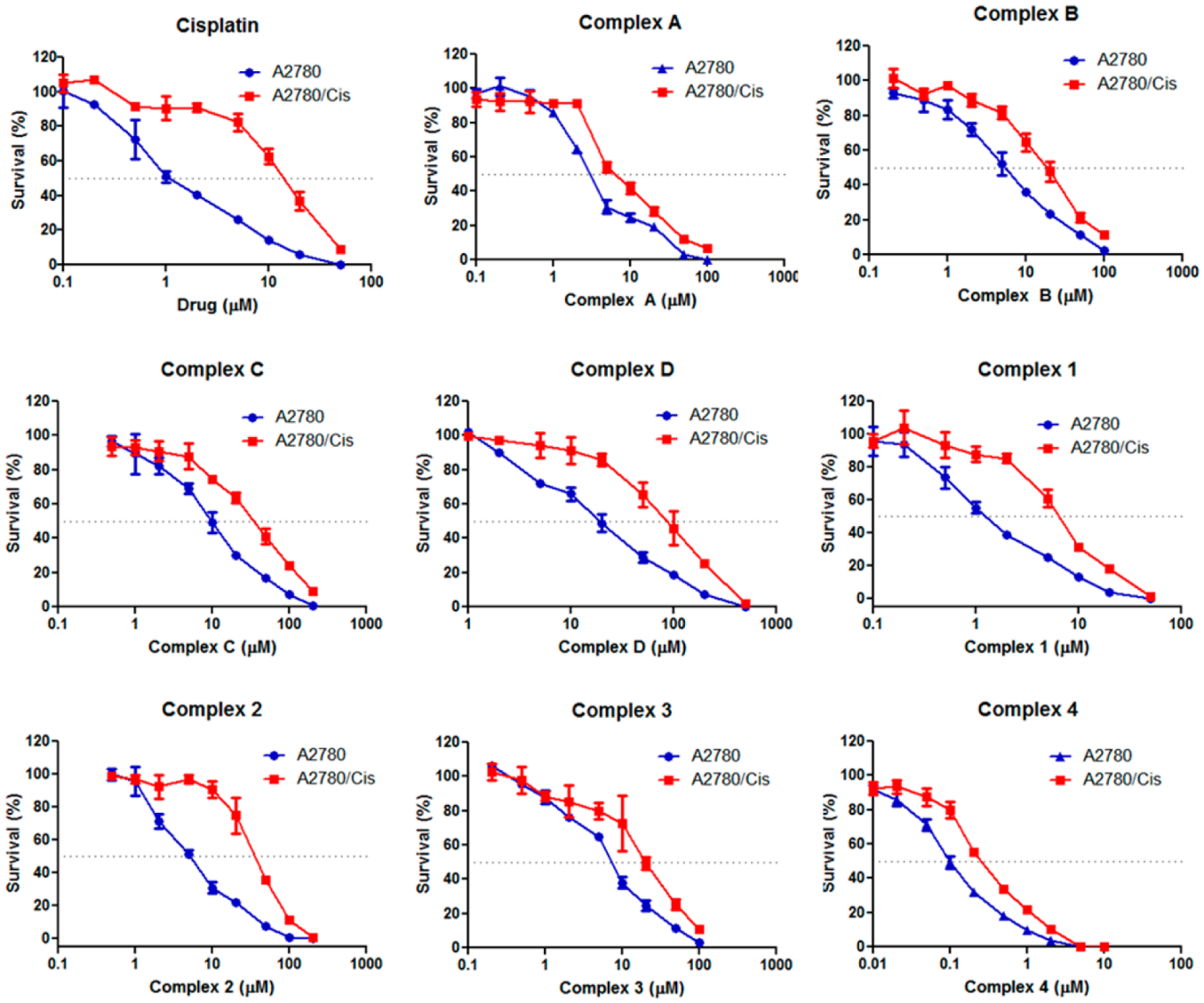

Figure 5. Dose-dependent drug efficacy studies for cisplatin, complexes **A**–**D** and **1**–**4** on A2780 and A2780/Cis tumor cells.

