# Synthesis and X-ray Crystal Structure of *trans,cis*-[Pt(OAc)$_2$I$_2$(en)]: A Novel Type of Cisplatin Analog That Can Be Photolyzed by Visible Light to DNA-Binding and Cytotoxic Species *in Vitro*

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An original approach intended to facilitate the intratumoral activation of Pt(IV) diamines by illumination with visible light to form photolysis products that irreversibly bind to DNA and are cytotoxic to human cancer cells is reported. The novel Pt(IV) complex *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] was prepared by the acetylation of *trans,cis*-[Pt(OH)$_2$I$_2$(en)] with acetic anhydride in $CH_2Cl_2$; *trans,cis*-[Pt(OH)$_2$I$_2$(en)] was synthesized by oxidation of [PtI$_2$(en)] with 30% aqueous $H_2O_2$. *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] crystallized from methanol as deep-red needles with $a = 9.029(4)$ Å, $b = 11.443(2)$ Å, $c = 12.822(2)$ Å, $\beta = 95.48(3)°$, monoclinic space group *Cc*, and $Z = 4$. The conformation of the acetato groups around the O−Pt−O axis deviated significantly from the conformation of the acetato groups in the X-ray crystal structure reported for the *cis*-dichloro analog, which may explain the very different aqueous solubilities of the two compounds. *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] and *trans,cis*-[Pt(OH)$_2$I$_2$(en)] displayed broad ligand-to-metal charge-transfer bands centered at $\lambda = 389$ and 384 nm, respectively ($\epsilon = 1372$ and 1425 $M^{-1}$ $cm^{-1}$, respectively), with tailing out to ca. 550 nm. When *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] was incubated with calf thymus DNA in the absence of light, no covalent binding of Pt to DNA was measurable after 6 h; however, irradiation with light of wavelengths > 375 nm resulted in 63 ± 13% of the platinum being covalently bound to DNA after 6 h, suggesting that a photoreduction to Pt(II) species took place. Although *trans,cis*-[Pt(OH)$_2$I$_2$(en)] was also labile to visible light, only 10 ± 2% DNA platination was observed after 6 h of illumination; however, covalent binding of Pt to DNA took place quantitatively when a reducing agent such as glutathione was added to the photolyzed incubations. These results provide evidence that the photolysis of the *trans*-dihydroxo analog resulted predominately in the substitution of the iodide ligands for water rather than a reduction of Pt(IV) to Pt(II). When protected from light, *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] and *trans,cis*-[Pt(OH)$_2$I$_2$(en)], both at a concentration of 10 $\mu$M, had half-lives of 6.6 ± 0.5 and 46.8 ± 8.8 h, respectively, at 37 °C in Eagle's minimum essential medium (EMEM) containing 5% fetal calf serum. When irradiated with light $\lambda_{irr} > 375$ nm, the half-lives were decreased by 24- and 53-fold for the diacetato- and dihydroxoplatinum(IV) complexes, respectively. Compared to the "dark" control, the *in vitro* treatment of TCCSUP human bladder cancer cells with *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] resulted in 35% greater growth inhibitory activity when during the first 1.5 h of drug exposure the cells were irradiated with light $\lambda_{irr} > 375$ nm. The photolysis of *trans,cis*-[Pt(OH)$_2$I$_2$(en)] with visible light resulted in a 22% enhancement of antiproliferative activity.

## Introduction

The inability of anticancer agents to specifically kill neoplastic cells means that toxicities will often be encountered at or near the dose required for therapeutic activity. One approach to restrict the cytotoxic effects of drugs to the tumor is through the use of visible light, which, because of advances in lasers and fiber optics,[1] can now be delivered directly to many localized tumors of epithelial origin. For example, photodynamic therapy (PDT), which involves the porphyrin-mediated conversion of triplet to singlet $O_2$ by visible light, has been attracting considerable attention for the treatment of a variety of cancers.[2]

There has also been some interest in developing photosensitive transition metal complexes for use in cancer chemotherapy. In this context, the effects of light on the interactions of chromium,[3] cobalt,[4] rhodium,[4c,5] and ruthenium[4c,6] complexes with DNA under carefully controlled *in vitro* conditions have been the subjects of recent investigations; however, to our knowledge there have been no reports about the effects of light on the cytotoxic activities of these complexes. We considered that platinum complexes, some of which are effective antitumor agents while others of which are photosensitive, might also be well suited for this application.

Pt(IV) complexes, compared to their Pt(II) counterparts, are extremely inert to substitution reactions,[7] and increasing evidence suggests that for Pt(IV) diamines to be active, they must first be reduced by biological reductants [e.g., ascorbate or glutathione (GSH)] to the corresponding Pt(II) antitumor agent.[7c,d,8] Thus, Pt(IV) complexes may be regarded as inactive prodrugs. The covalent binding of Pt(II) to DNA is widely consider responsible for the therapeutic activity of these drugs (for recent reviews see ref 9). We reasoned that if the rate of reduction of Pt(IV) to Pt(II) could be increased in and around the tumor relative to normal tissue, then

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**Table 1.** Important Bond Lengths (Å) and Bond Angles (deg) for *trans,cis*-[$Pt(OAc)_2I_2(en)$]

| | | | |
|---|---|---|---|
| Bond Lengths (Å) | | | |
| Pt−I(1) | 2.621(2) | Pt−I(2) | 2.632(2) |
| Pt−N(32) | 2.079(15) | Pt−N(31) | 2.100(15) |
| Pt−O(11) | 2.025(15) | Pt−O(21) | 2.032(13) |
| O(11)−C(11) | 1.261(22) | O(21)−C(21) | 1.213(22) |
| O(12)−C(11) | 1.272(26) | O(22)−C(21) | 1.308(23) |
| Bond Angles (deg) | | | |
| O(21)−Pt−O(11) | 170.92(69) | O(21)−Pt−I(2) | 86.83(43) |
| O(21)−Pt−N(32) | 94.78(59) | O(11)−Pt−I(2) | 86.85(46) |
| O(11)−Pt−N(32) | 90.94(61) | N(32)−Pt−I(2) | 174.59(46) |
| O(21)−Pt−N(31) | 86.83(54) | N(31)−Pt−I(2) | 92.66(41) |
| O(11)−Pt−N(31) | 86.96(57) | I(1)−Pt−I(2) | 91.02(5) |
| N(32)−Pt−N(31) | 82.29(61) | C(11)−O(11)−Pt | 126.36(1.38) |
| O(21)−Pt−I(2) | 92.24(38) | C(21)−O(21)−Pt | 125.21(1.25) |
| O(11)−Pt−I(1) | 94.38(41) | C(41)−N(31)−Pt | 107.23(1.07) |
| N(32)−Pt−I(1) | 94.07(45) | C(42)−N(32)−Pt | 110.05(1.20) |
| N(31)−Pt−I(1) | 176.14(42) | | |

a more effective, less toxic therapy would be achieved. Along these lines, Kido et al.[8f] showed recently that the combination of GSH with tetraplatin, a tetrachloroplatinum(IV) diamine, in a short-term (i.e., 2 h) *in vitro* treatment of L1210 cells leads to an increase in antiproliferative activity approaching that of the dichloroplatinum(II) diamine; that is the $IC_{50}$ value of tetraplatin decreases from 1.2 to 0.75 $\mu$M when 10 $\mu$M GSH is present.

