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THE EFFECT OF A NOVEL DINUCLEAR PLATINUM COMPLEX WITH BERENIL AND 2-PICOLINE LIGANDS ON GROWTH OF HUMAN BREAST CANCER CELLS

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Abstract: Evaluation of the cytotoxicity of a novel dinuclear platinum(II) complex of formula Pt_2(2-picoline)$_4$(berenil)$_2$ employing a MTT assay and inhibition of [^{3}H]thymidine incorporation into DNA in both MDA-MB-231 and MCF-7 breast cancer cells demonstrated that the complex was more potent anti-proliferative agent than cisplatin. Data from the ethidium displacement assay indicated that the complex showed specificity for AT base pairs of DNA. Our study showed that Pt_2(2-picoline)$_4$(berenil)$_2$ was a potent catalytic inhibitor of topoisomerase II in opposition to cisplatin.

Keywords: cisplatin, dinuclear platinum complexes, DNA-binding, breast cancer

The widespread clinical use of platinum compounds in cancer chemotherapy has prompted a search for new platinum agents. While there has been some success in lowering the toxicity of platinum drugs (carboplatin) and limited success in overcoming acquired cisplatin resistance (oxaliplatin), there has been little success in developing drugs that show activity in cancer cell lines that have a natural resistance to cisplatin and carboplatin. Many researchers have turned to multi-nuclear platinum complexes in an effort to overcome both natural and acquired resistance to cisplatin in human cancer cell lines (1, 2). The present study was undertaken to extend our recent findings related to antineoplastic activity of novel dinuclear platinum(II) complexes with berenil and amine ligands (3). Berenil (1,3-bis(4'-amidinophenyl)triazene) can exhibit intercalative, as well as minor groove binding, properties when it binds to both DNA and RNA duplexes, while also exhibiting a preference for DNA duplexes with unobstructed minor grooves. Berenil preferentially recognizes and binds to AT-rich DNA sequences and it is strong catalytic inhibitor of mammalian DNA topoisomerase II (4, 5). Based on this strategy and due to the high affinity of berenil for the minor groove, we are expecting that Pt_2(2-picoline)$_4$(berenil)$_2$ would localize in the vicinity of the DNA, and the combined effect resulting from platination and minor groove binding might confer cytotoxic activity of Pt_2(2-picoline)$_4$(berenil)$_2$. In this study, we have investigated the antiproliferative activity of Pt_2(2-picoline)$_4$(berenil)$_2$ (Fig. 1) in both MDA-MB-231 and MCF-7 human breast cancer cells. The mechanism of action of Pt_2(2-picoline)$_4$(berenil)$_2$ was studied by means of the relaxation assay of topoisomerase II,

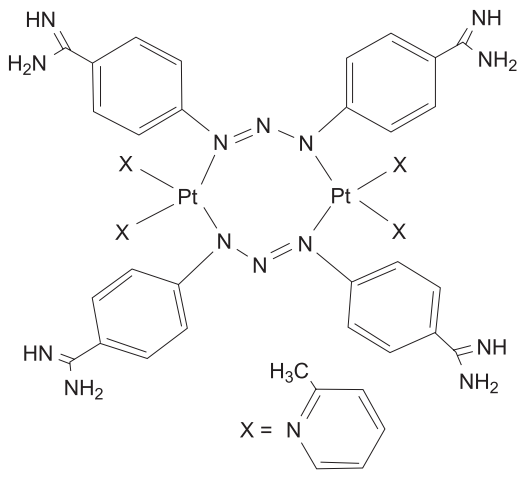

Figure 1. Structure of Pt_2(2-picoline)$_4$(berenil)$_2$

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and ethidium displacement assay using calf thymus DNA, T4 coliphage DNA, poly(dA-dT)$_2$ and poly(dG-dC)$_2$.