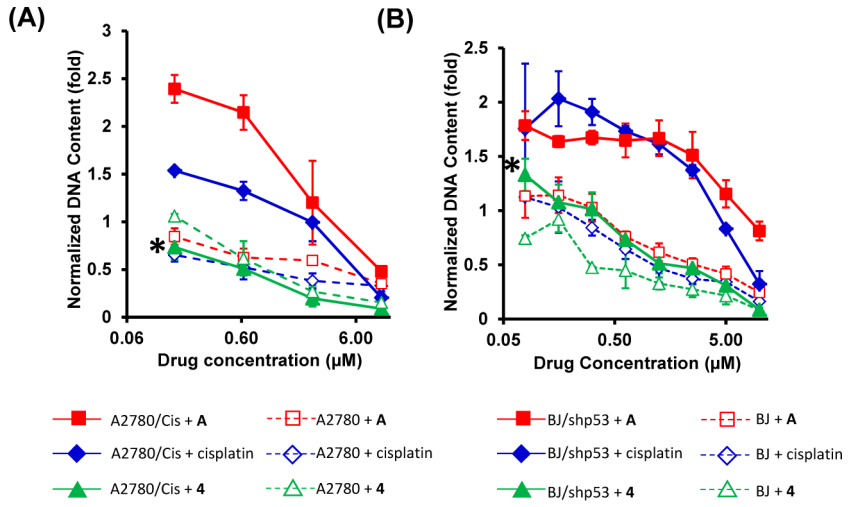

Figure 6. p53 status corroborates with prodrug types; DNA quantification of total cells in each group and concentrations of: (A) A2780 and A2780/Cis (left) and (B) BJ and BJ/shp53 cells (right); (*) denotes the significant reduction of DNA content after treatment with **4** when compared to the treatments with cisplatin and with **A** (one way ANOVA, $p < 0.05$).

dx.doi.org/10.1021/jm300580y | J. Med. Chem. 2012, 55, 7571–7582

with cisplatin, **A**, and **4**. BJ/shp53 was derived from nontumorgenic BJ cells that have suppressed expression of p53 through shp53. DNA content was quantified in the treated cells as expressed as a function of untreated controls as a more definitive measure of cell proliferation. The data showed that cisplatin and **A** significantly reduced the number of A2780 cells in a dose-dependent manner, whereas in the A2780/Cis cells, cisplatin and **A** kill cells at higher dosage ranges, illustrating the expected A2780/Cis resistance against cisplatin (Figure 6A). However, **4** was more effective in killing both A2780 and A2780/Cis cells compared to the cisplatin and **A** (Figure 6A). A similar observation was made in BJ and BJ/shp53 (Figure 6B), indicating that **4** operated via a pathway that was p53-independent in contrast to cisplatin and **A**.

DISCUSSION

The ability to control the nature of platinum(IV) carboxylate complexes through their axial ligand positions has been vital to their development as anticancer prodrugs since lipophilicity was identified early on to be an important factor determining their efficacies in vitro.[11] Initial attempts to tune lipophilicity relied exclusively on symmetric platinum(IV) bis-carboxylate scaffolds with identical axial ligands.[30−32] For example, homologous aliphatic carboxylates were added directly to both axial positions of platinum(IV) scaffolds. By increasing their chain length, lipophilicities of the complexes were enhanced with improved cytotoxic properties. Another approach involved functionalizing platinum(IV) bis-benzoate complexes with different substituents.[21] By altering the nature of these functional groups, cellular accumulation of the platinum(IV) complexes in cancer cells in vitro can be controlled. A more recent attempt employed platinum(IV) carboxylate scaffolds containing axial succinyl-carboxylic groups. Using classical coupling techniques, different ester and amide groups were readily added to improve the lipophilic properties of the target platinum(IV) complex.[19,33]