The well-documented photochemistry of Pt(IV) complexes[10] provided us with a theoretical basis to develop Pt(IV) prodrugs that can be photoreduced to cytotoxic Pt(II) species by visible light. In practice, localized cancers of the lung, esophagus, oral cavity, cervix, skin, and bladder, which are accessible for illumination and also generally sensitive to cisplatin,[11] would lend themselves to this type of treatment strategy.

We recently reported[12] on the Pt(IV) complex *trans,cis*-[$PtCl_2I_2(en)$], which because of the small optical electronegativity of iodide can be photolyzed with just visible light to species that bind to DNA and inhibit the growth of human cancer cells *in vitro*. A drawback with this compound, however, is the very limited stability in the presence of serum even in the absence of light.[12] In the present paper, the synthesis and structural characterization of the novel complex *trans,cis*-[$Pt(OAc)_2I_2$-(en)] are reported; to our knowledge this is the first Pt(IV) diamine bearing equatorial iodide ligands to be characterized by X-ray crystallography. In culture medium containing serum, *trans,cis*-[$Pt(OAc)_2I_2(en)$] has much better stability in the dark compared to *trans,cis*-[$PtCl_2I_2(en)$] but can also be photolyzed by visible light to Pt species that irreversibly bind to DNA as well as inhibit the growth of human cancer cells *in vitro*.

## Results

**Synthesis.** Until very recently[12] Pt(IV) diamines with square-planar iodide ligands have not been adequately characterized in the literature. The key intermediate in the synthesis of *trans,cis*-[$Pt(OAc)_2I_2(en)$] is *trans,cis*-[$Pt(OH)_2I_2(en)$], which had been reported once before, but the structural assignment was based solely on an elemental analysis.[13] We now know that an oxidation of [$PtI_2(en)$] with 30% aqueous $H_2O_2$ (pH 2.6) at room temperature for 3−4 d leads to a ca. 1:1 mixture of starting material and product (determined by $^1$H NMR), although elemental analysis of the crude product is correct when calculated for just *trans,cis*-[$Pt(OH)_2I_2(en)$]. By stirring the gray-green crude product in pyridine for 1 h at 70 °C, the Pt(II) starting material is converted to soluble Pt(II)−pyridine complexes while the Pt(IV) product remains as an insoluble, bright-yellow solid, which can be collected by filtration in a 56% yield. The acetylation of *trans,cis*-[$Pt(OH)_2I_2(en)$] is achieved by reacting the Pt(IV) complex with an excess acetic anhydride in a manner similar to that described for other *trans*-dihydroxoplatinum(IV) ethylenediamines.[14] Crude *trans,cis*-[$Pt(OAc)_2I_2(en)$] recrystallizes from methanol to give dark-red needles in a 41% yield.

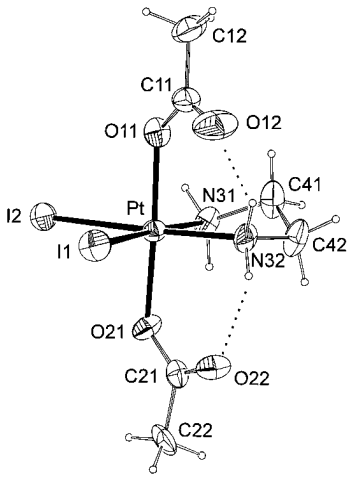


**Figure 1.** An ORTEP diagram of the X-ray crystal structure of *trans,cis*-[$Pt(OAc)_2I_2(en)$]. Dotted lines indicate possible hydrogen bonds.

**X-ray Crystallography.** The crystal structure of *trans,cis*-[$Pt(OAc)_2I_2(en)$] is illustrated in the ORTEP diagram in Figure 1 and confirms the expected coordination stereochemistry. Important bond lengths and angles are provided in Table 1, and a comparison of key bond lengths (Pt−O, Pt−N, Pt−Hal, C=O, C−O) and angles (N−Pt−N, N−Pt−Hal, Hal−Pt−Hal) with those recently reported for *trans,cis*-[$Pt(OAc)_2Cl_2(en)$] appears in Table 2. Although most key bond angles and distances are comparable for the two complexes, rather surprisingly, the conformations of the acetato ligands around the O−Pt−O axis are considerably different. For the dichloro analog, which has a $C_2$ symmetry axis, both carbonyl oxygens point in opposite directions and are in position to form a total of two intramolecular hydrogen bonds with a proton from each of the amine nitrogens.[15] On the other hand, the crystal structure of the diiodo analog is asymmetric because the carbonyl oxygens are oriented in the *same* direction (Figure 1). Both carbonyl oxygens of *trans,cis*-[$Pt(OAc)_2I_2(en)$]

**Table 2.** Compared Bond Angles and Distances for [$Pt(OAc)_2X_2(en)$] Complexes (X = Cl or I)

| | bond angles (deg) | | | bond distance (Å) | | | | |
|---|---|---|---|---|---|---|---|---|
| X | N−Pt−N | X−Pt−X | N−Pt−X | Pt−X | Pt−N | Pt−O | C−O | C=O |
| Cl[a] | 84.1 | 90.7 | 92.6 | 2.315 | 2.040 | 2.017 | 1.218 | 1.325 |
| I[b] | 82.29 | 91.02 | 94.07[c] | 2.621[c] | 2.079[c] | 2.025[e] | 1.216[e] | 1.272[e] |
| | | | 92.66[d] | 2.632[d] | 2.100[d] | 2.032[f] | 1.213[f] | 1.308[f] |

[a] Reference 15. [b] This work. [c] N−Pt−I. [d] N′−Pt−I′. [e] OAc. [f] OAc′.

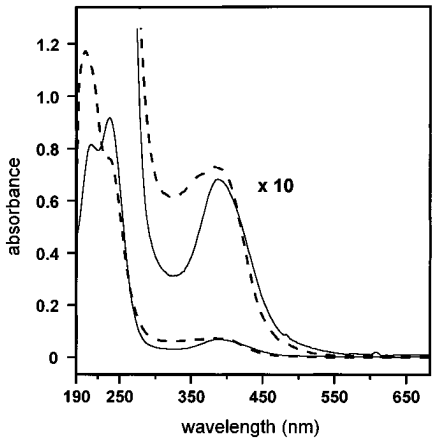


**Figure 2.** UV−vis spectra of *trans,cis*-[Pt(OH)$_2$I$_2$(en)] (- - -) and *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] (—), both at 50 $\mu$M in phosphate buffer (10 mM, pH 7.4).