EXPERIMENTAL

Chemistry

The structure of $Pt_2(2\text{-picoline})_4(berenil)_2$ was confirmed by ^1H and ^{13}C NMR spectra recorded on Bruker AC 200F apparatus (^1H – 200 MHz and ^{13}C – 50 MHz) in DMSO-d_6. Multiplicity of resonance peaks was indicated as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). Infrared spectra were recorded on a Specord 75 spectrometer (C. Zeiss, Jena) in KBr pellets (4000–450 cm^{-1}). Melting points were determined on Büchi 535 (Büchi; Flawil, Switzerland) melting-point apparatus and were uncorrected. Elemental analysis of C, H, and N was performed on a Perkin Elmer 240 analyzer (USA) and satisfactory results within ± 0.4% of calculated values were obtained.

Chemical synthesis of $Pt_2(2\text{-picoline})_4(berenil)_2$

K_2PtCl_4 (1 mmol) was dissolved in 50 mL of deionized water and filtered. KI (10 mmol) in 5 mL of water was added to it and the reaction mixture was stirred for 15 min. Then, 2-picoline (2 mmol) was added dropwise to the reaction mixture while stirring, to obtain a yellow precipitate, cis-[Pt(2-picoline)$_2$I$_2$]. The stirring was continued for further 30 min and the precipitate was then collected by filtration. This compound was dissolved in dimethylformamide and filtered. Cold water was added in excess to the filtrate to obtain a bright yellow precipitate, cis-[Pt(2-picoline)$_2$I$_2$], which was washed with water, ethanol and acetone and dried in vacuum. Cis-[Pt(2-picoline)$_2$I$_2$] (0.40 mmol) was suspended in 5 mL of an aqueous solution of silver nitrate ($AgNO_3$) (0.80 mmol). The reaction mixture was stirred for 24 h at room temperature in the dark. The AgI precipitate was filtered off and berenil (0.40 mmol) and a solution of 10% NaCl (5 mL) were added with constant stirring until a precipitate of $[Pt_2(2\text{-picoline})_4(berenil)_2]\times4HCl\times2H_2O$ was formed. Afterwards, the product was filtered off and washed with a small amount of diluted HCl, water, methanol, acetone and ether and dried under vacuum.

$[Pt_2(2\text{-picoline})_4(berenil)_2]\times4HCl\times2H_2O$

Yield = 54%, m.p. 232°C. ^{1}H-NMR (DMSO-d_6, δ, ppm): 9.35 (bs, 4H, amidine), 9.00 (bs, 4H, amidine), 8.50 (d, 4H, Ar), 7.66–7.73 (m, 8H, Ar), 7.24 (t, 4H, Ar) 7.02–7.15 (m, 16H, Ar), 2.53 (s, 12H, CH_3). ^{13}C-NMR (DMSO-d_6, δ, ppm): 164.1 (amidine), 158.0 (Py), 148.6 (Ar), 148.6 (Py), 136.0 (Py), 129.5 (Ar), 124.2 (Py), 122.0 (Ar), 120.9 (Py), 118.0 (Ar), 23.8 (CH_3). IR (KBr, cm^{-1}): 3061 (C-H aromatic), 2975 (C-H, aliphatic), 1664 (NCN), 1610 (CN, pyridine), 1600 (triazene), 1440 (CH_3), 1380 (CH_3), 1258 (triazene), 1158 (triazene), 534 (Pt-N), 470 (Pt-N), 327 (Pt-Cl), 317 (Pt-Cl). Analysis: calcd. for $C_{52}H_{56}N_{18}Pt_2\times4HCl\times2H_2O$: C, 41.49; H, 4.29; N 16.75%; found: C, 41.20; H, 4.28; N, 16.42%.