Our approach was to combine ligands with contrasting lipophilic properties onto the same platinum(IV) carboxylate scaffold, necessitating a new acylation approach that could introduce carboxylate ligands sequentially and yielding asymmetric platinum(IV) compounds with different axial ligands (Figure 2). Previously, asymmetric platinum(IV) complexes had been prepared via the treatment of platinum(IV) bis-hydroxyl precursors with mixed acid anhydrides, which yielded statistical mixtures of platinum carboxylates that were difficult to separate.[34] Galanski and Keppler showed that sterically encumbered equatorial amine ligands could be used to prepare exclusively mono-acylated platinum(IV) complexes in good yield.[35] This elegant approach was, however, not applicable for our work because the central theme was to design asymmetric platinum(IV) prodrugs bearing a template corresponding to known platinum(II) drug; changing the ammine ligands would inadvertently alter the integral [*cis*-$Pt(NH_3)_2$]-pharmacophore. Kidani described a stepwise replacement of axial chloride ligands of platinum(IV) complexes using silver carboxylates or, alternatively, axial carboxylate ligands using dilute HCl solution to achieve asymmetric complexes containing mixed carboxylate and chloride ligands.[36] More recently, Gibson reported a one-pot method of preparing platinum(IV) mono-acetates via oxidation of cisplatin and oxaliplatin using hydrogen peroxide in the presence of acetic acid.[37] The versatile reaction generated hydrophilic platinum(IV) mono-acetates that could be further derivatized. Our approach differed from these examples by providing access to lipophilic platinum(IV) carboxylates with axial aryl ligands. The key limitation, however, was the moderate recovery levels involved in preparing the mono-carboxylate complexes, which required reaction optimization. In this regard, we were successful in producing good yields of representative compounds **A** and **1**.

Lipophilicity, expressed as the logarithms of *n*-octanol/water partition coefficient Log P_{ow}, is a good estimate for the drugs' ability to penetrate cancer cells. It described the distribution of the drug between the polar water phase and nonpolar *n*-octanol phases and an important tool in predicting the transport and activity of the drugs.[38] Recently, Hall and co-workers developed a method to computationally derive Log P_{ow} values of platinum(IV) complexes using quantum mechanical calculations in order to predict their whole-molecule lipophilicity and concluded that there was a strong correlation between lipophilicity and drug accumulation.[39,40] Notably, complexes that were too lipophilic or hydrophilic showed little or poor bioavailability.[41] The asymmetric carboxylates described in this report exhibited Log P_{ow} that were intermediate of the hydrophobic platinum(IV) bis-benzoates or the hydrophilic bis-acetates, demonstrating the utility of this approach in achieving fine-tuning over their lipophilic properties. The increased hydrophilicity of the complexes also brought about improved aqueous solubility.

The putative mechanism of action of inert platinum(IV) prodrugs involved their conversion to cytotoxic platinum(II) species via chemical reduction. Recent work by Gibson using mammalian cellular extracts suggested that intracellular reduction of platinum(IV) carboxylate prodrugs may be mediated by electron transfer reactions with biomacromolecules under physiological conditions.[42,37,43] In its active platinum(II) state, cisplatin exerted its bioactivity by forming genomic Pt−DNA adducts that interfered with replication and transcription processes.[44] In particular, it reacted readily with guanosine via the nucleophilic N7-position, promoted by cooperative H-bonding interaction between the ammine ligand and exocyclic O6-position. Previously, bis-benzoate **4** was observed to only react with Na(DDTC) after treatment with a bioreductant such as ascorbic acid or glutathione to form yellow [$Pt(DDTC)_2$] in the same manner as cisplatin.[45] Without reduction, **4** remained unreactive toward DDTC, suggesting the chemical reactivity of platinum(II) drugs can be caged as the kinetically inert platinum(IV) carboxylate and triggered upon reduction.[46] Therefore, platinum(IV) carboxylates **A** and **1** were investigated using 5′-dGMP as a probe, as well as a model for DNA, and ascorbic acid as a biologically compatible reductant to evaluate if they could fulfill their roles as prodrugs akin to **4**. The reduced platinum complexes were functionally similar to cisplatin, forming the same types of mono- and bifunctional platinated 5′-dGMP adducts. In addition, these complexes exhibited good aqueous stability under various pH conditions. Taken together, the data suggested that the newly synthesized platinum(IV) carboxylates were functionally anticancer prodrugs of cisplatin.

The in vitro data against cisplatin-sensitive A2780 and cisplatin-resistant A2780/Cis cell lines suggested a link between lipophilicites and their efficacies in inhibiting cancer cell viability within this class of platinum(IV) complexes (Figure 5 and Table 3). Highly hydrophilic platinum(IV) complexes oxoplatin and **5** are poorly efficacious when compared to the lipophilic mono- and bis-carboxylates. In comparison, cisplatin,

dx.doi.org/10.1021/jm300580y | J. Med. Chem. 2012, 55, 7571−7582

which exhibit comparable Log P_{ow}, was much more efficacious. Furthermore, lipophilic bis-carboxylates **1**−**5** were generally more efficacious than mono-carboxylates **A**−**D** despite only minor structural changes. One possible explanation would be that the platinum(IV) carboxylates were taken up by cells via passive diffusion mechanisms and therefore affected by their intrinsic lipophilic properties. In contrast, active transport processes could be involved in the cell entry of cisplatin, notably by CTR1, which was previously implicated, thus explaining the higher efficacy levels despite its low lipophilicity.[5,47]

