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Dinuclear Platinum Complexes Containing Planar Aromatic Ligands to Enhance Stacking Interactions with Proteins

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Abstract

In an approach to design drugs with higher affinity for π - π stacking and electrostatic interactions with targeted biomolecules, complexes of the type $[\{cis\text{-Pt}(\text{A})_2(\text{L})\}_2\text{-}\mu\text{-}\{trans\text{-}1,4\text{-dach}\}](\text{NO}_3)_4$ ($(\text{A})_2 = (\text{NH}_3)_2$ or ethylenediamine (en), L = quinoline (quin) or benzothiazole (bztz), dach = *trans*-1,4-diaminocyclohexane) were synthesized. The quinoline complex, $[\{cis\text{-Pt}(\text{en})(\text{quin})\}_2\text{-}\mu\text{-}(\text{dach})](\text{NO}_3)_4$ (**9**) was synthesized from the precursor $\text{K}[\text{PtCl}_3(\text{quin})]$ (**1**) while the benzothiazole complexes, $[\{cis\text{-Pt}(\text{A})_2(\text{bztz})\}_2\text{-}\mu\text{-}(\text{dach})](\text{NO}_3)_4$ ($(\text{A})_2 = (\text{NH}_3)_2$ (**10**); $(\text{A})_2 = \text{en}$ (**11**)) were synthesized from the precursors *cis*- $[\text{Pt}(\text{A})_2\text{Cl}(\text{bztz})]$ ($(\text{A})_2 = (\text{NH}_3)_2$ (**7**); $(\text{A})_2 = \text{en}$ (**8**)). Their interactions with *N*-acetyltryptophan and a model pentapeptide (N-AcWLASW-OH) modeled on the pentapeptide recognition sequence of p53-mdm2 (FLASW) were examined by fluorescence spectroscopy. The dinuclear complexes were found to be significantly stronger quenchers of fluorescence than their mononuclear Pt analogs. Molecular modeling suggests a “sandwich” mode of binding and the flexibility of the dinuclear motif can allow design for more selective and stronger-binding complexes. Based on these results a further prototype, $[\{\text{Pt}(\text{en})(9\text{-EtGua})\}_2\text{-}\mu\text{-}\text{H}_2\text{N}(\text{CH}_2)_6\text{NH}_2]^{4+}$, incorporating the purine 9-Ethylguanine (9-EtG) as a stacking moiety was prepared which showed good cytotoxicity in A2780 and OsACL tumor cell lines.

Keywords

Platinum-heterocycle; tryptophan stacking; protein recognition

Introduction

Platinum-biomolecule interactions have been dominated by the study of bond-forming reactions with target (DNA) or metabolizing proteins such as Human Serum Albumin (HSA), generally considered as belonging to the field of chemistry. The pre-association through “non-covalent” interactions (strictly speaking non-coordinating) by electrostatic and/or H-bonding interactions is somewhat more characteristic of the biodisciplines, with the potential for ligand design and molecular recognition. Study of pre-association has led to new insights into the range of biomolecule reactions available to coordination compounds. Specifically from our own laboratory, non-covalent H-bonding interactions on polynuclear platinum compounds (PPCs) have described the phosphate clamp, a third mode of (ligand)-DNA interaction discrete from the classical minor groove binding and intercalation [1,2]. An unedited mode of cellular accumulation of PPCs identifying HeparanSulfateProteoGlycans (HSPGs) as receptors through initial non-covalent binding to oligosaccharides has

significant implications for new target discovery for coordination compounds [3,4]. Stacking π - π interactions play a fundamental role in biology and especially DNA/RNA-protein selective recognition. Design of metallated nucleobases in a formally substitution-inert MN_4 coordination sphere, such as $[M(\text{dien})(\text{nucleobase})]^{n+}$ ($M = \text{Pt(II)}$, $n=2$; $M = \text{Au(III)}$, $n=3$), has led to chemotypes capable of specific interactions with tryptophan-containing peptides, especially the HIV nucleocapsid protein HIVNCp7 using enhanced π - π stacking between the metallated purine/pyrimidine and the aromatic aminoacid [5,6].

