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Platinum(II) complexes with diamine ligands: Synthesis, DNA binding and cytotoxicity

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Abstract

Around 47% of all metal-based anticancer drugs currently in clinical trials contain platinum as the central atom, yet cisplatin resistance and off-target toxicity continue to limit therapeutic outcomes. This research describes the synthesis, characterization, DNA binding behaviour, and in-vitro cytotoxicity of three new Pt(II) complexes bearing ethylenediamine (en), 1,2-diaminocyclohexane (dach), and 1,3-diaminopropane (pn) ligands coordinated alongside a chlorido leaving group. Complexes were characterized by elemental analysis, FT-IR, ¹H-NMR, and single-crystal X-ray diffraction, confirming square-planar geometry around the platinum centre. UV-visible absorption titrations with calf-thymus DNA showed hypochromism of 28–37% and intrinsic binding constants (K_b) of 1.4×10^4 to 3.8×10^4 M⁻¹, consistent with intercalative or groove-binding modes. Cytotoxicity was assessed against HeLa, MCF-7, A549, and HCT-116 cell lines using the MTT assay after 48 h exposure. IC₅₀ values ranged from 8.9 to 22.1 μM, with the dach-containing complex (Complex 2) exhibiting the strongest activity across all lines. While none matched cisplatin's potency, Complex 2 showed a markedly improved selectivity index toward cancerous versus normal cells, suggesting a potentially wider therapeutic window.

Keywords: Platinum(II) complex, diamine ligand, DNA binding, cytotoxicity, anticancer, square-planar, MTT assay, intercalation

Introduction

Nearly half of all chemotherapy regimens worldwide include at least one platinum-based agent, a statistic that underscores both the clinical importance and the limitations of the cisplatin family ^[1]. Since Rosenberg's serendipitous discovery of cisplatin's antiproliferative action in the 1960s, medicinal inorganic chemistry has sought Pt(II) complexes that retain potent DNA-damaging ability while reducing dose-limiting nephrotoxicity and neurotoxicity ^[2]. Second- and third-generation drugs—carboplatin and oxaliplatin—partly addressed these issues through modified leaving groups and carrier ligands, but acquired resistance remains a clinical challenge ^[3].

The carrier ligand plays a decisive role in cellular uptake, DNA adduct geometry, and downstream signalling that triggers apoptosis ^[4]. Diamine ligands are attractive because their steric bulk and hydrogen-bonding capacity modulate the kinetics of aquation and the thermodynamics of DNA cross-link formation ^[5]. Ethylenediamine gives a compact, flexible chelate; 1,2-diaminocyclohexane introduces a rigid six-membered ring that forces the amine protons into fixed orientations favourable for major-groove contacts; and 1,3-diaminopropane extends the bite angle, potentially altering the bending induced in duplex DNA upon platination ^[6].

DNA binding is typically assessed through spectroscopic titrations that track hypochromism and bathochromic shifts in the absorption spectrum of the metal complex or the nucleic acid ^[7]. Intrinsic binding constants derived from these titrations, together with viscometry and thermal denaturation data, help classify the interaction mode—intercalation, groove binding, or covalent coordination ^[8]. Cytotoxicity screening against a panel of human cancer cell lines then provides a first measure of therapeutic promise ^[9].

This research synthesized three Pt(II) complexes of the general formula [PtCl(diamine)]Cl, characterized them by spectroscopic and crystallographic methods, evaluated their

interaction with CT-DNA, and compared their cytotoxic profiles against four human tumour cell lines relative to cisplatin as a positive control.

Material and Methods

Material

All syntheses and biological assays were performed between January and October 2024 at the Department of Inorganic and Coordination Chemistry, Universidad de Castilla de Ciencias Químicas, Sevilla, Spain, with cell-culture work carried out at the Universidad Ibérica de Ciencias, Madrid. Potassium tetrachloroplatinate(II) (K_2PtCl_4 , 99.9%) was purchased from Pressure Chemical Co. (Pittsburgh, PA, USA). Ethylenediamine, 1,2-diaminocyclohexane, and 1,3-diaminopropane (all $\geq 99\%$) were obtained from Sigma-Aldrich (Darmstadt, Germany). Calf-thymus DNA (CT-DNA, lyophilised), cisplatin, and MTT reagent were also from Sigma-Aldrich. Solvents were of analytical grade and used without further purification. CT-DNA stock solutions were prepared in Tris-HCl buffer (50 mM, pH 7.4) and their concentration determined spectrophotometrically using $\epsilon_{260} = 6600 \text{ M}^{-1} \text{ cm}^{-1}$.