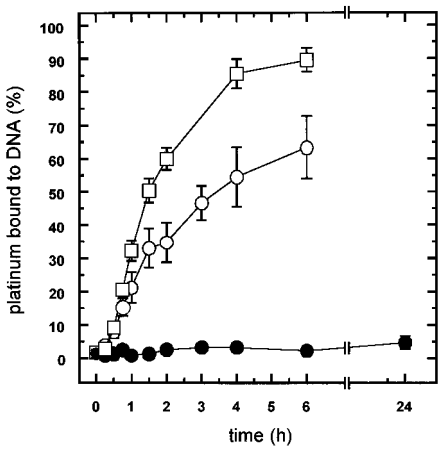


**Figure 3.** Influence of visible light (wavelengths > 375 nm) on the covalent binding of *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] to DNA. Conditions were as follows: *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] with light for 6 h, ○; *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] without light, ●; and [PtCl$_2$(en)] with light for 6 h, □. Solutions of 7.5 $\mu$M of Pt complex in the presence of 0.250 mg/mL DNA and 10 mM $NaClO_4$ (pH 6.8) were incubated at 37 °C. The amount of Pt bound to DNA (i.e. the *R* value) was determined by flammeless AAS. The percent of Pt bound is the *R* value divided by the maximal amount of Pt that can theoretically bind to DNA ($R_{max}$) and multiplied by 100. Data points are the means (±SD) of three independent experiments.

are within distance to form hydrogen bonds with one of the two protons from amine N32 (1.875 ± 80 and 2.018 ± 72 Å, respectively). On the other side of the molecule, however, both protons from amine N31 are available to hydrogen bond with water. This difference in the crystal structures may explain why *trans,cis*-[Pt(OAc)$_2$I$_2$-(en)] has considerably better water solubility than its *cis*-dichloro-analog.

**Electronic Spectra.** The UV−vis spectra of *trans,-cis*-[Pt(OH)$_2$I$_2$(en)] and *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] are compared in Figure 2; these spectra are dominated by moderately intense ligand-to-metal charge-transfer (LMCT) bands. Because of the relatively low optical electronegativity of iodide,[16] LMCT bands of comparatively low energy are observed for both compounds; that is at $\lambda_{max}$ = 384 nm ($\epsilon$ = 1416 $M^{-1}$ $cm^{-1}$) for *trans,cis*-[Pt(OH)$_2$I$_2$(en)] and at $\lambda_{max}$ = 389 nm ($\epsilon$ = 1372 $M^{-1}$ $cm^{-1}$) for *trans,cis*-[Pt(OAc)$_2$I$_2$(en)]. These very broad LMCT bands, which tail out to ca. 550 nm, come at wavelengths and intensities comparable to those reported for *trans*-[PtI$_2$(NH$_3$)$_4$]$^{2+}$ ($\lambda_{max}$ = 385 nm, $\epsilon$ = 2400 $M^{-1}$ $cm^{-1}$).[17]

**DNA Binding Studies.** It was anticipated that the Pt(IV) of *trans,cis*-[Pt(OAc)$_2$I$_2$(en)], because of the low-energy LMCT band, would be reduced when the complex was illuminated with just visible light. To test the hypothesis that (an) electrophilic Pt(II) species form(s) as a result of the photolysis of *trans,cis*-[Pt(OAc)$_2$I$_2$(en)], the ability of light of wavelengths > 375 nm to facilitate the covalent binding of Pt to calf thymus DNA was assessed. Figure 3 shows that during the first 6 h the platination of DNA occurs only when *trans,cis*-[Pt-(OAc)$_2$I$_2$(en)] is irradiated with light $\lambda_{irr}$ > 375 nm; after a 6 h illumination 62.7 ± 13.2% of the Pt is bound to DNA. Covalent binding of Pt to DNA in the nonphotolyzed incubations was first observed after a 24 h incubation, but even then the amount of Pt bond to DNA was only ca. 5% (Figure 3). Thus, while *trans,cis*-[Pt-(OAc)$_2$I$_2$(en)] only very slowly reacts with DNA, most of the photolysis products bind to DNA easily. The time-frame of DNA platination by [PtCl$_2$(en)], a known antitumor agent, is included for comparison (Figure 3); these kinetics are unaffected by visible light.

Interestingly, although *trans,cis*-[Pt(OH)$_2$I$_2$(en)] is also photolabile and can be completely photolyzed by a 6 h illumination with visible light, as can be seen in Figure 4A, very little (i.e., 10%) DNA platination has taken place even 24 h following photolysis. If, however, two reducing equivalents in the form of GSH are added to the photolyzed solutions, then within 24 h quantitative DNA platination takes place (Figure 4A). A quantitative platination of DNA also occurs when GSH (Figure 4A) is added to the nonphotolyzed complex. These results provide evidence that the photolysis of *trans,cis*-[Pt(OH)$_2$I$_2$(en)] results predominately in a complex that has the metal ion still in the Pt(IV) oxidation state, and must first be chemically reduced in order to form electrophilic Pt(II) species.

We also studied the kinetics of DNA platination after the addition of GSH to the incubations of *trans,cis*-[Pt-(OH)$_2$I$_2$(en)] at pH 6.8 and 37 °C (Figure 4B). Already 30 min following GSH addition, marked increases are observed in the DNA platination of both the photolyzed and nonphotolyzed incubations; however, as can be seen in Figure 4B the rate of DNA platinination is noticeably greater in the nonphotolyzed incubation compared to the photolyzed one. Three hours following the addition of GSH to the nonphotolyzed solutions a near quantitative platination of DNA is achieved, but 24 h is needed for GSH to facilitate the full DNA platination in the photolyzed incubations.

**Stability in Cell Culture Medium.** The stability of *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] in cell culture medium was investigated with and without irradiation with visible light. When protected from light and incubated at a concentration of 10 $\mu$M in Eagle's minimum essential medium (EMEM) containing 5% fetal calf serum (FCS) at 37 °C, *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] and *trans,cis*-[Pt-(OH)$_2$I$_2$(en)] were lost from the incubations in a pseudo-first-order manner with half-lives of 6.6 ± 0.5 and 46.8 ± 8.8 h (mean ± SD of three independent experiments), respectively. Under the same conditions but with irradiation by light $\lambda_{irr}$ > 375 nm, the diacetato- and dihydroxoplatinum(IV) complexes were lost from the culture medium in a first-order manner with half-lives of 0.28 ± 0.02 and 0.88 ± 0.11 h, respectively. Thus, the stabilities of the diacetato- and dihydroxoplatinum-