The modular nature of PPCs certainly enhances the “non-covalent” binding affinity to biomolecules over their mononuclear counterparts. In examining the utility of the metallated nucleobase $[\text{PtN}_3(\text{nucleobase})]^{2+}$ chemotype for π - π stacking interactions it is of interest to extend the nature of the π -stacking ligand to other N-heterocycles. In general N-heterocycles are not as effective as their purine/pyrimidine analogs, [7,8], and we therefore decided to examine the potential of a dinuclear motif to enhance biomolecule recognition by platination of inherently weak π - π stacking ligands quinoline and benzothiazole. Examination of the dinuclear compounds in fluorescence-based assays confirmed enhancement of stacking affinity for both the simple aminoacid N-acetyltryptophan (N-AcTrp, N-AcW) itself and incorporated into a pentapeptide of biological relevance N-ACWLDSW-OH based on the p53-mdm2 recognition site [9]. Molecular modeling indicated a possible sandwich structure as the source of stacking affinity augmentation. Comparison of binding to a model nucleobase 5'-GMP confirmed specificity for the aromatic aminoacid.

Experimental Section

Synthesis and Characterization

The starting complex K_2PtCl_4 was purchased from Engelhard Corporation, Seneca, SC while *cis*- $\text{PtCl}_2(\text{NH}_3)_2$ and $\text{PtCl}_2(\text{en})$ were synthesized by the literature method [10]. All other chemicals and solvents were purchased from Aldrich and used without purification. The pentapeptide N-AcWLDSW-OH was purchased from GenScript Corporation. NANOpure water was obtained from a Barnstead Ultrapure water system. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ.

^1H NMR measurements

Complexes **3** and **4** were recorded in $\text{DMF-}d_7$ solutions while all other Pt complexes were recorded in D_2O at 300 MHz on a Varian Oxford 300 NMR spectrometer at 293 K. Chemical shifts (δ , ppm) are referenced to TMS ($\text{Si}(\text{CH}_3)_4$) and TSP ($((\text{CH}_3)_3\text{Si}(\text{CD}_2)_2\text{CO}_2\text{Na})$) for $\text{DMF-}d_7$ and D_2O , respectively. The interactions between 5'-GMP and Pt complexes were monitored by NMR titrations of Pt complexes ($1 \times 10^{-3} \text{ M}$) with 5'-GMP (0 to 20 equivalents) in D_2O . The association constants (K_a) between 5'-GMP and Pt complexes were evaluated by the Benesi-Hildebrand equation [11]:

$$\frac{1}{\Delta\delta} = \frac{1}{\Delta\delta_c K_a} \cdot \frac{1}{[5'-\text{GMP}]} + \frac{1}{\Delta\delta_c} \text{ where } \delta = \delta_0 - \delta_c; \delta_c = \delta_0 - \delta_c \text{ where } \delta_0 = \text{chemical shift of H in Pt complex with no added 5'-GMP; } \delta = \text{chemical shift of H in Pt complex with added 5'-GMP and } \delta_c = \text{chemical shift of H in Pt complex when completely complexed by}$$

5'-GMP. The K_a values were determined by the linear regression analyses of the plots of $1/\delta$ (ppm⁻¹) versus $1/[5'\text{-GMP}]$ (M⁻¹).

Fluorescence measurements

Fluorescence spectra were measured on a Cary Eclipse fluorescence spectrophotometer and a 10 mm cell at room temperature. N-Acetyltryptophan and NAc-WLSAW-OH solutions (1.0×10^{-5} M) were buffered with 20 mM tris(hydroxymethyl)aminoethane-HCl (pH = 7.2). The excitation wavelength was set at 280 nm, while the emission spectra were recorded at 300 – 500 nm. The excitation and emission slitwidths were set at 20 and 1.5 nm, respectively. The absorbances at $\lambda_{\text{max}} = 360$ nm were monitored at a function of increasing Pt complex concentration (0 to 4.0×10^{-4} M). The association constant (K_a) between the tryptophan indole ring and Pt complex was evaluated by the Eadie-Hofstee equation [12]:

$\Delta F = -\frac{1}{K_a} \cdot \frac{\Delta F}{[Pt]} + \Delta F_c$, where $F = F_0 - F$. F_0 = fluorescence intensity of N-AcTrp or peptide; F = fluorescence intensity of N-AcTrp in the presence of Pt complex; $[Pt]$ = concentration of Pt complex $F_c = F_0 - F_\infty$; F_∞ = fluorescence intensity when N-AcTrp or peptide is completely complexed with Pt complex. K_a = association constant (M⁻¹). The K_a values were determined by the linear regression analyses of the plots of F versus $F/[Pt]$ (M⁻¹).

