Cisplatin is a classical Pt-based anticancer drug that is widely used for the treatment of a broad spectrum of tumors (1, 2). Its cytotoxicity is mediated mainly through interactions with DNA and inhibition of DNA synthesis and replication by formation of bifunctional interstrand and intrastrand cross-links. Despite its success, the clinical usefulness of cisplatin is limited by its severe side effects such as dose-dependent nephrotoxicity, nausea and vomiting, ototoxicity, neurotoxicity, and myelosuppression (1-3). The need for alternatives to cisplatin has consequently inspired further work towards the development of novel platinum-based drugs with improved and/or complementary properties. Some strategies have been applied during synthesis of new platinum drugs, such as the use of different ligands, in order to reduce side effects or increase the cytotoxicity potential of the drug (2, 3). Another strategy is the synthesis of dinuclear or trinuclear platinum complexes which may decrease the action of the cellular repair machinery by forming different types of complex-DNA adducts (3-7).

Recent work on the targeting of antitumor agents to DNA by the use of DNA minor groove-binding ligands has shown that this strategy can greatly enhance both the in vitro cytotoxicity and the in vivo antitumor activity of the alkylating moiety, when compared with untargeted compounds of similar reactivity (8-10). Based on this strategy and due to the high affinity of berenil for the minor groove of DNA, we are expecting that [Pt₂L₄(berenil)₂]Cl₄ complexes where L is piperidine (1), 4-picoline (2), 3-picoline (3) or isopropylamine (4) (Figure 1) would localize in the vicinity of the DNA, and the combined effect resulting from platination and minor groove binding might confer cytotoxic activity to these complexes.

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 (11, 12). Berenil preferentially recognizes and binds to AT-rich DNA sequences and it is strong catalytic inhibitor of mammalian DNA topoisomerase II (13). Moreover, the presence of an amidino moiety in berenil should confer hydrophilicity to these platinum complexes, that may facilitate transport across the cellular membrane, increase both intracellular drug accumulation and interaction with the DNA, thus improving their effectiveness.

In this study, we have investigated the antiproliferative activity of these novel Pt-berenil compounds 1-4 (Figure 1) in both MDA-MB-231 and

SYNTHESIS, DNA-BINDING AFFINITY AND CYTOTOXICITY OF THE DINUCLEAR PLATINUM(II) COMPLEXES WITH BERENIL AND AMINES LIGANDS

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Abstract: A series of platinum(II) complexes of formula [Pt₂L₄(berenil)]Cl₄·4HCl·2H₂O where L is piperidine (1), 4-picoline (2), 3-picoline (3) or isopropylamine (4) was prepared and their cytotoxicity have been tested against the growth of human breast cancer cells. Evaluation of the cytotoxicity of these compounds employing a MTT assay and inhibition of [3H]thymidine incorporation into DNA in both MDA-MB-231 and MCF-7 breast cancer cells demonstrated that these compounds were more active than cisplatin. Data from the ethidium displacement assay indicated that these compounds show moderate specificity for AT base pairs of DNA. Compounds 1-4 were also potent topoisomerase II inhibitors, with 50% inhibitory concentrations (IC₅₀) ranging from 5 to 50 µM.

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

363

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MCF-7 breast cancer cells. The mechanism of action of compounds 1-4 was studied employing the topoisomerase I/II inhibition assay, and ethidium displacement assay using calf thymus DNA. T4 coliphage DNA, poly(dA-dT)2 and poly(dG-dC)2 (14, 15).