Comparing the structurally similar **A**, **1**, and **4**, which differ only by one axial ligand (hydroxyl vs acetate vs benzoate, respectively), the effects of tuning the lipophilicity and solubility via the axial ligand position was apparent. Complexes **A** and **1** attained comparable efficacy levels in cancer cell growth inhibition to cisplatin, with asymmetric bis-carboxylate **1** benefitting from higher lipophilicity levels. At the same time, they exhibited moderate aqueous solubilities, unlike **4**, which was not directly soluble in water and would be more compatible for intravenous administration and further pharmaceutical development. In all cases, cross-resistance to cisplatin were observed in A2780/Cis albeit differences in resistance factors. Cisplatin-based resistance in A2780/Cis cells had been ascribed to enhanced cellular repair via mismatch repair mechanisms.[48] Because the platinum(IV) carboxylates described share the same pharmacophore and would exert their activity via DNA binding after intracellular reduction, in a manner similar to cisplatin, they would be affected by the same resistance mechanisms resulting in lower efficacies.

To bring our novel synthesized drugs closer to clinical relevance, we asked whether the status of important tumor suppressors like p53 might contribute to cancer cell responses toward our synthesized drugs, namely the highly cytotoxic bis-benzoate **4** and the less cytotoxic mono-benzoate **A** using cisplatin for comparison. p53 was chosen due to its prevalent mutated form in human tumors (Figure 6).[49] The in vitro data suggested that **4** acted through a mechanism that did not involve the initial resistance toward cisplatin. Considering that A2780 cells express functional p53 while A2780/Cis did not,[48] it highlighted the possibility that **A** worked through p53 to bring about cell death. Because p53 activates a milieu of antioxidant genes,[50] it was possible that in A2780 cells where the p53 pathway was still intact; having p53 also provided the activating environment for cisplatin and **A**, while in the A2780/Cis cells, reducing power was kept nominal due to a nonresponsive dysfunctional p53 pathway.

Because tumor-derived cells like A2780 cells may have underlying dysfunctional non-p53 related pathways that were responsible for our observations, we verified our findings in a human primary skin fibroblasts nontumor derived cell line, BJ cells that have suppressed expression of p53 through shp53 (BJ/shp53). As expected, we observed a similar trend in that **4** was as potent to cells independent of p53 status while **A** was more potent toward cells with functional p53 pathway (Figure 6B). Because BJ/shp53 cells are derived from the parental BJ cells and cell passage for both BJ and BJ/shp53 cell lines are the same, any differences toward **A** would then be attributable to p53. These findings in BJ and BJ/shp53 further supported the role that p53 plays in regulating prodrug activation. These observations also highlight the need to consider the transcriptomic state of the target cell in the design of prodrugs.

CONCLUSION

Asymmetric *trans*-platinum(IV) carboxylate complexes represent a new paradigm of platinum(IV) complexes containing both lipophilic and hydrophilic axial ligands by design in order to achieve a desirable pharmaceutical outcome. Through the asymmetric acylation strategy, the characteristics of the platinum(IV) complexes were improved, leading to novel prodrugs with better aqueous solubility that are equally efficacious against cancer cells in vitro as cisplatin. Among the panel of novel platinum(IV) complexes containing different axial ligands, mono-benzoate platinum(IV) complex **1** exhibited good aqueous solubility and activity profile against tested cancer cells, comparable to cisplatin. As with cisplatin, its activity was modulated by tumor suppressor p53 as evidenced in the cisplatin-resistant and p53-deficient cell lines. This new approach of preparing platinum(IV) carboxylates with asymmetric axial ligands could pave the way for a new generation of platinum(IV) with highly tuned properties.