Pharmacology

Materials

Dimethylformamide, K_2PtCl_4, KI, acetone, 2-picoline, diethyl ether, methanol, ethidium bromide, cisplatin, calf thymus DNA, T4 coliphage DNA, homopolymers poly(dA-dT)·poly(dA-dT), and poly(dG-dC)·poly(dG-dC), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and controlled process serum replacement I (CPSR1) were purchased from Sigma Chemical Co. (USA). Topoisomerase II, supercoiled pRYG DNA, and etoposide were purchased from TopoGEN (USA). Stock cultures of breast cancer MCF-7 and MDA-MB-231 were purchased from the American Type Culture Collection, Rockville, MD (USA). Dulbecco's minimal essential medium (DMEM) and fetal bovine serum (FBS) used in cell culture were products of Gibco (USA). Glutamine, penicillin and streptomycin were obtained from Quality Biologicals Inc. (USA). [^{3}H]Thymidine (6.7 Ci/mmol) was the product of NEN (USA).

Cell culture

Human breast cancer MDA-MB-231 and MCF-7 cells maintained in DMEM supplemented with 10% FBS, 50 U/mL penicillin, 50 mg/mL streptomycin at 37°C. Cells were cultured in Costar flasks and subconfluent cells were detached with 0.05% trypsin and 0.02% EDTA in calcium-free phosphate buffered saline, counted in hemocytometers and plated at 5×10^5 cells per well of 6-well plates (Nunc) in 2 mL of growth medium (DMEM without phenol red with 10% CPSR1). Cells reached about 80% of confluency at day 3 and in most cases such cells were used for the assays.

DNA synthesis assay

To examine the effect of the studied compounds on cells proliferation, MCF-7 and MDA-MB-231 cells were seeded in 6-well plates and grown as described above. Cells culture were incubated with varying concentrations of Pt_2(2-picol-

ine)$_4$(berenil)$_2$, cisplatin and 0.5 µC of [^{3}H]thymidine for 24 h at 37°C. The cells were then harvested by trypsinization and washed (with the cold phosphate-buffered saline) with centrifugation for 10 min at 1500 × g several times until the dpm in the washes were similar to the reagent control. Radioactivity was determined by liquid scintillation counting. [^{3}H]Thymidine incorporation was expressed as dpm/well.

Cell viability assay

The assay was performed according to the method of Carmichael using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (6). Confluent cells, cultured for 24 h with various concentrations of study compounds in 6-well plates, were washed three times with PBS and then incubated for 4 h in 1 mL of MTT solution (0.5 mg/mL of PBS) at 37°C in 5% CO_2 in an incubator. The medium was removed and 1 mL of 0.1 mol/L HCl in absolute isopropanol was added to attached cells. Absorbance of converted dye in living cells was measured at a wavelength of 570 nm. Cell viability of breast cancer cells cultured in the presence of ligands was calculated as a per cent of control cells.

Relaxation assay of topoisomerase II

Supercoiled pRYG DNA (0.5 mg) was incubated with 4 units of human topoisomerase II in the cleavage buffer (30 mM Tris-HCl (pH 7.8), 50 mM KCl, 10 mM $MgCl_2$, 3 mM ATP, 15 mM mercaptoethanol), in the presence of varying concentrations of the test compounds. Reactions were carried out at 37°C for 1 h and then terminated by the addition of 2 mL of 10% sodium dodecyl sulfate (SDS) and 2 mL of 50 µg/mL proteinase K. The reaction mixture was subjected to electrophoresis through a 0.8% agarose gel containing 0.5 mg/mL ethidium bromide in TBE buffer (90 mM Tris-borate and 2 mM EDTA). The gels were stained with ethidium bromide and photographed under UV light. The photographic negatives were scanned and the area representing supercoiled DNA, migrating as a single band at the bottom of the gel, was measured using UVI-KS4000i gel documentation and the analysis system (SyngenBiotech, San Carlos, CA, USA). The concentrations of the inhibitor that prevented 50% of the supercoiled DNA from being converted into relaxed DNA (IC_{50} values) were determined by averaging out the data from at least three experiments.