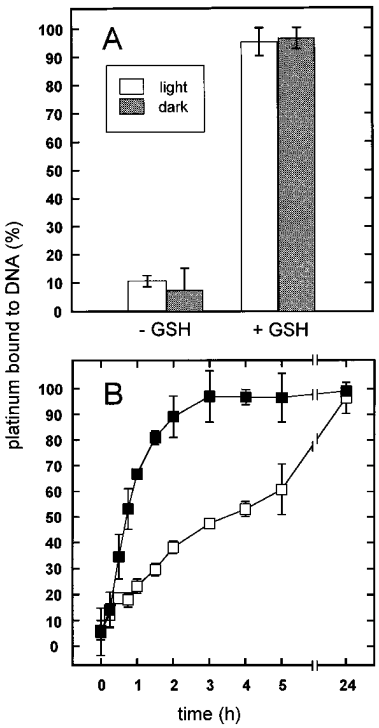


**Figure 4.** Effect of visible light and GSH on the covalent binding of *trans,cis*-[Pt(OH)$_2$I$_2$(en)] to DNA. (A) *trans,cis*-[Pt(OH)$_2$I$_2$(en)] (7.5 $\mu$M) was incubated with DNA 6 h at 37 °C in 10 mM PIPES buffer (pH 6.8), without or with illumination $\lambda_{irr}$ > 375 nm, and then the incubations were continued in the dark at 37 °C with and without 2 equiv of GSH. The level of DNA platination was determined 24 h following the addition of GSH. Columns represent the means (±SD) to three independent experiments. (B) Effect of photolysis on the kinetics of DNA platination by *trans,cis*-[Pt(OH)$_2$I$_2$(en)] (7.5 $\mu$M) in the presence of 2 equiv of GSH. GSH was added to the photolyzed (i.e., 6 h with $\lambda_{irr}$ > 375 nm) and nonphotolyzed solutions of Pt complex as indicated in (A) and then incubated further at 37 °C in the dark. Samples were drawn at the times indicated. Open symbols and closed symbols are for photolyzed and nonphotolyzed incubations, respectively. Symbols are the means (±SD) of three independent experiments.

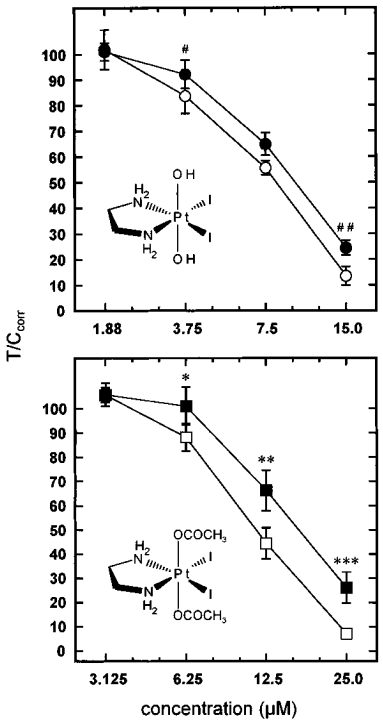


**Figure 5.** Effect of visible light on the growth-inhibitory activity of *trans,cis*-[Pt(OH)$_2$I$_2$(en)] and *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] on the TCCSUP human bladder cancer cell line at 37 °C. The cells were either incubated entirely in the dark (closed symbols) or illuminated with visible light (open symbols); illumination was for 1.5 and 3.5 h with *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] and *trans,cis*-[Pt(OH)$_2$I$_2$(en)], respectively. After a 24 h exposure to Pt complex, the medium was changed and the cells were grown an additional 4 d. For a definition of $T/C_{corr}$, see the Experimental Methods. Data points represent the means (±SD) of six independent experiments. Significant differences between light and dark experiments at the same concentrations are: *, #, ##, $p < 0.05$; ***, $p < 0.02$; **, $p < 0.005$ (two-sided, paired Student's *t*-test).

(IV) complexes in culture medium decrease by 24- and 53-fold, respectively, when irradiated with light $\lambda_{irr}$ > 375 nm.

Concerning possible products of either the chemical or photochemical reactions with *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] or *trans,cis*-[Pt(OH)$_2$I$_2$(en)], only [PtI$_2$(en)] was retained well enough by the RP-18 columns to be identifiable in our HPLC assays; however, we never observed this compound forming in incubations of either the diacetato- or dihydroxoplatinum(IV)-diamine in culture medium, with or without an illumination.

**Effects on Cancer Cell Growth.** The growth inhibitory activity of *trans,cis*-[Pt(OAc)$_2$I$_2$(en)], with and without an accompanying irradiation with visible light (i.e., wavelengths > 375 nm), was investigated on the TCCSUP human bladder cancer cell line. The same experimental setup was used here as was used in determining of the stabilities of the Pt(IV) complexes in culture medium. To assure the complete photolysis of *trans,cis*-[Pt(OAc)$_2$I$_2$(en)], an irradiation time of 1.5 h was chosen. After the cells were exposed to drug for 24 h, the medium was replaced with fresh medium and the cells grown an additional 4 d. Figure 5 shows that the photolysis of *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] with visible light leads to compounds that possess growth inhibitory activity against TCCSUP cells. When protected from light, cytotoxic species also form, but more activity is present when treatment with *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] is begun with 1.5 h of illumination. The mean (±SD) concentrations required to inhibit cell growth by 50% relative to an untreated control ($IC_{50}$) are 11.6 ± 1.7 and 16.5 ± 4.2 $\mu$M for experiments done with and without illumination, respectively; this difference is statistically significant (two-sided, paired Student's *t*-test: $p < 0.01$). Thus, *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] appears to function as a light-sensitive prodrug for some yet unknown cytotoxic species in the TCCSUP cell line.

As shown in Figure 5, a 3.5 h illumination of *trans,cis*-[Pt(OH)$_2$I$_2$(en)] with visible light, which is sufficient to bring about the complete photolysis of the Pt(IV) complex, results in a small but significant increase in antiproliferative activity. The $IC_{50}$ values for *trans,cis*-[Pt(OH)$_2$I$_2$(en)] were 7.3 ± 1.6 and 9.4 ± 2.2 $\mu$M for experiments done with and without illumination, respectively; this difference was statistically significant ($p < 0.05$). Visible light had no significant effect on the growth inhibitory activity of *trans,cis*-[Pt(OH)$_2$Cl$_2$(en)] in TCCSUP cells (results not shown).

## Discussion

These results show that *trans,cis*-[Pt(OH)$_2$I$_2$(en)] and *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] are both rapidly photolyzed with just visible light. Due to the small optical electronegativity of iodide, LMCT bands at about 400 nm are observed for iodoplatinum(IV)-amines.[12,17] The

photochemistry of Pt(IV) complexes usually originates from LMCT excitation[10] although excitation of the lower energy ligand-field (LF) transitions (i.e. d−d transitions) can also lead to reactions;[18] in either case, photochemical reductive eliminations, resulting in the formation of Pt(II) species, are frequently encountered (for examples see refs 18 and 19). On the other hand, light-induced substitutions of the Cl ligands of some Pt(IV) diamines for solvent (i.e., water) have also been reported (for examples see ref 20). Although we did not directly determine the structures of the photolysis products arising from either *trans,cis*-[$Pt(OH)_2I_2$(en)] or *trans,-cis*-[$Pt(OAc)_2I_2$(en)], the results of the DNA-binding experiments provide indirect evidence for the oxidation state of Pt in these species.