K[PtCl₃(quin)] (1)

Complex **1** was synthesized following published methods for mononucleoside [13] and monopyridine [14] platinum complexes with modification. A suspension of K₂PtCl₄ (2.0 g, 4.82 mmol) in 50 mL of anhydrous DMF was heated at 65 °C for 30 min or until all the platinum salt dissolved. Quinoline (570 μL, 4.82 mmol) was added and the dark orange solution was stirred for 16 h at 70 °C. After the volume of the orange solution was reduced to 5 mL under reduced pressure, the solution was stored at 4 °C for 12 h to induce the precipitation of KCl. The precipitate was filtered off, washed with 2 mL cold DMF. A 50 mL solution of acetone/Et₂O (1:2 v/v) was added and a bright yellow powder was filtered off, and washed with Et₂O. Crude **1** (1.54 g) was stirred in 100 mL 0.3 M HCl for 30 min at 80 °C. The insoluble pale yellow solid was filtered off and the dark yellow filtrate was concentrated to 5 mL. Acetone (20 mL) was added and the solution was allowed to evaporate at room temperature for 16 h during which time orange crystalline solids formed. **1** was filtered off, washed with Et₂O and dried *in vacuo*. Yield: 1.20 g (53 %). ¹H NMR (D₂O): δ 9.85 (d, 8.7 Hz, 1H), 9.29 (d, 5.7 Hz, 1H), 8.44 (d, 8.1 Hz, 1H), 8.02 (m, 2H), 7.74 (dd, 1H), 7.53 (dd, 1H). Anal. Calcd for C₉H₇NCl₃KPt: C, 23.01; H, 1.50; N, 2.98. Found: C, 23.12; H, 1.40; N, 2.98.

K[PtCl₃(bztz)] (2)

Benzothiazole (666 μL, 6.10 mmol) was added to a solution of K₂PtCl₄ (2.53 g, 6.10 mmol) in 50 mL anhydrous DMF which has been heated to 65 °C. The dark orange solution was stirred for 20 h at 70 °C during which time KCl was precipitated. The volume of the reaction mixture was concentrated to 5 mL under reduced pressure and the flask was stored at 4 °C for 12 h. KCl was filtered off and washed with 2 mL cold DMF. Addition of MeOH (15 mL)

followed by Et₂O (50 mL) to the dark orange filtrate resulted in the precipitation of an orange gel-like solid. This solid was filtered, collected and stirred in 30 mL 0.3 M HCl for 15 min at room temperature. The insoluble yellow residue, *cis*-PtCl₂(bztz)₂, that remained undissolved was filtered off and washed with 5 mL H₂O. The filtrate was evaporated to dryness and acetone was added to the residue and the insoluble yellow solid was again filtered off. A solution of isopropanol/Et₂O (30 mL; 1:2 v/v) was added to the filtrate and the mixture was allowed to evaporate for 16 h during which time an orange powder precipitated. The product was filtered off, washed with Et₂O and dried *in vacuo*. Up to three crops of **2** may be obtained from the filtrate. Total yield: 1.30 g (45 %). ¹H NMR (D₂O): δ 9.64 (s, 1H), 8.90 (d, 8.4 Hz, 1H), 8.07 (d, 8.1 Hz, 1H), 7.78 (dd, 1H), 7.64 (dd, 1H). Anal. Calcd for (C₇H₅NCl₃SKPt)·2(H₂O): C, 16.43; H, 1.77; N, 2.74. Found: C, 16.24; H, 1.69; N, 2.96.

[*cis*-Pt(Cl)(I)(quin)]₂(μ-1,4-dach) (**3**)

Mixed chloriodo mixed amine platinum(II) complexes were prepared following a published method [15]. The triaminemonochloroplatinum(II) complexes were prepared following published methods [16,17,18]. A solution of KI (2.10 g, 12.65 mmol) in 20 mL H₂O was added to a suspension of **1** (1.48 g, 3.15 mmol) in 60 mL H₂O. The mixture was stirred for 2 h during which time a light orange suspension formed. A solution of *trans*-1,4-diaminocyclohexane (1,4-dach, 270 mg, 2.36 mmol) in 20 mL H₂O was added and the mixture was stirred for 20 h at room temperature during which time a light yellow suspension formed. The yellow solid of **3** was filtered off, washed with H₂O (3 X 5 mL), EtOH (2 X 5 mL), Et₂O (2 X 5 mL) and dried *in vacuo*. Yield: 1.50 g (88 %). ¹H NMR (DMF-d₇): δ 9.79 (d, 9.0 Hz, 2H), 9.54 (m, 2H), 8.62 (d, 7.8 Hz, 2H), 8.11 (d, 8.1 Hz, 2H), 8.04 (m, 2H), 7.76 (m, 2H), 7.66 (m, 2H), 5.22 (br, 2H, NH₂), 5.09 (br, 2H, NH₂), 2.20 (br, 2H, H₁' of dach), 2.06 (br, 4H, H_e of dach), 1.21 (br., 4H, H_a of dach). Anal. Calcd for C₂₄H₂₈N₄Cl₂I₂Pt₂: C, 26.51; H, 2.60; N, 5.15. Found: C, 26.30; H, 2.50; N, 5.00.