**EXPERIMENTAL**

**Chemistry**

The structures of all the compounds were confirmed by 'H and 13C NMR spectra recorded on Brucker AC 200F apparatus (1H – 200 MHz and 13C – 50 MHz) in DMSO-d6. Infrared spectra were recorded on a Specord 75 spectrometer as KBr pellets (4000-450 cm⁻¹). Melting points were determined on Büchi 535 melting-point apparatus and were uncorrected. Elemental analysis of C, H, and N was performed on a Perkin Elmer 240 analyzer and satisfactory results within ± 0.4% of calculated values were obtained. Multiplicity of resonance peaks are indicated as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). Dimethylformamide, K₂PtCl₄, KI, acetone, isopropylamine, piperidine, diminazene aceturate (berenil), 3-picoline, 4-picoline, diethyl ether and methanol were purchased from Sigma Chemical Co (St. Louis, MO, USA).

**General synthetic method for [Pt₂(amine)₄(berenil)₂]Cl₄•4HCl•2H₂O**

K₂PtCl₄ (415 mg, 1 mmol) was dissolved in 50 mL of deionized water and filtered. KI (1.66 g, 10 mmol) in 5 mL of water was added to it and the reaction mixture was stirred for 15 min. Then, amine (2 mmol) was added dropwise to the reaction mixture, which was stirred for 15 min. To the filtrate an excess of cold water was added to obtain a yellow precipitate, cis-[Pt₂(isopropylamine)₂I₂]Cl₄•4HCl•2H₂O. Afterwards, the product was filtered off and washed with small amount of diluted HCl, water, methanol, acetone and ether and dried under vacuum. cis-[Pt₂(isopropylamine)₂I₂]Cl₄ formed. Afterwards, the product was filtered off and washed with small amount of diluted HCl, water, methanol, acetone and ether and dried under vacuum.

**[Pt₂(piperidine)₂(berenil)₂]Cl₄•4HCl•2H₂O (1)**

Yield = 59%, m.p. 247-249°C. 'H-NMR (DMSO-d₆) δ (ppm): 1.5 (m, 16H, -CH₂CH₂-), 2.74 (t, 8H, N-CH₂), 9.35 (bs, 4H, amidine), 9.00 (bs, 4H, amidine), 7.92 (d, 4H, Ar) 7.64 (d, 4H, Ar). ¹³C-NMR (DMSO-d₆) δ (ppm): 164.1 (amide), 150.1 (Ar), 129.5 (Ar), 121.9 (Ar), 118.0 (Ar), 47.9 (CH₂-N), 27.8 (CH₃), 25.9 (CH₂). IR (KBr, cm⁻¹): 3188 (NH aliphatic), 1664 (NCN), 1600 (triazene), 1258 (triazene), 1158 (triazene), 534 (Pt-N), 520 (Pt-N), 470 (Pt-N), 327 (Pt-Cl), 317 (Pt-Cl). Analysis: Calcd. for C₅₂H₅₆N₁₈Pt₂•4HCl•2H₂O: C, 39.13; H, 4.24; N, 17.21%.

**[Pt₂(4-picoline)₂(berenil)₂]Cl₄•4HCl•2H₂O (2)**

Yield = 42%, m.p. 225°C. 'H-NMR (DMSO-d₆) δ (ppm): 8.45 (d, 4H, Ar), 7.37, (d, 4H, Ar), 2.40 (s, 6H, CH₃), 9.35 (bs, 4H, amidine), 9.00 (bs, 4H, amidine), 7.92 (d, 4H, Ar) 7.64 (d, 4H, Ar). ¹³C-NMR (DMSO-d₆) δ (ppm): 164.1 (amide), 150.6 (Ar), 150.1 (Py), 147.0 (Py), 129.5 (Ar), 124.2 (Py), 121.9 (Ar), 118.0 (Ar), 20.3 (CH₃). IR (KBr, cm⁻¹): 3061 (C-H aromatic), 2960 (C-H, aliphatic), 1664 (NCN), 1610 (CN, pyridine), 1600 (triazene), 1440 (CH₂), 1380 (CH₂), 1258 (triazene), 1158 (triazene), 534 (Pt-N), 470 (Pt-N), 327 (Pt-Cl), 317 (Pt-Cl). Analysis: Calcd. for C₃₈H₆₆N₁₈Pt₂•4HCl•2H₂O: C, 41.49; H, 4.29; N 16.75. Found: C, 41.21; H, 4.24; N, 16.31%.