In the case of *trans,cis*-[$Pt(OAc)_2I_2$(en)], photolysis leads to the formation of Pt species that platinate DNA at a rate comparable to that of [$PtCl_2$(en)] (Figure 3), strongly suggesting that the majority of photolysis products are Pt(II) species. The products expected from a photoreduction of *trans,cis*-[$Pt(OAc)_2I_2$(en)] would be $I_2$ and [$Pt(OAc)_2$(en)]. A direct verification of $I_2$ in these studies is made complicated because $I_2$ is itself labile to visible light, resulting in species that will react with [$PtCl_2$(en)], for example.[12]

Whether [$Pt(OAc)_2$(en)] forms photolytically and then reacts with DNA has not yet been shown; however, it has been reported that aqueous solutions of *cis*-[$Pt(OAc)_2(NH_3)_2$] come to equilibrium with *cis*-[$Pt(OH_2)(OAc)(NH_3)_2]^+$ and acetate.[21] An analogous equilibrium exists for cisplatin, and it is the reactive aquachloroplatinum(II) species that platinates DNA.[9b,22] Furthermore, the hydrolysis of chloro- and acetatoplatinum(II) amines have been reported to progress at comparable rates; that is, the observed rate constant for the first hydrolysis of *cis*-[$Pt(Pr^iNH_2)_2(ClCH_2CO_2)_2$] without added acid ($4.38 \times 10^{-5}$ $s^{-1}$ at 25 °C)[23] is within a factor of 2 of the rate constant for the hydrolysis of cisplatin under similar conditions ($2.5 \times 10^{-5}$ $s^{-1}$ at 25 °C).[24] (This comparison should be viewed with some caution, however, because the rates of these types of hydrolysis reactions are often sensitive to changes in pH.[23,25]) Recent NMR work also indicates that deoxynucleotides (i.e., 5′-GMP) can react directly with carboplatin, a cisplatin analog bearing a chelating bis(carboxylato) ligand, by displacement of one of the coordinated carboxylato oxygens by the N7-guanine nitrogen, resulting in the opening of the chelated ring.[26] Thus, it is reasonable to assume that [$Pt(OAc)_2$(en)], either directly or indirectly through the hydrolysis product, will bind covalently to DNA.

Interestingly, the photolysis of *trans,cis*-[$Pt(OH)_2I_2$-(en)] leads to very little (i.e., ca. 10%) DNA platination; however, the addition of GSH to the photolyzed incubations results in a quantitative platination of DNA within 24 h (Figure 4). The most likely explanation for these results is that light first brings about the substitution of one or, more probably, both of the iodide ligands for water but with very little photoreduction; upon chemical reduction (i.e., by GSH), reactive Pt(II) species form that bind to DNA. Although GSH undergoes facile substitution reactions with various Pt(II) complexes,[27] because of the low concentrations of GSH (15 $\mu$M) compared to DNA (250 mg/L) in these incubations, substitutions of Pt(II) species with GSH would be expected to proceed at rates slower than those with DNA. Moreover, glutathione disulfide (GSSG), the expected product resulting from Pt(IV) reduction, is even less reactive toward Pt(II) complexes than GSH.[28]

An alternative explanation for the lack of DNA binding following the photolysis of *trans,cis*-[$Pt(OH)_2I_2$-(en)] is that the loss of $I_2$ would give [$Pt(OH)_2$(en)], which because of the inertness of the Pt−OH groups to substitutions would not be expected to bind covalently to DNA.[29] However, because buffered solutions (10 mM PIPES) at pH 6.8 were used in the photolysis experiments, any [$Pt(OH)_2$(en)] that might have formed would have established a rapid equilibrium with the highly reactive [$Pt(OH)(OH_2)$(en)]$^+$;[25,30] thus, had [$Pt(OH)_2$(en)] been produced photolytically, we should have detected much more platinum bound to DNA. Moreover, the results in Figure 3 show that [$PtCl_2$(en)] *via* its hydrolysis product can effectively platinate DNA under the conditions of the assay. Another explanation for the poor DNA binding could be that the photoreduction of *trans,cis*-[$Pt(OH)_2I_2$(en)] leads to [$Pt(OH)(OH_2)$(en)]$^+$ but then dimerizes to form hydroxo-bridged dinuclear Pt species {e.g., $[PtOH(en)]^+_2$}, which may have a different reactivity toward DNA than does [$Pt(OH)(OH_2)$(en)]$^+$.[31] This also seems unlikely given that such hydroxo-bridged Pt species have only been observed in concentrated solutions of platinum complex (e.g., see refs 21 and 32); at lower cisplatin concentrations (e.g., 2 mM) there was no evidence for Pt dimer or oligomer formation.[25] At the concentrations of Pt complex used in our investigations (i.e., 7.5 $\mu$M), it can be assumed that the formation rate of such dimers will be insignificant compared to the rate of the irreversible reaction of [$Pt(OH)(OH_2)$(en)]$^+$ with DNA.

Thus, it appears that the nature of the *trans*-ligand plays an important role in directing between the photoreduction and photosubstitution pathways.

The type of *trans*-ligand also affects greatly the "dark" stability of iodoplatinum(IV) diamines in culture medium. For the complexes investigated thus far, their "dark" stability increases as follows: *trans,cis*-[$PtCl_2I_2$-(en)] < *trans,cis*-[$Pt(OAc)_2I_2$(en)] < *trans,cis*-[$Pt(OH)_2I_2$-(en)]; at a Pt concentration of 10 $\mu$M, we have determined (by RP-HPLC) half-lives of $0.78 \pm 0.14$, $6.6 \pm 0.5$, and $46.8 \pm 8.8$ h (mean ± SD), respectively, for these complexes in cell culture medium (EMEM with 5% FCS, 37 °C). This order of stability parallels the decreasing ease of Pt(IV) reduction reported for the comparable series of *cis*-dichloroplatinum(IV) complexes.[15]

A difference in biological activity is also observed between *trans,cis*-[$Pt(OAc)_2I_2$(en)] and *trans,cis*-[$Pt(OH)_2I_2$(en)] (Figure 5). Without illumination, *trans,-cis*-[$Pt(OH)_2I_2$(en)] is ca. 2-fold more potent at inhibiting the *in vitro* growth of cancer cells than *trans,cis*-[$Pt(OAc)_2I_2$(en)]. This result is surprising because in culture medium *trans,cis*-[$Pt(OH)_2I_2$(en)] is chemically more stable (i.e., by 7-fold), presumably to reduction, than the diacetato homolog. Ongoing studies are aimed at understanding this apparent contradiction.

Regarding the illumination experiments with cancer cells, the results presented in Figure 5 show that the photolysis products of both *trans,cis*-[$Pt(OAc)_2I_2$(en)] and *trans,cis*-[$Pt(OH)_2I_2$(en)] possess somewhat greater growth inhibitory activity against TCCSUP cells compared to their nonphotolyzed complexes. In the case of *trans,cis*-[$Pt(OH)_2I_2$(en)], a large increase in antiproliferative activity had not been anticipated upon illumina-

tion because the photolysis of this compound results in only a sparse formation of Pt species that bind to DNA (Figure 4). For *trans,cis*-[$Pt(OAc)_2I_2$(en)], however, a larger difference between illuminated and nonilluminated incubations might have been expected on the basis of the observations that the Pt complex is 24-fold less stable in culture medium when illuminated and that photolysis leads to a strong formation of DNA-binding Pt species (Figure 3).