EXPERIMENTAL SECTION

Materials. Unless otherwise noted, all procedures were carried out without taking precautions to exclude air and moisture. All solvents and chemicals were used as received without further treatment. K_2PtCl_4 was obtained from both Precious Metals Online and Strem Chemicals. Cisplatin, oxoplatin, and **4**−**5** were synthesized and purified accordingly to literature procedures.[21,22,26,51,52] RPMI 1640 medium was purchased from Invitrogen (Carlsbad, CA, USA). Penicillin, streptomycin, and thiazolyl blue tetrazolium bromide (MTT) were all obtained from Sigma Chemical Co (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Hyclone (Thermo Scientific Inc., Logan, UT, USA). The ultrapure water used was purified by a Milli-Q UV purification system (Sartorius Stedim Biotech SA, Aubagne Cedex, France). All other solvents and chemicals were of analytical grade or HPLC grade obtained from commercial sources.

Instrumentation. ^{1}H and $^{195}Pt\{^{1}H\}$ NMR spectra were recorded on a Bruker ACF 300 and Bruker AMX 500 spectrometer, and the chemical shifts (δ) were internally referenced by the residual solvent signals relative to tetramethylsilane for ^{1}H and externally referenced using K_2PtCl_4 for $^{195}Pt\{^{1}H\}$. Mass spectra were measured using a Finnigan MAT LCQ or Bruker Ultimate 3000 ion trap ESI mass spectrometer. UV spectra were recorded on a Shimadzu UV-1800 UV spectrophotometer using 1 cm path-length quartz cuvettes. Platinum concentration determination was performed using inductively-coupled plasma optical emission spectroscopy (ICP-OES) by CMMAC, NUS. Elemental analyses of selected platinum compounds were carried out on the Perkin-Elmer PE 2400 elemental analyzer by CMMAC, NUS.

HPLC Studies. Studies on the reaction of platinum compounds with 5′-dGMP were conducted using analytical HPLC on a Agilent 1200 series DAD using a Phenomenex Luna C18 (5 μM, 100 Å, 250 mm × 4.60 mm i.d.) column at rt at a flow rate of 1.0 mL/min with 254 and 280 nm UV detection. The gradient eluent conditions were as follows: 5−7% solvent A for 20 min followed by 80% solvent B for 5 min, where solvent A was NH_4OAc buffer (10 mM, pH 7.0) and solvent B was MeOH. The purity of platinum(IV) compounds was determined using analytical HPLC on a Shimadzu Prominence using a Shimpack VP-ODS C18 (5 μM, 120 Å, 250 mm × 4.60 mm i.d.) column at rt at a flow rate of 1.0 mL/min with 254 and 280 nm UV detection. The gradient eluent conditions were as follows: 20−80% solvent A for 20 min followed by 80% solvent B for 5 min, where solvent A was NH_4OAc buffer (10 mM, pH 3.8) and solvent B was MeCN. Eluted fractions, besides peaks arising from 5′-dGMP, were collected and analyzed by ESI-MS.

X-ray Diffraction Studies. X-ray data were collected with a Bruker AXS SMART APEX diffractometer using Mo Kα radiation at 223(2) K with the SMART suite of Programs.[53] Data were processed and corrected for Lorentz and polarization effects using SAINT

software[54] and for absorption effects using the SADABS software.[55] Structural solution and refinement were then carried out using the SHELXTL suite of programs.[56] The structure was solved by direct methods. Non-hydrogen atoms were located using difference maps and were given anisotropic displacement parameters in the final refinement. All H atoms were put at calculated positions using the riding model.

Synthesis of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(CO$_2$C$_6$H$_5$)OH] (A). Benzoic anhydride (100 mg, 440 μmol) was added to *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$] (100 mg, 300 μmol) in DMSO (20 mL). The reaction was stirred at rt for 12 h and filtered to remove unreacted starting materials. The filtrate was lyophilized and washed with acetone (3 × 10 mL) and cold DMF (1 × 5 mL) and dried in vacuo to yield the product as a white precipitate. Yield: 99 mg (75%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.88 (d, 2 H, Ar−H), 7.47 (t, 1 H, Ar−H), 7.39 (t, 2 H, Ar−H), 6.06 (m, 6 H, NH$_3$, $^1J_{HN}$ = 52.2 Hz, $^2J_{HPt}$ = 52.9 Hz). ^{195}Pt{^{1}H} NMR (107.6 MHz, DMSO-d_6): δ 1022.2 ppm. ESI-MS (negative ion mode): m/z = 436.9 [M − H]$^-$. Purity (HPLC): 94.1% at 254 nm and 93.4% at 280 nm, respectively; retention time (t_r) = 6.9 min. Anal. Calcd. for **A**·1.25(DMF), C$_7$H$_{12}$Cl$_2$N$_2$O$_3$Pt·(C$_3$H$_7$NO)$_{1.25}$: C, 24.38; H, 3.95; N, 8.60. Found: C, 24.71; H, 4.00; N, 8.60.