Ethidium displacement assay

Fluorescence was measured using a Hitachi spectrophotometer F-2500 FL (Tokyo, Japan) at room temperature. The DNA-ethidium complex was excited at 546 nm and the fluorescence was measured at 595 nm. 2 mL DNA solution (A_{260} = 2) in 10 mM Tris-HCl (pH 7.4) and 75 mM NaCl buffer solution were added to 2 mL of ethidium bromide (5.0×10^{-6} M) in 10 mM Tris-HCl (pH 7.4) and 75 mM NaCl buffer solution, and the maximum fluorescence was measured. Aliquots of a 10 mM stock of the test compound solution were then added to the DNA-ethidium solution, and the fluorescence was measured after each addition until a 50% reduction of fluorescence occurred. Theoretical curves were fit to the fluorescence intensity data points with nonlinear least-squares computer routines. The apparent binding constant was calculated from K_{EtBr}[EtBr] = K_{app}[drug], where [drug] = the concentration of the

Table 1. Viability of MCF-7 and MDA-MB-231 cells treated for 24 h with different concentrations of Pt_2(2-picoline)$_4$(berenil)$_2$ and cisplatin.

Concentration (µM)	Viability of cells (% of control)[a]			
	Pt_2(2-picoline)$_4$(berenil)$_2$		cisplatin	
	MCF-7	MDA-MB-231	MCF-7	MDA-MB-231
0	100	100	100	100
5	92 ± 2	88 ± 2	98 ± 2	98 ± 2
10	80 ± 2	75 ± 2	96 ± 2	96 ± 2
20	59 ± 2	49 ± 2	84 ± 2	76 ± 2
40	42 ± 2	33 ± 2	76 ± 2	70 ± 2
60	25 ± 2	16 ± 2	66 ± 2	60 ± 2
80	6 ± 2	6 ± 2	60 ± 2	52 ± 2
100	4 ± 2	4 ± 2	47 ± 2	40 ± 2

[a] Mean values ± SD from three independent experiments done in duplicate are presented

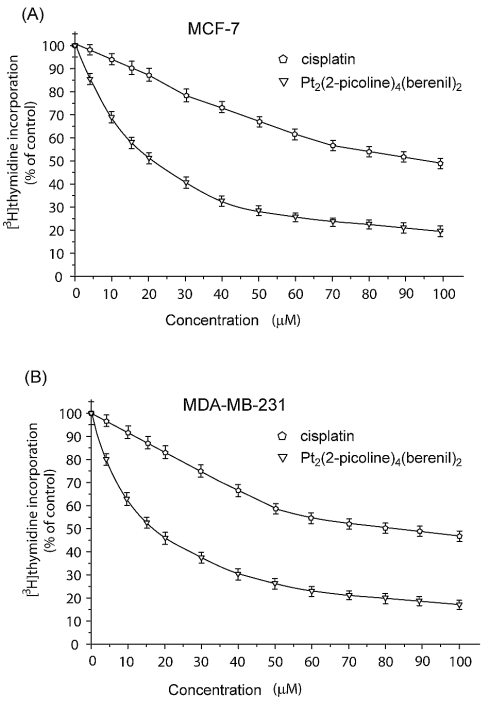

Figure 2. Antiproliferative effects of cisplatin and $Pt_2(2\text{-picoline})_4(berenil)_2$ in cultured breast cancer MCF-7 (A) and MDA-MB-231 (B) cells as measured by inhibition of [^{3}H]thymidine incorporation into DNA. The mean values ± SD of 3 independent experiments (n = 4) done in duplicates are presented

test compound at a 50% reduction of fluorescence and K_{EtBr} was known (7).

Statistical analysis

Statistical analyses were carried out using Origin 7.5 software (OriginLab, USA). Statistical evaluation of the untreated control cells along with drug- and solvent-treated cells was calculated using one-way ANOVA (analysis of variance). A probability of 0.05 or less was deemed statistically significant.