A number of reasons could explain why this difference in activity (i.e., between light and dark incubations) was not larger. For example, intracellularly, where GSH concentrations for the TCCSUP cell line are reported to be 3.19 mM,[33] *trans,cis*-[$Pt(OAc)_2I_2$(en)] will be reduced through processes not requiring light. Reduction of Pt(IV) would also be expected to take place in culture medium,[34] and because the cells are treated for 24 h with Pt complex, there is time for a substantial fraction of the complex to be reduced to cytotoxic Pt(II) species. It should be noted that we did not attempt to optimize the cell culture conditions with respect to either a drug incubation time before illumination, illumination period, drug exposure time, or drug concentration in order to maximize the difference between the light and the dark experiments. This optimization was not done because an extrapolation from such an *in vitro* experiment to an *in vivo* situation, where a very different pharmacokinetic−pharmacodynamic relationships would exist, was considered of limited utility at this stage in the project.

A further interesting property of *trans,cis*-[$Pt(OAc)_2I_2$(en)], although not related to its photochemistry, is the reasonably good solubility in water. This is in contrast to *trans,cis*-[$Pt(OAc)_2Cl_2$(en)], which in our hands has very poor aqueous solubility. The opposite trend is found with the Pt(II) complexes [$PtCl_2$(en)] and [$PtI_2$(en)], where the dichloro complex has considerably better aqueous solubility than the diiodo one. On the other hand, from RP-HPLC work we observe that *trans,cis*-[$Pt(OAc)_2I_2$(en)] is better retained by a RP-18 column than the dichloro analog, indicating that the diiodo analog is more lipophilic. An explanation for the difference in water solubility may be a result of the dissimilar crystal structures of the two Pt(IV) complexes. The X-ray crystal structure of *trans,cis*-[$Pt(OAc)_2Cl_2$(en)] shows each of the carbonyl oxygens oriented in opposite directions and hydrogen bonded with a proton from different amines.[15] On the other hand, in the crystal structure of *trans,cis*-[$Pt(OAc)_2I_2$(en)] (Figure 1) the carbonyl oxygens are oriented, rather surprisingly, in the same direction and are close enough (ca. 2 Å) to form hydrogen bonds with the protons from one of the amines; however, the other coordinated $NH_2$ is fully available to hydrogen bond with a solvent water molecule.

Recently, there has been a resurgence of interest in Pt(IV) complexes following the discoveries that some Pt(IV) *cis* mixed-amine prodrugs are orally active as antitumor agents[35] and that Pt(IV) *trans* mixed-amine complexes have antitumor activity *in vivo*.[36] The results presented here show that through the proper choice of coordinating groups, Pt(IV) complexes can be rendered labile to visible light, yielding photolysis products which have DNA-binding and cytotoxic properties. We do not yet know the structures of the photolysis products of *trans,cis*-[$Pt(OAc)_2I_2$(en)] that are causing cell growth inhibition, but the data presented suggests that the majority are Pt(II) ethylenediamine species. Whether such photolysis processes will actually lead to improved *in vivo* efficacy of Pt(IV) complexes remains to be answered; nevertheless, our findings indicate that there are interesting possibilities for developing Pt(IV) prodrugs that can be photoactivated in and around a tumor. A detailed investigation of the structure−activity relationships of iodoplatinum(IV) diamines is in progress.

## Experimental Methods

**Chemicals.** $K_2PtCl_4$ was from Degussa AG (Frankfurt a. M., FRG). Reagent-grade chemicals were from Merck (Darmstadt, FRG). Calf thymus DNA, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), piperazine-*N*,*N*′-bis[2-ethanesulfonic acid] (PIPES), L-glutamine, reduced GSH, and EMEM were from Sigma (Deissenhofen, FRG). HPLC-grade methanol and acetonitrile and HPLC-grade *N*,*N*′-dimethylformamide (DMF) were from Baker Chemicals (Deventer, Holland) and Aldrich (Steinheim, FRG), respectively. Water was deionized with a Millipore Milli-Q Water System (Eschborn, FRG). Plastic cell culture materials were from Falcon (Becton Dickinson, Heidelberg, FRG), and FCS was from Serva (Heidelberg, FRG).

**Equipment.** $^1$H NMR were recorded with a Bruker ARX400 instrument at 400.1 MHz in a solvent of DMF-$d_7$ and TMS as the internal reference. Liquid secondary-ion mass spectra (liquid SIMS) were recorded with a Finnigan MAT-95 spectrometer. The UV−vis spectra were measured with a Kontron Uvikon 930 spectrophotometer (Eching b. München, FRG). Flammeless atomic absorption spectroscopy (AAS) was done with a Spectr AA 30 instrument equipped with a graphite tube atomizer GTA 96 and a data station DS15 (Varian, Darmstadt, FRG). The partition tubes (coated)-GTA were also from Varian. The HPLC system consisted of two Altex 110A pumps, an Altex 420 microprocessor controller (Beckman, Fullerton, CA), a Rheodyne 7125 sample injector fitted with a 500 $\mu$L injection loop, a Rheodyne 7000 switching valve attached to a 5701 pneumatic actuator, and a L-4500 Diode-Array Detector (Merck, Darmstadt, FRG). The apparatus for the irradiation of microtiter plates with visible light has been described elsewhere;[12] briefly, a 1000 W halogen lamp mounted 100 cm above the temperature-controlled microtiter plates was used as a light source, and a 5 mm G375 cutoff filter (Schott Glaswerk, Mainz, FRG) was used to remove light of wavelengths < 375 nm. Elemental analyses were performed by the Microanalysis Lab at the University of Regensburg.

**Synthesis of (Ethylenediamine)diiodoplatinum(II) {[$PtI_2$(en)]}.** An aqueous solution of 1.04 g (2.5 mmol) of $K_2PtCl_4$ was allowed to react with 4.15 g of KI (25 mmol) for 30 min at room temperature. Addition of a solution of 170 $\mu$L (2.5 mmol) of ethylenediamine in 10 mL of water resulted in orange-yellow precipitate, which was continuously collected by suction filtration, washed with water, and dried overnight over $P_2O_5$. The product was recrystallized from a 0.25 mM KI solution to give orange crystals, which were collected, washed with water, and dried over $P_2O_5$. The yield was 922 mg (73%): UV−vis ($H_2O$) $\lambda_{max}$ ($\epsilon$) 346 (268), 293 (342), 195 nm (18676 $M^{-1}$ $cm^{-1}$). Anal. Calcd for $C_2H_8N_2I_2Pt$: C, 4.72; H, 1.58; N, 5.50. Found: C, 4.71; H, 1.53; N, 5.44.