[*cis*-Pt(Cl)(I)(bztz)]₂(μ-1,4-dach) (**4**) was synthesized following the procedure of **3** except **2** (546 mg, 1.15 mmol), KI (770 mg, 4.64 mmol) and 1,4-dach (130 mg, 1.14 mmol) were used. Yield: 577 mg (91 %). ¹H NMR (DMF-d₇): δ 10.03 (d, 10.5 Hz, 2H), 8.94 (d, 8.1 Hz, 2H), 8.28 (d, 7.8 Hz, 2H), 7.78 (dd, 2H), 7.64 (dd, 2H), 5.20 (br, 2H, NH₂), 5.12 (br, 2H, NH₂), 2.25 (br, 2H, H₁' of dach), 2.16 (br, 4H, H_e of 1,4-dach), 1.27 (br., 4H, H_a of 1,4-dach). Anal. Calcd for C₂₀H₂₄N₄Cl₂I₂S₂Pt₂: C, 21.85; H, 2.20; N, 5.10. Found: C, 22.09; H, 2.18; N, 5.05.

cis-[PtCl(quin)(NH₃)₂](NO₃) (**5**)

To a suspension of *cis*-[PtCl₂(NH₃)₂] (1.0 g, 3.33 mmol) in 45 mL anhydrous DMF was added AgNO₃ (566 mg, 3.33 mmol), and the mixture was stirred at room temperature for 24 h in the dark. The precipitated AgCl was filtered off through a pad of Celite, and quinoline (394 μL, 3.33 mmol) was added to the pale yellow filtrate. After the resulting dark yellow solution was stirred at room temperature for 24 h, the solution was evaporated to dryness under reduced pressure. H₂O (60 mL) was added to the brown residue. An insoluble yellow solid was filtered off, and the pale yellow filtrate was treated with activated carbon (500 mg) and stirred for 10 min. After the solution was passed through a pad of Celite, the resulting pale yellow filtrate was evaporated to dryness and EtOH (20 mL) was added. The insoluble

yellow solid was filtered off and the resulting colorless filtrate again evaporated to dryness. To the pale yellow oily residue that remained, was added 5 mL EtOH. After the mixture was placed in a sonication bath for 30 min, an off-white solid was precipitated. The crude **5** was filtered off, washed with Et₂O and recrystallized in a minimum quantity of MeOH at 4 °C. White powdery **5** formed, which was filtered off, washed with Et₂O and dried *in vacuo*. Yield: 616 mg (40 %). ¹H NMR (D₂O): δ 9.61 (d, 1H, 9.0 Hz), 9.27 (d, 1H, 5.7 Hz), 8.57 (d, 1H, 8.4 Hz), 8.07 (m, 2H), 7.80 (dd, 1H, 7.2 Hz), 7.61 (dd, 1H, 4.8 Hz). Anal. Calcd for C₉H₁₃N₄ClO₃Pt: C, 23.72; H, 2.88; N, 12.29; Cl, 7.78. Found: C, 23.33; H, 2.74; N, 12.07; Cl, 7.64.

[PtCl(quin)(en)](NO₃) (**6**) was synthesized using the method for **5** except [PtCl₂(en)] (300 mg, 0.92 mmol), AgNO₃ (156 mg, 0.92 mmol) and quinoline (110 μL, 0.92 mmol) were used. The final product was recrystallized from 10 mL solution of EtOH/acetone (1:4, v/v) at 4 °C. Yield: 250 mg (56 %). ¹H NMR (D₂O): δ 9.54 (d, 1H, 9.0 Hz), 9.25 (d, 1H, 5.7 Hz), 8.57 (d, 1H, 9.0 Hz), 8.05 (m, 2H), 7.79 (dd, 1H, 7.8 Hz), 7.61 (dd, 1H, 5.4 Hz), 2.75 (m, (CH₂)₂ of en). Anal. Calcd for C₁₁H₁₅N₄ClO₃Pt: C, 27.42; H, 3.14; N, 11.63; Cl, 7.36. Found: C, 27.55; H, 3.01; N, 11.45; Cl, 7.35.