Synthesis of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(CO$_2$C$_6$H$_4$OCH$_3$)OH] (B). The compound **B** was prepared in accordance with the method used for **A** using 30 mg of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$] starting material Yield: 13 mg (30%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.83 (d, 2 H, Ar−H), 6.93 (d, 2 H, Ar−H), 6.05 (m, 6 H, NH$_3$, $^1J_{HN}$ = 51.1 Hz, $^2J_{HPt}$ = 52.4 Hz), 3.80 (s, 3 H, Ar-OCH$_3$) ppm. ^{195}Pt{^{1}H} NMR (107.6 MHz, DMSO-d_6): δ 1025.4 ppm. ESI-MS (negative ion mode): m/z = 466.9 [M − H]$^-$. Purity (HPLC): 95.1% at 254 nm and 95.3% at 280 nm; t_r = 8.6 min.

Synthesis of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(CO$_2$C$_6$H$_4$NC$_2$H$_6$)OH] (C). The compound **C** was prepared in accordance with the method used for **A** using 30 mg of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$]. Yield: 4 mg (9%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.20 (m, 3 H, Ar−H), 6.83 (d, 1 H, Ar−H), 6.08 (m, 6 H, NH$_3$, $^1J_{HN}$ = 51.7 Hz, $^2J_{HPt}$ = 52.3 Hz), 2.90 (s, 6 H, Ar-NCH$_3$) ppm. ^{195}Pt{^{1}H} NMR (107.6 MHz, DMSO-d_6): δ 1024.9 ppm. ESI-MS (negative ion mode): m/z = 479.9 [M − H]$^-$. Purity (HPLC): 95.7% at 254 nm and 95.7% at 280 nm; t_r = 9.0 min.

Synthesis of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(CO$_2$C$_{10}$H$_6$COOH)OH] (D). Napthalic anhydride (95 mg, 480 μmol) was added to *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(OH)$_2$] (60 mg, 180 μmol) in DMF (5 mL). The reaction was stirred at 80 °C for 6 h, treated with deionized water (20 mL), and cooled at 4 °C for 12 h. The reaction mixture was filtered through Celite to remove unreacted starting material and the solvent removed in vacuo. The residue was extracted with water (3 × 20 mL) and the aqueous extract lyophilized to yield an off-white product. Yield: 38 mg (40%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.85 (d, 1 H, *o*-Ar−H), 7.78 (d, 1 H, *o*-Ar−H), 7.59 (d, 1 H, Ar−H), 7.45−7.38 (m, 3 H, Ar−H), 6.31 (m, 6 H, NH$_3$). ^{195}Pt{^{1}H} NMR (107.6 MHz, DMSO-d_6): δ 1015.8 ppm. ESI-MS (negative ion mode): m/z = 530.9 [M − H]$^-$. Purity (HPLC): 95.0% at 254 nm and 95.2% at 280 nm; t_r = 7.5 min.

Synthesis of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(CO$_2$C$_6$H$_5$)(CO$_2$CH$_3$)] (1). Compound **A** (50 mg, 114 μmol) was stirred at rt in acetic anhydride (5 mL) for 12 h. The reaction mixture was lyophilized and washed with diethyl ether (2 × 5 mL) to yield **1** as an off-white product after drying in vacuo. Yield: 52 mg (95%). Single crystals suitable for X-ray diffraction were grown from vapor diffusion of diethyl ether into a solution of **1** in acetone. ^{1}H NMR (300 MHz, acetone-d_6): δ 7.95 (d, 2 H, Ar−H), 7.51 (t, 1 H, Ar−H), 7.40 (t, 2 H, Ar−H), 6.57 (m, 6 H, NH$_3$, $^1J_{HN}$ = 53.9 Hz, $^2J_{HPt}$ = 53.3 Hz), 1.96 (s, 3 H, −COOCH$_3$) ppm. ^{195}Pt{^{1}H} NMR (107.6 MHz, acetone-d_6): δ 1123.0 ppm. ESI-MS (negative ion mode): m/z = 478.92 [M − H]$^-$. Purity (HPLC): 97.3% at 254 nm and 100% at 280 nm; t_r = 9.1 min. Anal. Calcd. for **1**, C$_9$H$_{14}$Cl$_2$N$_2$O$_4$Pt: C, 22.51; H, 2.94; N, 5.83. Found: C, 22.33; H, 2.95; N, 5.79.