**[Pt₂(3-picoline)₂(berenil)₂]Cl₄•4HCl•2H₂O (3)**

Yield = 46%, m.p. 228°C. 'H-NMR (DMSO-d₆) δ (ppm): 2.35 (s, 6H, CH₃), 7.37 (m, 2H, Ar), 7.64 (d, 4H, Ar), 7.77 (d, 2H, Ar), 7.92 (d, 4H, Ar), 8.54 (d, 2H, Ar), 8.62 (s, 2H, Ar), 9.00 (bs, 4H, amidine), 9.35 (bs, 4H, amidine). ¹³C-NMR (DMSO-d₆) δ (ppm): 164.1 (amide), 150.1 (Ar), 129.5 (Ar), 121.9 (Ar), 118.0 (Ar), 150.5 (Py), 147.5 (Py), 133.9 (Py), 123.0 (Py), 136.8 (Py), 133.9 (Py), 19.0 (CH₃). IR (KBr, cm⁻¹): 3061 (C-H aromatic), 2960 (C-H, aliphatic), 1664 (NCN), 1610 (CN, pyridine), 1600 (triazene), 1440 (CH₂), 1380 (CH₂), 1258 (triazene), 1158 (triazene), 534 (Pt-N), 470 (Pt-N), 327 (Pt-Cl), 317 (Pt-Cl). Analysis: Calcd. for C₃₈H₆₆N₁₈Pt₂•4HCl•2H₂O: C, 41.49; H, 4.29; N 16.75. Found: C, 41.14; H, 4.26; N, 16.44%.

**[Pt₂(isopropylamine)₂(berenil)₂]Cl₄•4HCl•2H₂O (4)**

Yield = 54%, m.p. 216-218°C. 'H-NMR (DMSO-d₆) δ (ppm): 1.30 (d, 12H, CH₂), 2.90 (m, 2H, CH aliphatic), 5.30 (bs, 4H, amine), 9.35 (bs, 4H, amidine), 9.00 (bs, 4H, amidine), 7.92 (d, 4H, Ar) 7.64 (d, 4H, Ar). ¹³C-NMR (DMSO-d₆) δ (ppm): 164.1 (amide), 150.1 (Ar), 129.5 (Ar), 121.9 (Ar),
PHARMACOLOGY

Materials

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) were purchased from Sigma Chemical Co. (USA). Topoisomerase I/II was purchased from Amersham Pharmacia Biotech. Stock cultures of breast cancer MCF-7 and MDA-MB-231 were purchased from the American Type Culture Collection, Rockville, MD. 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). [3H]thymidine (6.7 Ci/mmol) was the product of NEN (USA).

Cell culture

Human breast cancer MDA-MB-231 and MCF-7 cells were maintained in DMEM supplemented with 10% fetal bovine serum (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 studied compounds on cells proliferation MCF-7 and MDA-MB-231 cells were seeded in 6-well plates and grown as described above. Cell proliferations were incubated with varying concentrations of compounds 1-4, cisplatin and 0.5 μC of [3H]thymidine for 24 h at 37°C. The cells were then harvested by trypsinization and washed (with 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. [3H]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) (16). Confluent cells, cultured for 24 h with various concentrations of studied 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% CO2 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 I and II