cis-[PtCl(bztz)(NH₃)₂]Cl (7**)**

A suspension of *cis*-[PtCl₂(NH₃)₂] (1.0 g, 3.33 mmol) in 100 mL H₂O was heated at 65 °C until all solid dissolved. Benzothiazole (364 μL, 3.33 mmol) was added and heating continued at 65 °C for 16 h. The cloudy light yellow solution was passed through a pad of Celite and the filtrate was evaporated to dryness. After adding MeOH (20 mL) to the residue, a yellow solid remained insoluble and was filtered off. Concentration of the filtrate resulted in the precipitation of a white solid. Crude **7** was filtered off, washed with Et₂O and recrystallized from a minimal amount of MeOH at 4 °C. White powdery **7** formed, and was filtered off, washed with Et₂O and dried *in vacuo* at room temperature. Yield: 650 mg (45 %). ¹H NMR (D₂O): δ 9.54 (s, 1H), 8.73 (d, 1H, 8.7 Hz), 8.00 (d, 1H, 7.8 Hz), 7.67 (dd, 1H, 8.4 Hz), 7.54 (dd, 1H, 7.8 Hz). Anal. Calcd for C₇H₁₁N₃Cl₂SPt: C, 19.32; H, 2.55; N, 9.65; Cl, 16.29. Found: C, 19.03; H, 2.54; N, 9.44; Cl, 16.25.

[PtCl(bztz)(en)]Cl (**8**) was synthesized using the method for **7** except [PtCl₂(en)] (1.20 g, 3.68 mmol) and benzothiazole (400 μL, 3.66 mmol) were used. Yield: 950 mg (56 %). ¹H NMR (D₂O): δ 9.69 (s, 1H), 8.87 (d, 1H, 8.4 Hz), 8.18 (d, 1H, 8.1 Hz), 7.84 (dd, 1H, 7.8 Hz), 7.72 (dd, 1H, 7.7 Hz), 2.77 (br, 4H, (CH₂)₂). Anal. Calcd for C₉H₁₃N₃Cl₂SPt: C, 23.44; H, 2.84; N, 9.11. Found: C, 22.96; H, 2.81; N, 8.74.

[(Pt(en)(quin))₂(μ-1,4-dach)](NO₃)₄ (9**)**

To a solution of **3** (484 mg, 0.45 mmol) in 30 mL DMF was added AgNO₃ (302 mg, 1.78 mmol), and the solution was stirred at room temperature for 24 h in the dark. The precipitated AgCl and AgI were filtered off through a pad of Celite and ethylenediamine (60 μL, 0.90 mmol) was added to the pale yellow filtrate. After the solution was stirred at room temperature for 24 h, DMF was removed under reduced pressure. H₂O (30 mL) was then added to dissolve the dark yellow residue. The solution was treated with 0.5 g activated carbon and stirred for 1 h. The carbon was filtered off through a pad of Celite and the

resulting colorless filtrate was evaporated to dryness. EtOH was added to the oily residue and a white powder precipitated upon sonication. **9** was filtered off and dried *in vacuo* at room temperature. Yield: 155 mg (30 %). ^1H NMR (D_2O): δ 9.40 (d, 2H, 8.7 Hz), 9.30 (br, 2H), 8.69 (d, 2H, 8.1 Hz), 8.17 (d, 2H, 8.4 Hz), 8.10 (m, 2H), 7.87 (m, 2H), 7.70 (m, 2H), 2.80 (m, 8H, $(\text{CH}_2)_2$), 2.39 (m, 2H, H_1' of dach), 2.16 (m, 4H, H_e of dach), 1.10 (m, 4H, H_a of dach). Anal. Calcd for $\text{C}_{28}\text{H}_{44}\text{N}_{12}\text{O}_{12}\text{Pt}_2\cdot 4\text{H}_2\text{O}$: C, 27.96; H, 4.35; N, 13.97. Found: C, 27.88; H, 3.91; N, 13.98.

$[\{\text{cis-Pt}(\text{NH}_3)_2(\text{bztz})\}_2(\mu\text{-1,4-dach})](\text{NO}_3)_4$ (10**)**