Synthesis of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(CO$_2$C$_6$H$_5$)-(CO$_2$C$_3$H$_6$COOH)] (2). Compound **A** (38 mg, 86.7 μmol) was stirred with glutaric anhydride (12 mg, 105 μmol) in DMF (10 mL) at 60 °C for 12 h. The reaction mixture was cooled to rt and added diethyl ether (40 mL), which yielded a white precipitate. The precipitate was collected by centrifugation and washed with diethyl ether (3 × 10 mL) and dichloromethane (10 mL). The product was dried in vacuo to yield a white solid. Yield: 13.8 mg (29%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.88 (d, 2 H, Ar−H), 7.52 (t, 1 H, Ar−H), 7.42 (t, 2 H, Ar−H), 6.64 (m, 6 H, NH$_3$), 2.28 (m, 4 H, −COOCH$_3$), 1.70 (m, 2 H, −CH$_2$*CH$_2$*CH$_2$−) ppm. ^{195}Pt{^{1}H} NMR (DMSO-d_6, 107.6 MHz): δ 1195.7 ppm. ESI-MS (negative ion mode): m/z = 550.9 [M − H]$^-$. Purity (HPLC): 95.4% at 254 nm and 95.9% at 280 nm; t_r = 9.7 min.

Synthesis of *cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(CO$_2$C$_6$H$_5$)-(CO$_2$C$_6$H$_4$COOH)] (3). The compound was prepared in accordance with the method used for **2** using 30 mg of compound **A** as starting material. Yield: 15.7 mg (39%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.91 (d, 1 H, Ar−H), 7.44−7.54 (m, 8 H, Ar−H), 6.69 (br, 6 H, NH$_3$) ppm. ^{195}Pt{^{1}H} NMR (107.6 MHz, DMSO-d_6): δ 1175.7 ppm. ESI-MS (negative ion mode, MeOH): m/z = 584.9 [M − H]$^-$. Purity (HPLC): 97.1% at 254 nm and 97.7% at 280 nm, respectively; t_r = 10.8 min.

Aqueous Stability of Compounds. Complexes **1**, **4**, and **A** were dissolved in 1.5 mL of NH$_4$OAc (10 mM, pH 5.5), PBS (pH 6.0), and PBS (pH 7.4) in 2 mL microtubes and agitated at 37 °C for 96 h. The initial and final platinum(IV) samples were analyzed using analytical HPLC on a Shimadzu Prominence using a Shimpack VP-ODS C18 (5 μM, 120 Å, 250 mm × 4.60 mm i.d.) column at rt at a flow rate of 1.0 mL/min with 254 and 280 nm UV detection. The gradient eluent conditions were as follows: 5−90% solvent A for 30 min, where solvent A was ultrapure water and solvent B was MeCN.

Log P_{ow} Determination. The Log P_{ow} determination of platinum(IV) compounds were conducted using the shake flask method.[57] Platinum(IV) compounds were dissolved in 0.9% NaCl w/v ultrapure water (presaturated with *n*-octanol for 96 h and left to stand overnight). The solutions were sonicated and filtered through Celite to remove undissolved platinum(IV) compounds. The initial concentrations of platinum content were determined by ICP-OES. Subsequently, the platinum(IV) solutions were added an equal volume of *n*-octanol (presaturated with 0.9% NaCl w/v ultrapure water for 96 h and left to stand overnight). The heterogeneous mixtures were shaken vigorously for 2 h before centrifuging at 4200*g* for 15 min to achieve phase separation. The final concentration of platinum content in the aqueous phase was determined again by ICP-OES, and the water−octanol partition coefficient was calculated. All experiments were done in triplicates.

Cell Culture. The human ovarian carcinoma cells A2780 and A2780/Cis were provided by Prof. Paul Dyson (EPFL). Both cell lines were cultured in complete RPMI 1640 medium containing 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum. To retain resistance, 1 μM cisplatin need to be added to the media for A2780/Cis every 2−3 passages. The two cell lines were grown at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Experiments were performed on cells within 10 passages. Viable cells were counted using the trypan blue exclusion method.

