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Synthesis, Characterization, Interaction with DNA, and Antitumor Activity of a *cis*-Dichloroplatinum(II) Complex Linked to an Intercalator *via* one Methylene Chain

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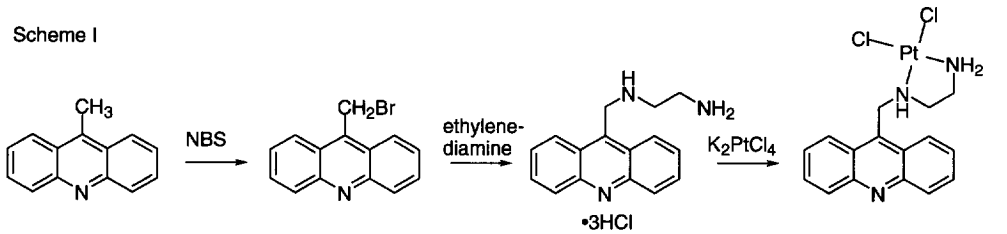
Abstract: A cisplatin-type complex tethered to acridine intercalator *via* one methylene chain has been synthesized. Its structure was elucidated by X-ray crystallography. A permeability into cell, interaction with calf thymus DNA (ct-DNA), and antitumor activity against HeLa and P388 cells were studied. © 1997 Elsevier Science Ltd.

Cisplatin, *cis*-Diamminedichloroplatinum(II) (*cis*-DDP), is one of the most effective anticancer agents that are clinically active to solid tumors.[1] Cytotoxicity of this drug is regarded as the consequence of making a covalent 1,2-intrastrand adduct with N7 of guanine base of DNA.[2] A lot of derivatives of *cis*-DDP having DNA attractive regions were investigated and their affinity with DNA and cross-link ability were studied.[3] Though intercalator-linked platinum complex has been also extensively studied, the tethered chain was somehow restricted to long methylene chain taking account of the shirking of the steric disadvantage.[3b,c] Shorter linkage, however, should be expected to produce the possibilities of (i) modulation of covalent binding rate due to noncovalent intercalation, (ii) improved sequence selectivity of platination by compatible binding, and (iii) increasing selectivity of photocleavage site of platinated DNA in the case of photoactivable intercalator.

In this communication we report the preparation, structural characterization, interaction with calf thymus DNA (ct-DNA), and biological activities of a platinum complex tethered to an acridine intercalator *via* one methylene chain.

Scheme I



1 **2** **3** **4**

Synthesis and Structure Determination of **4**

[9-(2-aminoethyl)aminomethylacridinio]dichloroplatinum(II) (**4**) was synthesized from 9-methylacridine[4] in the route shown in scheme I. All the compounds obtained (**1**-**4**) were characterized by ^{1}H and ^{13}C-NMR, IR, MS, and elemental analyses.[5]

Recrystallization of **4** from DMF afforded single crystals suitable for X-ray crystallography. Elucidated structure was shown in Fig. 1.[6] The coordination around platinum is almost square planar as the related platinum complexes reported earlier.[3] The Pt-N and Pt-Cl distances (Pt-N1, 2.02(2); Pt-N2, 2.05(2); Pt-Cl1, 2.298(5); Pt-Cl2, 2.313(5) Å) are also typical values as *cis*-diaminodichloroplatinum(II) complex.[3] Figure 1b demonstrates the existence of intermolecular π-π stacking interaction and hydrogen-bond association between terminal aliphatic nitrogen atom and chlorine atom.