To a solution of **7** (200 mg, 0.460 mmol) in 30 mL DMF was added AgNO_3 (156 mg, 0.918 mmol), and the solution was stirred at room temperature for 20 h in the dark. After AgCl was filtered off through a pad of Celite, *trans*-1,4-diaminocyclohexane (26 mg, 0.230 mmol) was added to the colorless filtrate. After the solution was stirred for 24 h, it was evaporated to dryness under reduced pressure and H_2O (50 mL) was added to the resulting brown residue. Activated carbon (0.25 g) was added to the mixture, stirred for 30 min and filter off through a Celite pad. The colorless filtrate was evaporated to dryness and EtOH was added to the residue. A clear and homogenous solution was obtained after heating the solution at reflux for 30 min. A white powder was obtained after 16 h at 4 °C. **10** was filtered off and dried *in vacuo*. Yield: 150 mg (60 %). ^1H NMR (D_2O): δ 9.80 (br, 2H), 8.80 (m, 2H), 8.19 (d, 2H, 8.1 Hz), 7.83 (m, 2H), 7.73 (m, 2H), 2.38 (m, 2H, H_1' of dach), 2.12 (m, 4H, H_e of dach), 1.04 (m, 4H, H_a of dach). Anal. Calcd for $\text{C}_{20}\text{H}_{36}\text{N}_{12}\text{O}_{12}\text{S}_2\text{Pt}_2\cdot 2\text{H}_2\text{O}$: C, 21.32; H, 3.58; N, 14.92. Found: C, 21.06; H, 3.32; N, 14.78; Cl.

$[\{\text{Pt}(\text{en})(\text{bztz})\}_2(\mu\text{-1,4-dach})](\text{NO}_3)_4$ (**11**) was synthesized following the method of **10** using **8** (500 mg, 1.084 mmol), AgNO_3 (368 mg, 2.166 mmol) and *trans*-1,4-diaminocyclohexane (62 mg, 0.543 mmol). Yield: 300 mg (48 %). ^1H NMR (D_2O): δ 9.79 (br, 2H), 8.76 (m, 2H), 8.19 (d, 2H, 8.1 Hz), 7.83 (m, 2H), 7.72 (m, 2H), 2.76 (m, 8H, $(\text{CH}_2)_2$), 2.67 (m, 2H, H_1' of dach), 2.17 (m, 4H, H_e of dach), 1.12 (m, 4H, H_a of dach). Anal. Calcd for $(\text{C}_{24}\text{H}_{40}\text{N}_{12}\text{O}_{12}\text{S}_2\text{Pt}_2)\cdot 4(\text{H}_2\text{O})$: C, 23.73; H, 3.98; N, 13.86. Found: C, 23.68; H, 3.53; N, 13.64.

Results and Discussion

Synthesis of Dinuclear Pt Complexes

The synthetic schemes for quinoline, **9**, and the benzothiazole **10** and **11** derivatives are given in Figure 1. The monoamine complexes **1** and **2** were prepared by the addition of one equivalent of quinoline and benzothiazole to DMF solutions of K_2PtCl_4 , respectively. While the products *cis*- $[\text{PtCl}_2\text{X}]$ (X = quin or bztz) were also formed, their insolubility in acetone and H_2O allows for separation from the monoamine complexes. In considering potential applications to protein binding and especially π - π stacking a rigid backbone was considered desirable and we therefore used the 1,4-diaminocyclohexane (1,4-dach) as bridging ligand to impart some degree of conformational inflexibility to the molecules.

The reactions of $\text{K}[\text{PtCl}_3\text{X}]$ with 1,4-dach failed to produce complete reactions and pure $\{\text{cis-PtCl}_2\text{X}\}_2(\mu\text{-1,4-dach})$ could not be obtained despite variation of solvents (MeOH and

H₂O) and reaction temperatures (reaction heated to 60°C). Addition of KI to K[PtCl₃X] leads to the initial formation of *trans*-[PtI₂CIX][−] which in turn accelerates the formation of the dinuclear complexes **3** and **4**. Addition of AgNO₃ to a DMF solution of **3** followed by the addition of en leads to the formation of **9**.

Analogous reactions of [*cis*-Pt(Cl)(I)(bztz)]₂(μ-1,4-dach) (**4**) with AgNO₃ followed by NH₄OH or en led to dark blue solutions. ¹H NMR spectra of the residue indicated that the benzothiazole ligand had decomposed (no signals). An alternative method for the preparation of **10** is given in Figure 1. Addition of benzothiazole to *cis*-PtCl₂(NH₃)₂ or PtCl₂(en) results in the displacement of one chloride ligand by the planar amine forming **7** and **8**, respectively. Removal of the second chloride by addition of AgNO₃ followed by 1,4-dach produced the dinuclear complexes **10** and **11**.

¹H NMR spectra

The ¹H NMR spectrum of **9** in D₂O is shown in Figure S1. The two identical Pt units gave one set of quinoline peaks. As a result of the rapid fluctuation of 1,4-dach, the axial and equatorial protons are seen as broad peaks. The 1,4-dach protons were identified based on [¹H, ¹H] COSY assignments: H_a is correlated with both H_c and H₁' while H_c and H₁' are not correlated. Complexes **3**, **5**, **10** and **11** display comparable spectra.