RESULTS

$Pt_2(2\text{-picoline})_4(berenil)_2$ (Fig. 1) was prepared by a modification of the procedure described (3, 8). Briefly, K_2PtI_4 obtained from K_2PtCl_4 was reacted with two equivalents of 2-picoline to form cis-[$Pt(2\text{-picoline})_2I_2$]. Cis-[$Pt(2\text{-picoline})_2I_2$] was reacted with 2 equiv of $AgNO_3$ in aqueous solution. The AgI precipitate was filtered off and then two equivalents of berenil and NaCl in water were added and the reaction mixture was mixed for several hours until a precipitate of $Pt_2(2\text{-picoline})_4(berenil)_2$ was formed. The chemical structure of $Pt_2(2\text{-picoline})_4(berenil)_2$ was proved by NMR, IR and elemental analysis.

The cell viability of breast cancer cells was measured by the method of Carmichael et al. (6) using tetrazolinum salt in order to compare cytotoxicity of $Pt_2(2\text{-picoline})_4(berenil)_2$ and cisplatin (Table 1). $Pt_2(2\text{-picoline})_4(berenil)_2$ was found to decrease the number of viable cells in both estrogen receptor-positive (MCF-7) and estrogen receptor-negative (MDA-MB-231) breast cancer cells. Although the cytotoxicity was concentration-dependent in both cell lines, it was more pronounced at shorter times in MDA-MB-231 than in MCF-7. $Pt_2(2\text{-picoline})_4(berenil)_2$ in both MDA-MB-231 and MCF-7 was proved to be more potent than cisplatin, with IC_{50} values of 20 ± 2 and 32 ± 2 mM, respectively, compared to 82 ± 2 and 93 ± 2 mM for cisplatin.

We measured DNA synthesis in the presence of $Pt_2(2\text{-picoline})_4(berenil)_2$ and cisplatin (Fig. 2). All of the study compounds showed concentration dependent activity, yet with different potency. Furthermore, the profiles of DNA synthesis obtained were similar between MCF-7 and MDA-MB-231 (Fig. 2). The concentrations of $Pt_2(2\text{-picoline})_4(berenil)_2$ needed to inhibit [^{3}H]thymidine incorporation into DNA by 50% (IC_{50}) in MDA-MB-231 after 24 hours was found to be 16 ± 2 mM, suggesting higher cytotoxic potency compared to cisplatin (IC_{50} = 86 ± 2 mM). The concentrations of $Pt_2(2\text{-picoline})_4(berenil)_2$ and cisplatin needed to 50% reduction in [^{3}H]thymidine incorporation into DNA in breast cancer MCF-7 (IC_{50}) after 24 hours were 21 ± 2 mM and 98 ± 2 mM, respectively.

Although there is some evidence to suggest that other biological targets are important in the mechanism of cisplatin, it is generally accepted that the antitumor activity of platinum drugs can be ascribed to interactions between the complex and DNA (1–3). The binding affinities of dinuclear or trinuclear platinum complexes to DNA is expected to depend on the size and electron density of the interacting aromatic rings as well as the combined effect of hydrophobic and hydrophilic interactions (1, 2). Flatness of the coordinated aromatic rings of berenil and 2-picoline and the additional stabilization by the cooperativity between stacking, hydrogen bonding, and electrostatic interactions will also affect the DNA binding affinity. The binding affinities of $Pt_2(2\text{-picoline})_4(berenil)_2$ and berenil to calf thymus DNA, T4 coliphage DNA, and synthetic polymers poly(dA-dT)$_2$ and poly(dG-dC)$_2$ were compared by using the ethidium displacement assay (7). Table 2 summarizes the results for those ligands that did affect the fluorescence due to the intercalated ethidium at pH 7.4. The large apparent binding