**Synthesis of *trans,cis*-Dihydroxodiiodo(ethylenediamine)platinum(IV) {*trans,cis*-[$Pt(OH)_2I_2$(en)]}.** To 14.5 mL of a 30% $H_2O_2$ solution (pH 2.6) was added an aqueous suspension of 0.80 g (1.57 mmol) of [$PtI_2$(en)], and the reaction mixture was stirred at room temperature for 3 days in the dark. The resulting gray-green precipitate was collected by suction filtration, washed with water and dried *in vacuo*. To remove nonreacted starting material, the crude product was stirred for 1 h in pyridine at 70 °C protected from light. The Pt(II) species was soluble in hot pyridine while the Pt(IV) product was not. The bright-yellow product was collected by suction filtration under red-light, washed with $CHCl_3$, and dried overnight over $P_2O_5$. The yield was 473 mg (56%): UV−vis ($H_2O$) $\lambda_{max}$ ($\epsilon$) 384 (1416), 237 (15 620), 203 nm (20 896 $M^{-1}$ $cm^{-1}$); IR (Nujol) $\nu$ 3480 (OH), 3210 and 3150 (NH), 1030

**Table 3.** Summary of Crystallographic Data for trans,cis-[Pt(OAc)$_2$I$_2$(en)]

| | |
|---|---|
| formula | $C_6H_{14}I_2N_4Pt$ |
| mol wt | 627.08 |
| *a*, Å | 9.029(4) |
| *b*, Å | 11.443(2) |
| *c*, Å | 12.822(2) |
| α, deg | 90.00 |
| *β*, deg | 95.48(3) |
| *γ*, deg | 90.00 |
| *V*, Å$^3$ | 1318.7(7) |
| cryst syst | monoclinic |
| space group | *Cc* (No. 9) |
| *Z* | 4 |
| $D_c$, g/cm$^3$ | 3.159 |
| radiation | Mo Kα (0.710 69 Å) |
| temp, deg | 297(1) |
| total reflns obsd | 1999 |
| $R^a$ | 0.1006 (for all 1999 data) |
| $R_w{}^b$ | 0.0778 |

$^a$ $R = \sum||F_o| - |F_c||/\sum|F_o|$. $^b$ $R_w = [\sum(w|F_o - F_c|)^2/\sum w|F_o|^2]^{1/2}$.

(strong), 1058 (strong), 990 (strong) cm$^{-1}$; positive-ion liquid SIMS (glycerol/$H_2O$/acetic acid) *m/z* (rel int) (M − OH$^-$) 525 (23), (M + H$^+$) 543 (43), ([PtI$_2$(en)] + H$^+$ + glycerol) 601 (48), (M + H$^+$ + glycerol) 635 (45); 400 MHz $^1$H NMR ($D_2O$) *δ* 2.62 (1H, CH$_2$, $^3J_{Pt-N-C-H}$ = 15 Hz). Anal. Calcd for $C_2H_{10}N_2I_2O_2$-Pt: C, 4.42; H, 1.86; N, 5.16. Found: C, 4.60; H, 1.69; N, 4.99.

**Synthesis of *trans,cis*-Diacetatodiiodo(ethylenediamine)platinum(IV)** {***trans,cis*-[Pt(OAc)$_2$I$_2$(en)]**}. To a suspension of 0.16 g (0.29 mmol) *trans,cis*-[Pt(OH)$_2$I$_2$(en)] in 11 mL of $CH_2Cl_2$ was added 4 mL of acetic anhydride, and the reaction mixture was stirred at room temperature for 4 d protected from light. The resulting red precipitate was collected by suction filtration under red light, washed with $CH_2Cl_2$, and dried over $P_2O_5$. The product was recrystallized from methanol, yielding 73 mg (41%) of dark-red needles: UV−vis ($H_2O$) $\lambda_{max}$ ($\epsilon$) 389 (1372), 236 (18 518), 211 nm (16 572 M$^{-1}$ cm$^{-1}$); IR (Nujol) *ν* 3220 and 3170 (NH), 1600 (C=O), 1356 (strong), 1289 (strong) cm$^{-1}$; positive-ion liquid SIMS (DMSO/glycerol/water/acetic acid) *m/z* (rel int) (M − OAc$^-$): 567 (34), (M + H$^+$) 627.1 (94), (M + H$^+$ + DMSO) 705.1 (23), (M + H$^+$ + glycerol) 719.2 (16); 400 MHz $^1$H NMR (DMF-$d_7$) *δ* 8.54 (2H, NH$_2$), 2.75 (2H, CH$_2$), 1.86 ppm (3H, CH$_3$). Anal. Calcd for $C_6H_{14}N_2I_2O_2Pt$: C, 11.48; H, 2.23; N, 4.47. Found: C, 11.42; H, 2.16; N, 4.47.

**X-ray Crystallography**. Table 3 contains a summary of the conditions of data collection and results for the structure. A single crystal (approximate dimensions 50 × 80 × 160 *μ*m) was used for the data collection on an Enraf-Nonius CAD-4 diffractometer (MoKα radiation, *λ* = 0.710 69Å, graphite monochromator in incident beam). The lattice parameters were refined from 25 carefully centered reflections (SET 4 mode of CAD-4 software) in the range 5.77° < *θ* < 23.82°. A monoclinic C-centered cell was found and 3990 intensities were measured for 2° < *θ* < 30° in *ω* − 2*θ* scan mode, scan width 1.2 + 1.4 tan *θ*. Three standard reflections every 200 data showed only small random fluctuations and indicated no loss of intensity throughout data collection. An analysis of systematic absences showed the reflection condition *h*0*l*: *h*,*l* = 2*n* leading to the possible space groups *Cc* (No. 9) and *C*2/*c* (No. 15). Statistical tests strongly suggested the absence of a center of inversion and consequently the acentric space group *Cc*. Merging of the 3901 observed intensities (sin Θ/*λ*)$_{max}$ = 0.7038Å$^{-1}$; −12 < *h* < 12, −16 < *k* < 16, 0 < *l* < 17) gave according to space group *Cc* 1999 unique reflections ($R_{int}$ = 0.0923, $R_{sigma}$ = 0.1137). The structure was solved by direct methods (program SIR92[37]), followed by successive difference-Fourier synthesis (program SHELXL93[38]). In the least-squares refinement $F^2$ values were used to refine an overall scale factor, positional parameters, and isotropic displacement parameters. When the molecule was established, all H atoms were refined as riding atoms with fixed $U = 1.5U_{eq}$ of the bonded atom. After the last isotropic refinement cycle a numerical correction for absorption was applied to the original data set (program DIFABS;[39] correction factors min = 0.509, max = 1.0; $R_{int}$, $R_{sigma}$ after merging of the corrected data were 0.0797, 0.1014 respectively). The anisotropic refinement of the 139 variables (including an extinction parameter) confirmed the acentric space group *Cc*, resulting without strong correlations in $R1 = 0.0386$ for 1370 reflections with $F_o > 4\sigma(F_o)$ and $R1 = 0.1006$, w$R2 = 0.0778$ and GOF = 1.032 for all data. The ratio mean shift/error was 0.000 in the final refinement cycle. The final difference-Fourier map was featureless (max, min = 1.77, −2.14 e/Å$^3$). The correctness of the absolute structure was established by the Flack parameter $x = 0.003(13)$.[40]

**DNA Platination Experiments**. Complete details of these experiments can be found elsewhere.[12] Briefly, 7.5 *μ*M solutions of Pt complex in the presence of 250 *μ*g/mL DNA and 10 mM $NaClO_4$ (pH 6.8) were incubated in 96-well microtiter plates that were housed in an aluminum block apparatus that could be maintained at 37 °C. The experiments were done with and without 6 h of irradiation at $\lambda_{irr}$ > 375 nm. Figure 3 gives the times at which samples were taken. Sample aliquots (in duplicate) of 200 *μ*L were removed, and the DNA was precipitated and washed (3×) as previously described[12] before being hydrolyzed for 2 days with 0.5% $HNO_3$ at 70 °C in the water bath. The concentration of the DNA bases in each sample was determined by UV spectrometry, and the platinum concentration was measured by using flameless AAS.