Fluorescence quenching of *N*-acetyltryptophan

Fluorescence quenching of NAcTrp occurs when the indole ring forms a stacking complex with other aromatic molecules by electron transfer from the excited state of the indole ring to an aromatic system [19,20,21]. The strength of the stacking interactions were calculated from Eadie-Hofstee plots (Figure 3 and Table 1).

The K_a values show that there is an enhancement of NAcTrp stacking for the dinuclear Pt(II) complexes compared to the mononuclear Pt(II) analogs including compounds studied earlier such as [Pt(dien)(quin)]²⁺[7]. The intensity of quenching decreases in the order of **9** > **10** > **11** > **6** > **8** > **5** ≈ **7**. In particular, the dinuclear complex containing quinoline (K_a = 8900 ± 500 M^{−1} for **9**) has also greatly enhanced interactions over those containing benzothiazole ligands (K_a = 5400 ± 300 for **10** and 4200 ± 300 for **11**).

We then examined the potential application to a more relevant biological system. The pentapeptide motif FLDSW is a critical recognition component of the p53-mdm2 interaction [9]. We therefore compared the tryptophan results with a model peptide N-AcWLDSW to examine potential stacking interactions of mono- and dinuclear complexes **5** – **11** in a more complex system, Figure 4. Fluorescence studies gave K_a values of 2500 ± 500, 2900 ± 300, 1400 ± 500, 3000 ± 400, 12000 ± 1000, 7800 ± 400 and 5100 ± 300 for complexes **5**, **6**, **7**, **8**, **9**, **10** and **11**, respectively (Table 1). These value emphasize that dinuclear Pt complexes, and again particularly the quinoline complex **9**, are better quenchers of fluorescence than their mononuclear analogues and that there is enhancement over free tryptophan in all cases.

The quinoline system showed the highest quenching, and significantly enhanced over that of the single aminoacid. In contrast, there is little or no difference between N-AcTrp and N-AcWLDSW for the mononuclear compounds. To explain this unusual enhancement,

molecular modeling showed that the nature of the molecule (**9**) actually allows for “sandwiching” of one tryptophan rather than binding to both aminoacids simultaneously, even allowing for a relatively flexible conformation in the pentapeptide, Figure 5A. Calculation of F-W distances in the p53 recognition sequence show that while suitable linker design may accommodate binding to both aromatic aminoacids, the relatively short distance is likely to also favor a “sandwich” binding for the tryptophan moiety Figure 5B.

Interaction of dinuclear Pt(II) complexes containing planar aromatic ligands with 5'-GMP

Given that, within this series, the 4+ quinoline derivative, **9**, has highest binding affinity and the potential explanation, it was of interest to study its interaction with 5'-GMP, which is -2 charged. Figure S2 shows the ^1H NMR spectra of **9** as increasing concentrations of 5'-GMP are added. The upfield shifts of both the quinoline resonances and the H_8 of 5'-GMP are indicative of aromatic ring-current effects [20]. Furthermore, no covalent bonding of the Pt center to the N_7 of guanine moiety of 5'-GMP was observed as this would lead to a downfield shift of the H_8 resonance. The chemical shifts of other protons not involved in stacking interactions in both species remain unchanged. The interaction between **9** and 5'-GMP is indeed electrostatic because increasing the ionic strength of the solutions with NaClO_4 leads to diminished upfield shifts of stacking protons. Similar results were also observed when 5'-GMP is reacted with **10**. The association constants, K_a , for the interactions of the quinoline protons in **9** and benzothiazole protons in **10** with 5'-GMP were determined by the Benesi-Hildebrand Plot (see Figure 6A). The K_a values for each proton, given in Figure 6B, indicate that the strongest interactions were observed for the proton furthest away from the Pt center. Values are significantly less than for N-AcTrp, confirming earlier results that PtN_4 coordination spheres of this type are unlikely to have high affinity for nucleic acids [22].

Conclusions

The results show that use of a dinuclear motif can enhance stacking ability of relatively weak ligands such as quinoline. In the case of the pentapeptide N-ACWDSW-OH the molecular model suggests that the enhanced stacking is likely to be the result of a sandwich rather than binding to both Trp simultaneously. This new mode of binding further expands the potential for design of “non-covalent” interactions. The linker and ancillary ligands give scope for design of specific compounds with potential to inhibit the p53-mdm2 interaction, an initial consideration of this work, but the enhancement of stacking even for the one tryptophan moiety is of relevance.