PBR322 plasmid DNA (0.083 mg) was incubated with 1 unit of human topoisomerase I (reaction buffer: 50 mM Tris-HCl (pH 7.9), 1 mM EDTA, 0.5 M NaCl, 1 mM dithiothreitol) or human...
topoisomerase II (reaction buffer: 10 mM Tris-HCl (pH 7.9), 1 mM ATP, 50 mM KCl, 5 mM MgCl₂, 50 mM NaCl, 0.1 mM EDTA, and 15 mg/mL bovine serum albumin) in the presence of varying concentrations of the test compound. The mixture was incubated at 37°C for 1 h and the reaction was terminated by addition of 2 mL of 10% SDS and 2 mL of proteinase K (1 mg/mL). 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. For the quantitative determination of topoisomerase concentration activity, photographic negatives were scanned and the area representing supercoiled DNA, 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. For the quantitative determination of topoisomerase concentration activity, 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 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\textsubscript{50} values) were determined by averaging 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. To 2 mL of ethidium bromide (5.0×10\textsuperscript{-6} M) in 10 mM Tris-HCl (pH 7.4), 75 mM NaCl buffer solution, containing 25 mL of DNA

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Figure 3. Cytotoxic effects of cisplatin and compounds 1-4 on the cultured breast cancer MCF-7 (A) and MDA-MB-231 (B) cells as measured by inhibition of \textsuperscript{[\textit{H}]thymidine incorporation into DNA. The mean values ± S.D. of 3 independent experiments (n = 4) done in duplicates are presented.
solution \((A_{260} = 2)\) was added, and the maximum fluorescence was measured. Aliquots of 10 mM test compound stock solution were then added to the DNA-ethidium solution, and the fluorescence was measured after each addition until a 50% reduction of fluorescence had occurred. Theoretical curves were fit to the fluorescence intensity data points with nonlinear least-squares computer routines. The apparent binding constant was calculated from 

\[
K_{\text{EtBr}}[\text{EtBr}] = K_{\text{app}}[\text{drug}],
\]

where \([\text{drug}] = \) the concentration of the test compound at a 50% reduction of fluorescence and \(K_{\text{EtBr}}\) is known (15).

Statistical analysis

In all experiments, the mean values for three assays ± standard deviations (S.D.) were calculated. The results were submitted to statistical analysis using the Student’s t-test. Differences were considered significant when \(p < 0.05\). Mean values, the standard deviations and the number of measurements in the group (n) are presented in the Figures.

RESULTS

We have synthesized several \([\text{Pt} \_2L_4(\text{berenil})]_2\text{Cl}_4\) complexes where \(L\) is piperidine (1), 4-picoline (2), 3-picoline (3) or isopropylamine (4) (Figure 1). The compounds 1-4 were prepared by a modification of the procedure described (17, 18). Briefly, \(\text{K}_2\text{PtI}_4\) obtained from \(\text{K}_2\text{PtCl}_4\) was reacted with two equivalents of amine (piperidine, 3-picoline, 4-picoline, or isopropylamine) to form cis-\([\text{Pt}(\text{amine})_2\text{I}_2]\). [\(\text{Pt}(\text{amine})_2\text{I}_2]\] was reacted with 2 equivalents of \(\text{AgNO}_3\) in aqueous solution. The \(\text{AgI}\) precipitate was filtered off and then two equivalents of berenil and \(\text{NaCl}\) in water were added and the reaction mixture was mixed for several hours until a precipitate of \([\text{Pt}_2\text{(amine)}_4(\text{berenil})_2]\text{Cl}_4\) was formed. All the compounds were synthesized in reasonable yield (60-40%) and were characterized by IR, \(^1\text{H}\) NMR, \(^{13}\text{C}\) NMR, and elemental analysis.

Cell viability of breast cancer cells was measured by the method of Carmichael et al. (16) using tetrazolium salt (Figure 2). Although growth inhibition was concentration-dependent in either cell line, it was more pronounced at shorter times, in MCF-7 than MDA-MB-231 (Figure 2). In terms of reduction in cell viability, the compounds rank in both MCF-7 and MDA-MB-231 cells in the order 1 > 3 > 2 > 4 > cisplatin. The most cytotoxic metal complexes are those that contain piperidine and 3-picoline ligands. Among the derivatives, compound 4 in both MDA-MB-231 and MCF-7 proved to be only slightly more potent than cisplatin, with IC\(_{50}\) values of 65 ± 2 and 70 ± 2 mM, respectively, compared to 85 ± 2 and 99 ± 2 mM for cisplatin. In contrast, compound 1 is clearly much more active and showed a high level of cytotoxic potency, IC\(_{50}\) 9 ± 2 and 17 ± 2 mM in MCF-7 and MDA-MB-231, respectively. Compound 1, the most active of the series, is approximately 10 times more potent than cisplatin.