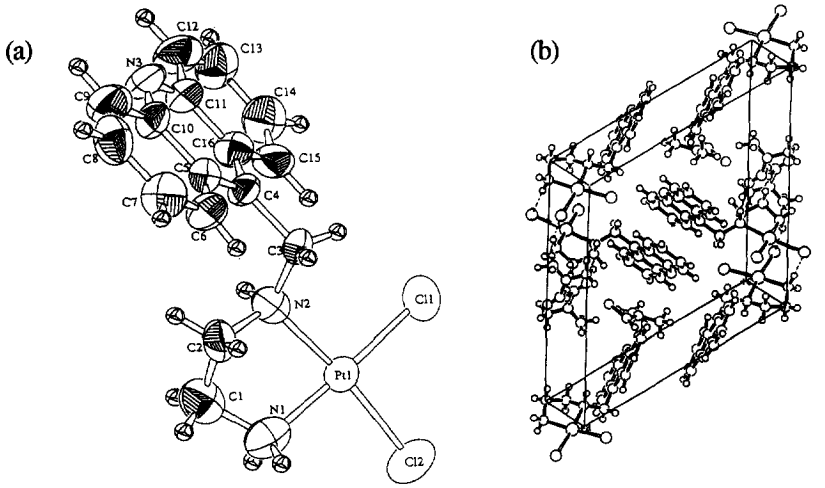


Figure 1. Structure of Pt complex **4**: (a) Molecular structure; (b) Unit cell packing. Dotted lines show hydrogen bond.

Evaluation of Penetration Activity of **3** *and* **4** *into Cell*

The permeability of fluorescent compounds **3** and **4** into cell were investigated by fluorescent microscopic analyses. Human uterine cancer cell (HeLa cell) was incubated in the presence of 3.8×10^{-5} M of ligand **3** or platinum complex **4** for 2 hours in the growth media (MEM contains 10 % Fetus Bovine Serum) and was analyzed by fluorescent microscopy. As a result, no fluorescence from nucleus of cell was observed in the cases of neither **3** nor **4** in contrast with acridine orange used as the reference. Moreover, in the concentration range of 10^{-6}-10^{-5} M, **4** did not inhibit the growth of the cell. These results indicate that **3** and **4** could not be taken into nucleus of HeLa cell and didn't affect the cell survival in the present experimental condition.

Evaluation of DNA Binding Activity of 3 and 4

The fluorescent titration spectra of **3** and **4** with ct-DNA were shown in Fig. 2. Binding of the **3** and **4** with the ct-DNA double helix was found to quench the acridine fluorescence significantly. The electronic absorption spectrum of **3** in the presence of ct-DNA also exhibited a little red-shift and strong decrease in the peak intensity, while **4** did not. These spectral changes were considered to intercalative binding and the noncovalent association constants K_{ass} were estimated using McGhee-von Hippel equation.[7] The K_{ass} value obtained from the fluorescent titration (3.4×10^4 M^{-1}) corresponded with that from absorption spectral change (3.6×10^4 M^{-1}) in **3** within experimental error. The binding number of metal-free ligand was estimated to 3.9. The association constant and binding number of platinum complex (**4**) obtained from fluorescence measurement are 4.3×10^4 M^{-1} and 1.7, respectively. During the DNA titration experiment, no additional spectral change due to covalent binding of cisplatin moiety with DNA was detected.