In this latter respect we have studied extensively the use of $[\text{Pt}(\text{dien})(9\text{-EtGua})]^{2+}$ (9-EtGua = 9-Ethylguanine) as a prototype for Trp stacking in HIVNCp7 [5,7,8]. In general the use of the purine enhances stacking over N-heterocycles but the dinuclear compound **9** is significantly more effective than $[\text{Pt}(\text{dien})(9\text{-EtGua})]^{2+}$ [5,8,21]. Synthetic routes to the 9-EtGua analog of **9** are not as yet available but synthesis of a model compound from the known $[\{\text{PtCl}(\text{en})\}_2\mu\text{-NH}_2(\text{CH}_2)_6\text{NH}_2]^{4+}$, [23], by displacement of the Pt-Cl bond with 9-EtGua gave compound **12**, Figure 7. Preliminary studies show similarly enhanced binding to both NAcTrp and the pentapeptide with K_a of approx. $3 \times 10^4 \text{ M}^{-1}$, a full order of

magnitude greater than the mononuclear analog [5]. A 1:1 adduct of **12**:N-AcWLASW-OH is observed by ESI Mass Spectrometry. Compound **12** in standard MTT assays, [3], showed an IC₅₀ of approximately 1.5 μ M in the highly cisplatin-sensitive A2780 ovarian cancer cell line while the corresponding value in OsACL, an osteosarcoma containing wtp53 and which overexpresses mdm2 protein, [24], is approximately 20 μ M. The interpretation of these results with respect to the proposed target may be explored more fully with protein inhibition and expression assays [25].

An inherent question in our “non-covalent” targeting approach mimicking the Trp-RNA/DNA reaction of nature is how we can modulate binding affinity. The potential for design of more specific inhibitors is worthy of further study as the dinuclear motif offers significant flexibility in design. Further, the suggested “sandwich” mode is to our knowledge a unique feature accessible to coordination compounds and may find analogy in use of bis-intercalating agents to enhance DNA stacking interactions. Future studies will focus on this aspect of “non-covalent” dinuclear Pt compounds using stacking and electrostatic interactions to target specific protein sequences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Synthetic schemes for dinuclear N-heterocycle quinolone compounds with rigid backbone linkers.

Figure 2.
Synthetic schemes for dinuclear N-heterocycle benzothiazole compounds with rigid backbone linkers.

Figure 3.
Eadie-Hofstee Plot - Fluorescence Quenching of *N*-Acetyltryptophan by **9**.

Figure 4.
Sequence of pentapeptide NAcWLDSW-OH used in quenching studies.

Figure 5.

A: Molecular model of the interaction of Complex 9 with N-AcWLDSW, based on B: recognition sequence of p53-mdm2 [9].

Figure 6.

A: Benesi-Hildebrand plot for determination of K_a for H_3 of **9** with 5'-GMP. **B:**

Association constants, K_a , for planar amine protons of quinoline and benzothiazole of **9** and **10** with 5'-GMP.

Figure 7.
Potential dinuclear 9-EtGua-based compounds ($[{\{PtCl(en)\}_2\mu-NH_2(CH_2)_6NH_2}]^{4+}$,
Compound 12) for enhanced tryptophan stacking.

Table 1

Fluorescence Quenching of *N*-Acetyltryptophan and *N*-AcWLASW-OH peptide by Pt(II) Complexes Containing Planar Aromatic Ligands.

Complexes	K_a (M^{-1}) <i>N</i> -Acetyltryptophan	K_a (M^{-1}) WLASW peptide
<i>cis</i> -[PtCl(quin)(NH ₃) ₂](NO ₃) (5)	1700 ± 300	2500 ± 500
[PtCl(quin)(en)](NO ₃) (6)	3000 ± 300	2900 ± 300
<i>cis</i> -[PtCl(bztz)(NH ₃) ₂]Cl (7)	1700 ± 500	1400 ± 500
[PtCl(bztz)(en)]Cl (8)	2500 ± 500	3000 ± 400
[{Pt(en)(quin)} ₂ -μ-(1,4-dach)](NO ₃) ₂ (9)	8900 ± 500	12000 ± 1000
[{ <i>cis</i> -Pt(NH ₃) ₂ (bztz)} ₂ -μ-(1,4-dach)](NO ₃) ₂ (10)	5400 ± 300	7800 ± 400
[{Pt(en)(bztz)} ₂ -μ-(1,4-dach)](NO ₃) ₂ (11)	4200 ± 300	5100 ± 300