constants for T4 coliphage DNA for Pt_2(2-picoline)$_4$(berenil)$_2$ gave evidence of their minor-groove selectivity, because the major groove of T4 coliphage DNA was blocked by α-glycosylation of the 5-(hydroxymethyl)cytidine residues (9). The homopolymer DNA-binding data reported in Table 2 characterize the affinity of the Pt_2(2-picoline)$_4$(berenil)$_2$ and berenil for a more limited set of DNA-binding sites and can give an indication of base-sequence specificity for DNA-binding molecules. Pt_2(2-picoline)$_4$(berenil)$_2$ was found to interact with a GC base pair though the binding affinity was weak compared with that for an AT base pair (Table 2). The binding constant obtained here for binding of Pt_2(2-picoline)$_4$(berenil)$_2$ to poly(dC-dG)$_2$ polymer was almost 25 smaller than the association constant for binding of Pt_2(2-picoline)$_4$(berenil)$_2$ to poly(dA-dT)$_2$. Berenil bound to DNA in ethidium bromide displacement assays equivalent to that of Pt_2(2-picoline)$_4$(berenil)$_2$ (Table 2).

The ability of Pt_2(2-picoline)$_4$(berenil)$_2$ to inhibit topoisomerase II activity was quantified by measuring the action on supercoiled pRYG DNA substrate as a function of increasing concentration of the ligands by means of agarose gel electrophoresis. In this assay, cisplatin as a control was ineffective. It was found that Pt_2(2-picoline)$_4$(berenil)$_2$ caused the topoisomerase II inhibitory activity with 50% inhibitory concentrations (IC_{50}) of 20 mM (Table 2), which was better than that of berenil (IC_{50} = 40 mM). Thus, the topoisomerase II inhibiting activity may contribute to the cytotoxicity of Pt_2(2-picoline)$_4$(berenil)$_2$.

DISCUSSION

The widespread clinical use of platinum compounds in cancer chemotherapy has prompted a search for new platinum agents (1–3). The present study was undertaken to find out about the antineoplastic activity of the novel dinuclear platinum complex of formula Pt_2(2-picoline)$_4$(berenil)$_2$ (Fig. 1). The choice of 2-picoline as amino ligand was related to the interesting cytotoxic properties shown by the analogue platinum(II) complex cis-[$PtCl_2(NH_3)$(2-picoline)] (AMD473) which is currently in phase I of clinical trials (10). The ligand reduces rapidly the detoxification of thiol-containing molecules; moreover, AMD473 has been proved to be active, toward both some tumor cell lines intrinsically resistant to cisplatin and some human ovarian carcinoma xenograph sublines characterized by an acquired resistance to the reference drug after prolonged treatment (10). As well as overcoming cisplatin resistance from deactivation by thiol-containing proteins, AMD-473 has also been shown to overcome other modes of resistance such as reduced cellular uptake and enhanced DNA repair. Our experimental studies demonstrated that Pt_2(2-picoline)$_4$(berenil)$_2$ treatment prevented the exponential growth and decreased the number of viable cells in both estrogen receptor-positive and estrogen receptor-negative breast cancer cells. Since the antiproliferative effect of Pt_2(2-picoline)$_4$(berenil)$_2$ is independent of the estrogen receptor status of the breast cancer cells, this potent inhibitor is a potential pharmacological agent for the treatment of both hormone responsive and nonresponsive breast cancer cells.

Owing to the preliminary study presented in this paper, it was difficult to establish definite modes of DNA-binding by Pt_2(2-picoline)$_4$(berenil)$_2$. However, the potentially polyfunctional dinuclear complexes may lead to the formation of unique types of Pt–DNA adducts (2). Cisplatin binding produces a rigid bend in DNA of 30–35^o directed toward the major groove, and a localized unwinding of the DNA double helix of 13^o (11). The N(7) guanine atom is in the DNA major groove, making it more exposed and more easily accessible; as a consequence, it represents the preferred, if not exclusive, site of attack by the metal center leading to the formation of interstrand and/or intrastrand cross-links (11, 12). Conversely, the DNA binding of dinuclear platinum complexes was generally characterized by flexible, non-directional DNA adducts, a greater percentage of interstrand to intrastrand adducts and the ability to induce DNA conformational changes to both A- and Z type DNA (1, 2). The binding strength of dinuclear or trinuclear platinum complexes to DNA is expected to depend on

Table 2. DNA binding and topoisomerase II inhibitory effect of berenil and Pt_2(2-picoline)$_4$(berenil)$_2$.