Inhibition of Cell Viability Assay. Drug effects on exponentially growing tumor cells were determined using MTT assay as described previously.[58] A2780 and A2780/Cis cells were seeded at a density of 6000 cells/100 μL per well in 96-well plates and incubated for 24 h. Thereafter, tumor cells were exposed to drugs at different concentrations in RPMI medium without FBS and antibiotics. Compounds were dissolved as DMSO stock solutions and serially diluted with DMSO to a series of decreasing concentrations. The final concentration of DMSO in medium was 1% (v/v), and such concentration showed little cytotoxicity to both strains of cells when incubated for 6 h. The drug-containing medium was removed by aspiration, and fresh drug-free complete medium with FBS and antibiotics was added and the cells were incubated for additional 66 h. At 72 h after drug addition, the medium was aspirated, replaced with 100 μL of MTT solution (0.5 mg/mL in PBS), and incubated for a further 4 h at 37 °C. The medium was aspirated, and the purple formazan precipitate dissolved in 100 μL of DMSO. UV−vis absorbance was measured at a 595 nm using a microplate reader

dx.doi.org/10.1021/jm300580y | J. Med. Chem. 2012, 55, 7571−7582

(Tecan). Experiments were performed in triplicates for each drug concentration and carried out independently at least three times. Cytotoxicity was evaluated with reference to the IC_{50} value, which was defined as the concentration needed for a 50% reduction of survival based on the survival curves. IC_{50} values were calculated from dose–response curves (cell survival vs drug concentration) obtained in repeated experiments and adjusted to actual [Pt] administered which was determined using ICP-OES.

Packaging shp53 Retrovirus and Transduction of BJ Cells. EcoPack 2–293 (Clontech, CA, USA) cells at 80% confluency were transfected with retroviral plasmids encoding either shp53 or empty vector control.[59] Media containing virus was collected and clarified 48 h post transfection. BJ cells (ATCC, VA, USA) expressing the ecotropic receptor were plated at 80% confluence the evening before transduction. Equal volumes of viral media were mixed with growth media containing Polybrene (8 μg/mL final concentration) and added directly to BJ cells to obtain BJ/shp53 cells. Control BJ at the same passage was transduced with empty vector virus at the same experiment. Cells were selected with 2 μg/mL of blasticidine for positively transduced cells for one week.

Sensitivity to p53-Proficient and p53-Deficient Cell Lines. A2780, A2780/Cis, BJ, and BJ shp53 cells were grown in 96-well plates with initial seeding density of 4000 cells/well. Compounds were introduced to the cells for 6 h, and afterward the cells were washed twice with PBS. The cells were further incubated for 66 h. Complete RPMI medium and DMSO-supplemented RPMI were introduced also to the vehicle controls. Following 72 h from the start of drug treatments, the cells were lysed by adding 0.1% Triton-X100 and then were shaken for 30 min on ice. Subsequently, the cells were freeze thawed for five cycles (30 min for each steps of freezing or thawing). The lysate were added into 1× SBYR Green solution, and the DNA contents were measured with Tecan Infinite 200 microplate reader at excitation/emission wavelength 485/535 nm. Experiments were done in triplicates.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR and HPLC chromatographs of compounds **A**–**D** and **1**–**3**, stability studies in aqueous media, binding studies to 5′-dGMP, and X-ray crystallographic data in CIF format for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Paul J. Dyson (EPFL) and Lucienne Juillerat-Jeanneret (CHUV) for their gifts of cell lines and to Lip Lin Koh (NUS), Geok Kheng Tan (NUS), and Yimian Hong (NUS) for performing the X-ray crystallographic analysis. We also acknowledge the generous funding provided by Singapore Ministry of Education (R143-000-411-133 and R143-000-512-112 to A.W.H. and R279-000-321-133 to D.T.L.) and Singapore Ministry of Health's National Medical Research Council under the New Investigator Grant scheme (to D.T.L.).

■ ABBREVIATION LIST

FDA , Food and Drug Administration; HCl , hydrochloric acid; DMF , dimethylformamide; DMSO , dimethyl sulfoxide; RP-HPLC , reverse phase–high performance liquid chromatography; ICP-OES , inductively coupled plasma-optical emission spectrometry; DDTC , diethyldithiocarbamate; 5′-dGMP , 5′-guanosine-2′-deoxymonophosphate; IC_{50} , half-maximal inhibitory concentration

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