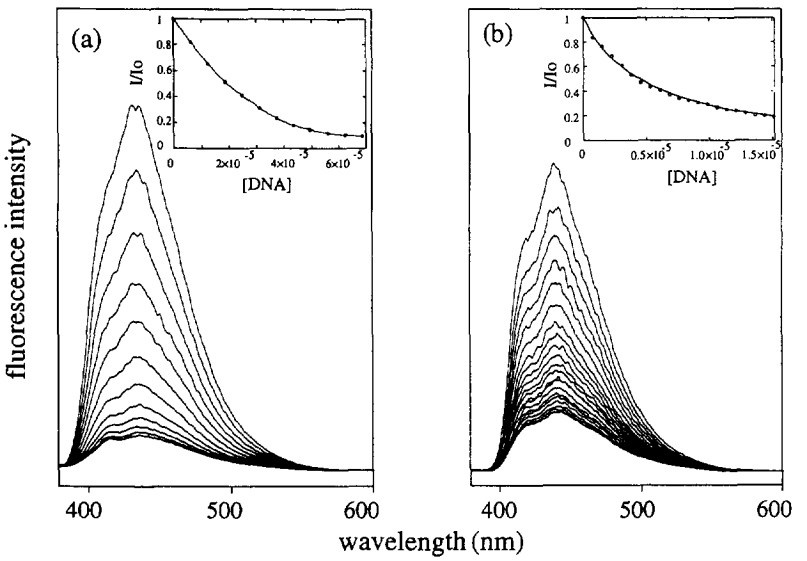


Figure 2. Fluorescence spectra of **3** and **4** in the presence of increasing amount of ct-DNA. (a) for **3**, in 1 mM sodium cacodylate buffer contains 4 mM of NaCl. [**3**] = 1.25×10^{-5} M, [DNA] = 0 - 6.86×10^{-5} M, upon excitation at 363 nm. Inset: plot of the emission titration data monitored at 426 nm. (b) for **4**, in 1 mM sodium cacodylate buffer contains 4 mM of NaCl and 20 % DMF. [**4**] = 1.25×10^{-5} M, [DNA] = 0 - 1.52×10^{-5} M, upon excitation at 360 nm. Inset: plot of the emission titration data monitored at 430 nm.

Antitumor Activity of 4 Against P388 Cell in Mice

10^6 P388 cells were intraperitoneally (ip) transplanted into CDF_1 mice, then the platinum complex **4** was given by the ip method two times on the days 1 and 5. The mean survival time of the treated group (T) was compared with that of untreated control group (C). As the results, this

compound was found to have a small pharmacological effect (T/C value = 113 % at 200 mg/kg dose). It must be noticed that no toxicity to exterminate the mice was observed even in the highest dose (the same T/C value at 400 mg/kg). The observed very small biological effect is considered to a consequence of an insufficient penetrative activity of the complex.

In conclusion, the cisplatin-type complex **4** tethered to acridine intercalator *via* one methylene chain reported here is regarded as the possible candidate to modulation drug of *cis*-DDP in the cancer chemotherapy. The ability of **4** to cross-link with double stranded DNA is under investigation.

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References and Notes

1. For recent review, Pil, P.; Lippard. S. J. In *Encyclopedia of Cancer*, Academic Press, New York, 1997; vol. 1, pp 392 - 410.

2. For X-ray crystallographic analyses of *cis*-DDP guanine adducts, (a) Takahara, P. M.; Rosenzweig. A. C.; Frederich, C. A.; Lippard, S. J. *Nature*, **1995**, *377*, 649. (b) Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *Science*, **1985**, *230*, 412.

3. (a) Gibson, D.; Gean, K.-F.; Ben-Shoshan, R.; Ramu. A.; Ringel, I.; Katzhendler, J. *J. Med. Chem.* **1991**, *345*, 414. (b) Palmer, B. D.; Lee, H. H.; Johnson, P.; Baguley, B.; Wickham, G.; Wakelin, L. P. G.; McFadyen, W. D.; Denny, W. A. *ibid.*, **1990**, *33*, 3008. (c) Bowler, B. E.; Ahmed, K. J.; Sundquist, W. I.; Hollis, L. S.; Whang, E. E.; Lippard, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 1299.

4. Tsuge, O.; Nishinohara, M.; Tashiro, M. *Bull. Chem. Soc. Jpn.* **1963**, *36*, 1477.

5. Elemental analysis for **4**: Calcd for $C_{16}H_{17}N_3Cl_2Pt$: C, 37.15; H, 3.31; N, 8.12. Found: C, 37.09; H, 3.79; N, 8.03.

6. Crystal Data for **4**•DMF: triclinic, $P\bar{1}$, a = 15.007(6) Å, b = 15.597(4) Å, c = 10.398(3) Å, α = 98.51(3) °, β = 96.79(3) °, γ = 114.61(2) °, V = 2144(1) Å^3, Z = 4, λ(Mo-Kα) = 0.71069 Å, R = 0.053, R_w = 0.063, GOF = 0.74. Data were collected on a Rigaku AFC7R diffractometer at room temperature. The structure was solved by the direct method (SHELEXS86) and refined by full matrix least squares analysis.

7. Liu, H.-Q.; Peng, S.-M.; Che, C. M. *J. Chem. Soc. Chem. Commun.* **1995**, 509.

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