Ligand	ct DNA[a] ($K_{app} \times 10^5$ M^{-1})	T4 DNA[a] ($K_{app} \times 10^5$ M^{-1})	poly(dA-dT)$_2$ [a] ($K_{app} \times 10^5$ M^{-1})	poly(dG-dC)$_2$ [a] ($K_{app} \times 10^5$ M^{-1})	Inhibition of topo[b] II (mM)
berenil	6.5	6.3	76.5	3.5	40
Pt_2(2-picoline)$_4$(berenil)$_2$	7.0	6.3	76.1	3.0	20

[a] The error for berenil and Pt_2(2-picoline)$_4$(berenil)$_2$ is ± 0.2 x 10^5 M^{-1} . [b] topo = topoisomerase

the size and electron density of the interacting aromatic rings as well as the combined effect of hydrophobic and hydrophilic interactions (1, 2).

In Pt_2(2-picoline)$_4$(berenil)$_2$ complex, berenil rather than platinum may dominate the DNA sequence specificity and ultimately dictate the sites of covalent attachment of the metal on DNA (Table 2). Flatness of the coordinated aromatic rings of berenil and 2-picoline and the additional stabilization by the cooperativity between stacking, hydrogen bonding, and electrostatic interactions will also affect the DNA binding affinity. Structures of DNA complexes of berenil, for example, reveal that it bound to the DNA minor groove at the central AATT sequence. The presence of the guanine NH_2 sterically impedes berenil from binding across GC pairs. Pt_2(2-picoline)$_4$(berenil)$_2$ was found to interact with a GC base pair though the binding affinities were weak compared with that for an AT base pair (Table 2). Since calf thymus DNA contained random sequences and therefore fewer AT sites than poly(dA-dT)$_2$, the selectivity of Pt_2(2-picoline)$_4$(berenil)$_2$ was further demonstrated by its much weaker binding to calf thymus DNA compared to poly(dA-dT)$_2$. The binding of Pt_2(2-picoline)$_4$(berenil)$_2$ in the minor groove of DNA may prevent binding of regulatory proteins or transcription factors to DNA promotors, as it was shown for other aromatic amidines (13, 14). Our study demonstrated that Pt_2(2-picoline)$_4$(berenil)$_2$ was a potent catalytic inhibitor of topoisomerase II (Table 2). This topological enzyme binds at least in part to AT rich sequences in the minor groove (14, 15). The AT base pair specificity shown by Pt_2(2-picoline)$_4$(berenil)$_2$ (Table 2) could be the result of the preferential binding of highly electropositive Pt_2(2-picoline)$_4$(berenil)$_2$ to the most electronegative region of DNA, rich in AT bases in the minor groove (16). The ability of Pt_2(2-picoline)$_4$(berenil)$_2$ can inhibit the activity of topoisomerase II (Table 2) due to the blockade of the enzyme binding to DNA. Topoisomerase II, identified as a major scaffold protein of mitotic chromosomes being present in the interphase nuclear matrix, is a homodimeric nuclear enzyme essential for DNA functioning, in particular negative supercoiling of DNA organized as superhelix, a process necessary for replication, recombination, and transcription of nuclear DNA (14, 15). Several widely used anticancer agents, including doxorubicin and other anthracyclines, amsacrine, etoposide and mitoxantrone target topoisomerase II are thought to be cytotoxic by virtue of their ability to stabilize a covalent topoisomerase II-DNA intermediate (17). It is now important to determine which adducts are responsible for the excellent cytotoxicity observed for Pt_2(2-picoline)$_4$(berenil)$_2$, the flexible, non-directional DNA intrastrand adducts, the long-range DNA interstrand adducts or DNA minor groove adducts. The mechanism by which the berenil moiety of Pt_2(2-picoline)$_4$(berenil)$_2$ contributes to the anticancer activity is being investigated at present.

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