To analyze if the inhibition in cell viability was due to decreased cell proliferation, we measured DNA synthesis in presence of compounds 1-4 and cisplatin (Figure 3). All of the tested 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 (Figure 3). The concentrations of 1, 2, 3 and 4 needed to inhibit \(^{3}\text{H}\)thymidine incorporation into DNA by 50% (IC\(_{50}\)) in MDA-MB-231 were found to be 14 ± 2 mM, 20 ± 3 mM, 18 mM and 32 ± 2 mM, respectively, suggesting higher cytotoxic potency compared to cisplatin (IC\(_{50}\) = 90 ± 2 mM). The concentrations of 1, 2, 3, 4 and cisplatin needed to 50% reduction in \(^{3}\text{H}\)thymidine incorporation into DNA in breast cancer MCF-7 (IC\(_{50}\)) were found to be 9 ± 2 mM, 14 ± 2 mM, 11 ± 2 mM, 24 mM and 105 ± 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

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<th>Ligand</th>
<th>Calf thymus DNA* ((K_{\text{app}} \times 10^5 \text{M}^{-1}))</th>
<th>T4 DNA* ((K_{\text{app}} \times 10^5 \text{M}^{-1}))</th>
<th>poly(dA-dT)* ((K_{\text{app}} \times 10^5 \text{M}^{-1}))</th>
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*The error for berenil and compounds 1-4 is ± 0.2 \(\times 10^5\) M\(^{-1}\).
antitumor activity of platinum drugs can be ascribed to interactions between the complex and DNA (1-3). The binding strength 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 (3, 19).

Flatness of the coordinated aromatic rings of berenil and the additional stabilization by the cooperativity between stacking, hydrogen bonding, and electrostatic interactions will also affect the DNA binding affinity (20). In \([\text{Pt}_2(\text{amine})_4(\text{berenil})_2]Cl_4\) complexes, berenil rather than platinum may dominate the DNA sequence specificity and ultimately dictate the sites of covalent attachment of the metal to DNA.

The binding affinities of compounds 1-4 and berenil to calf thymus DNA, T4 coliphage DNA, and synthetic polymers poly(dA-dT)\(_2\) and poly(dG-dC)\(_2\) were compared using the ethidium displacement assay (14, 15). Table 1 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 1-4 gave evidence of their minor-groove selectivity, because the major groove of T4 coliphage DNA is blocked by \(\alpha\)-glycosylation of the 5-(hydroxy-methyl)cytidine residues (21). The homopolymer DNA-binding data reported in Table 1 characterize the affinity of compounds 1-4 for a more limited set of DNA-binding sites and can give an indication of base-sequence specificity for DNA-binding molecules. Compounds 1-4 were found to interact with a GC base pair though the binding affinity was weak compared with that for an AT base pair (Table 1).

The binding constant obtained here for binding of 1 to poly(dC-dG)\(_2\) polymer is almost 25 times smaller than the association constant for binding of 1 to poly(dA-dT)\(_2\). Berenil was bound to DNA in ethidium bromide displacement assays equivalent to that of compounds 1-4 (Table 1).