For the experiments with GSH, *trans,cis*-[Pt(OH)$_2$I$_2$(en)] (7.5 *μ*M) was incubated with DNA (250 *μ*g/mL) for 6 h in 10 mM $NaClO_4$, 10 mM PIPES-buffer (pH 6.8) at 37 °C without and with illumination by light $\lambda_{irr}$ > 375 nm. The "light" and "dark" incubations were each divided, half receiving 2 molar equiv of GSH (relative to Pt complex) and the other half receiving no GSH. The incubations were continued in the dark at 37 °C, and sample aliquots (in duplicate) of 200 *μ*L were removed at the times indicated in Figure 4. DNA platination was determined as described above.

The *R* bound value (*R* = Pt/DNA nucleotide mole ratio) was calculated by dividing the concentration of Pt covalently bound to DNA by the concentration of DNA bases in each sample. The % DNA platination was estimated by dividing each *R* value by the maximal *R* value theoretically possible ($R_{max}$) and multiplying by 100.

**Stability Studies of the Platinum Complex in Cell Culture Medium**. The cell culture medium consisted of EMEM, 0.29 g/L glutamine, and 5.96 g/L HEPES (pH 7.4) with 5% FCS (v/v). The experiments were done with and without light $\lambda_{irr}$ > 375 nm. Under red light, stock solutions of the Pt(IV) complexes in DMF were added to culture medium in a 1000-fold dilution so that the starting concentration of the Pt(IV) complexes was 10 *μ*M, 100 *μ*L of this solution was added to each well of the microtiter plates, and the plates were either illuminated or incubated protected from light at 37 °C; during the course of irradiation the microtiter plate was housed in the aluminum block-filter apparatus at 37 °C. Immediately following the addition of the platinum complex to the cell culture medium and at specified times, a 0.55 mL aliquot was removed from the plate. A two-column HPLC assay with UV−vis detection was used to quantify both Pt(IV) complexes in the cell culture medium. For *trans,cis*-[Pt(OAc)$_2$I$_2$(en)], the HPLC arrangement was composed of a 50 × 4.6 mm strong cation-exchange column (Nucleogel SCX 1000-8/46; Macherey-Nagel, Düren, FRG) and a 4 × 250 mm reversed-phase (RP18) column (Nucleosil 100-5C$_{18}$; Macherey-Nagel), which was protected by a 4 × 30 mm Nucleosil 120-7C$_{18}$ precolumn. Both columns were kept at room temperature. The ion-exchange and RP-18 columns were connected in series via a switching valve. A mobile phase of 50% (v/v) $H_2O$/methanol was delivered by two pumps at a flow rate of 0.5 mL/min for each column. The sample (500 *μ*L) was first loaded onto the ion-exchange column; the platinum complex was retained by the ion-exchange column, but most of the components of the culture medium were not and passed through into the drain instead. After 2.4 min the direction of the mobile phase flow was automatically switched to back-flush the platinum complex onto the RP-18 column. The effluent from the RP-18 column was monitored at 256 nm. *trans,cis*-[Pt(OAc)$_2$I$_2$(en)] eluted at 12.5 min. The detector response (peak height) was linear over the investigated concentration range, and the detection limit was 2.8 nmol.

A comparable setup was used to determine the relative concentration of *trans,cis*-[$Pt(OH)_2I_2$(en)] in culture medium; however, in place of the cation exchanger a Merck 4 × 125 mm LiChrosorb RP-18 (7 $\mu$m) column was used and the analytical column was a 4 × 250 mm LiChrosorb RP-18 (7 $\mu$m). Under red light, Pt complex was dissolved directly into the culture medium to a concentration of 10 $\mu$M and incubated at 37 °C with or without illumination. The injection volume was 100 $\mu$L. The mobile phase was 5% acetonitrile in water (v/v), and the flow direction was switched 3.5 min after loading the probe onto the first column. The retention time was 15.8 min. Detection was done at 240 nm, and the detector response was linear with a 0.03 nmol limit of detection.

**Determination of Cell Growth Inhibitory Activity**. The human bladder cancer cell TCCSUP was obtained from the American Type Culture Collection (Rockville, MD) and used between the 65th and 80th passages. Culture medium consisted of EMEM containing 10% (v/v) FCS, 5.96 g/L HEPES, and 0.29 g/L L-glutamine adjusted to pH 7.4 with 5 N NaOH. The cells were plated in 96-well plates with 100 $\mu$L of culture medium per well and incubated at 37 °C in a humidified atmosphere of air for 48 h, after which time the cells were growing as a monolayer and actively dividing. For the diacetatoplatinum(IV) complex, stock solutions at 500 times the final concentration where made with DMF as a solvent. Under red light, the stock DMF solutions were diluted 500 times into culture medium containing no FCS, and then each well received 100 $\mu$L of culture medium at 16 wells per concentration. Cells which received medium containing only DMF served as the untreated controls; DMF has no effect on cell growth at this concentration (i.e., 13 mM). Because the dihydroxoplatinum(IV) complex has poor solubility in DMF, concentrated DMF stock solutions could not be prepared. Instead, the dihydroxo complex was dissolved directly in culture medium (without FCS) to give the highest stock concentration (i.e., 60 $\mu$M), and then this solution was sterile filtered. Filtration had no effect on the concentration of Pt complex. Culture medium containing *trans,cis*-[$Pt(OH)_2I_2$(en)] was serially diluted into culture medium to give the desired stock dilutions, and then to each well was added 100 $\mu$L of these stock solutions at 16 wells per concentration. (The compounds remained in solution even at the highest concentrations.)

The microtiter plates were illuminated with wavelengths > 375 nm for 1.5 and 3.5 h for *trans,cis*-[$Pt(OAc)_2I_2$(en)] and *trans,cis*-[$Pt(OH)_2I_2$(en)], respectively, as described previously[12] and incubated an additional 24 h at 37 °C in the presence of drug. The medium was then replaced by 200 $\mu$L of fresh medium containing 5% FCS, and the cells were allowed to grow an additional 4 days. For the "dark" controls, an identical sequence was followed except that the cells were not illuminated. Cell growth was determined with an assay that estimates the cell number (or density) by staining cellular components with crystal violet.[41] $T/C_{corr}$ values were determined according to the equation: $(T - C_0) \div (C - C_0) \times 100$, where $T$ is the absorbance of crystal violet in the treated cells, $C$ the absorbance of the controls, and $C_0$ the absorbance of the cells at the time when drug was added.[42] The concentration of drug that inhibited the cell growth by 50% ($IC_{50}$ value) compared to the untreated control was calculated by least-squares analysis of the $T/C_{corr}$ values versus the logarithm of the drug concentration.

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**Supporting Information Available:** Tables of final atomic positional parameters and equivalent isotropic displacement factors (Table 1S), anisotropic displacement factors (Table 2S), and bond distances and angles (Table 3S) for *trans,cis*-[$Pt(OAc)_2I_2$(en)] (6 pages). Ordering information is given on any current masthead page.

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