The ability of compounds 1-4 to inhibit topoisomerase I and II activity was quantified by measuring the action on supercoiled pBR322 DNA substrate as a function of increasing concentration of the ligands by the use of agarose gel electrophoresis. These results demonstrated that 1-4 have topoisomerase II inhibitory activity with 50% inhibitory concentrations (IC\(_{50}\)) ranging from 5 to 50 mM (Table 1). None of compounds 1-4 inhibited the topoisomerase I mediated relaxation of supercoiled DNA at a concentration of 150 mM (data not shown). Compound 1 was the most potent topoisomerase II inhibitor, with 50% inhibitory concentration (IC\(_{50}\)) of 5 mM. The result of DNA binding studies revealed that 1 does have a greater DNA binding affinity, which correlates with its greater potency relative to 2, 3 and 4 as a topoisomerase II inhibitor.

**DISCUSSION**

There is a continuing interest in development of new platinum complexes, which are less toxic and have a broader activity spectrum than cisplatin (1-3). Polynuclear platinum(II) complexes have attracted considerable attention because they represent a new class of anticancer agents which possess potent and distinct biological activity from cisplatin (3-7). The multinuclear complexes appear to offer the greatest potential as they are generally highly cytotoxic and maintain their activity in platinum resistant cells (3-7). Variation in the nature of the amine can have a significant effect on the activity and toxicity of these complexes. Several platinum complexes with N-heterocyclic ligands, such as imidazole, thiazole, pyridine and purine derivatives, have been reported (22-24).

In breast cancer studies, estrogen responsive and nonresponsive breast cancer cell lines have been extensively used for elucidation of factors responsible for cell growth and for developing new strategies to inhibit cell growth. Our experimental studies have demonstrated that treatment with compounds 1-4 prevented the exponential growth and decreased the number of viable cells in both estrogen receptor positive and estrogen receptor-negative breast cancer cells. Because the antiproliferative effect of compounds 1-4 is independent of the estrogen receptor status of the breast cancer cells, these potent inhibitors are potential pharmacological agents for the treatment of both hormone responsive and nonresponsive breast cancer cells. These data suggest that \([\text{Pt}_2(\text{amine})_4(\text{berenil})_2]Cl_4\) complexes might be more effective than cisplatin in breast tumor cells. A more definite conclusion on the activity of these new platinum(II) complexes will be obtained by their testing in more tumor cell lines. These tests are in progress and their results will be reported in our future communication.

Previous studies have shown that compounds with planar N-heterocycles and cyclic amines can mainly form monofunctional adducts, 1,3-intrastrand bifunctional adducts, and interstrand bifunctional adducts (3, 24, 25). They can bind monofunctionally to DNA with a rate similar to that of cisplatin, then the rearrangement to bifunctional adducts is also similar to that observed in the case of cisplatin and it is a process relatively slow (24, 25).
Owing to the preliminary nature of the assay work presented in this paper, it is difficult to establish definite modes of DNA-binding by the dinuclear complexes. However, the potentially polyfunctional dinuclear and trinuclear complexes may lead to the formation of unique types of Pt-DNA adducts.

We have shown in the present report that compounds 1-4 are potent catalytic inhibitors of topoisomerase II. This topological enzyme binds at least in part to AT rich sequences in the minor groove (26, 27). Topoisomerase II which has been 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 (26-28). Several widely used anti-cancer agents, including doxorubicin and other anthracyclines, amsacrine, etoposide and mitoxantrone target topoisomerase II and are thought to be cytotoxic by virtue of their ability to stabilize a covalent topoisomerase II-DNA intermediate (26, 28). It is probable that deregulation of DNA replication and transcription by inhibition of topoisomerase II activity contribute significantly to the cytotoxicity of [Pt₂(amine)₄(berenil)₂]Cl₄ complexes in addition to primary drug-DNA reaction products.

The results of our studies suggest that further work should be conducted to better define the limits of the structure-activity relationships among [Pt₂(amine)₄(berenil)₂]Cl₄ complexes. Our group is currently examining a range of biological interactions that these compounds may undergo inside the cell to better understand their biochemistry and mechanism of action.

REFERENCES


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