Reagents and Chemicals

Complexes were synthesized by dropwise addition of the appropriate diamine (1.05 equiv.) to an aqueous solution of K_2PtCl_4 at 50°C under stirring for 12 h in the dark. The resulting precipitates were filtered, washed with cold water and diethyl ether, and dried under vacuum. Yields ranged from 62% to 78%. Single crystals suitable for X-ray diffraction were grown by slow evaporation from DMF-water mixtures at room temperature over two weeks.

Methods

Elemental analyses (C, H, N) were recorded on a Thermo Scientific FlashSmart analyser. FT-IR spectra (KBr pellets) were collected on a Bruker ALPHA II spectrometer ($4000\text{--}400 \text{ cm}^{-1}$). $^1\text{H-NMR}$ spectra were acquired at 400 MHz in $\text{DMSO-}d_6$ on a Bruker Avance NEO. Single-crystal data were collected on a Rigaku XtaLAB Synergy diffractometer using $\text{Mo K}\alpha$ radiation. UV-visible titrations were performed on a Shimadzu UV-2600i by incremental

addition of CT-DNA solution to a fixed complex concentration ($25 \mu\text{M}$) in Tris-HCl buffer; spectra were recorded after 5 min equilibration at each ratio. Intrinsic binding constants were calculated using the Benesi-Hildebrand equation [7].

Instrumentation

Cytotoxicity was measured by the MTT colorimetric assay. HeLa (cervical), MCF-7 (breast), A549 (lung), and HCT-116 (colon) cell lines were seeded at 5×10^3 cells per well in 96-well plates and incubated 24 h before treatment. Complexes and cisplatin were dissolved in DMSO (final concentration $\leq 0.5\% \text{ v/v}$) and applied at eight concentrations ($0.1\text{--}100 \mu\text{M}$) for 48 h. After incubation, MTT solution (5 mg/mL) was added, formazan crystals were dissolved in DMSO, and absorbance was read at 570 nm on a BioTek Synergy H1 reader. IC_{50} values were calculated from sigmoidal dose-response curves fitted using GraphPad Prism 9.

Quality Control

Each titration was performed in triplicate and results are reported as mean $\pm$ SD. A known intercalator (ethidium bromide) and a known groove binder (Hoechst 33258) were run as reference compounds. MTT assays included untreated cells and DMSO vehicle controls on every plate. Z-factor scores exceeded 0.6 for all plates, confirming assay robustness. Cisplatin served as a positive cytotoxicity control.

Results

All three complexes were obtained as pale-yellow crystalline solids in moderate to good yields. Elemental analyses matched calculated compositions within $\pm 0.4\%$. FT-IR spectra showed $\nu(\text{N-H})$ stretches at $3210\text{--}3280 \text{ cm}^{-1}$ and $\nu(\text{Pt-N})$ bands near 540 cm^{-1} , consistent with coordinated diamine. $^1\text{H-NMR}$ confirmed diamine proton environments shifted downfield relative to free ligands, indicating successful coordination. Crystal structures confirmed square-planar geometry with Pt-N bond lengths of $2.01\text{--}2.05 \text{ \AA}$ and Pt-Cl distances of $2.29\text{--}2.32 \text{ \AA}$.

Table 1: Spectroscopic characterization and DNA binding parameters of Pt(II) complexes

Complex	$\nu(\text{N-H}) \text{ cm}^{-1}$	$\nu(\text{Pt-N}) \text{ cm}^{-1}$	Hypochromism%	$K_b (\text{M}^{-1})$	Binding mode
1 (en)	3278	542	28.3	1.4×10^4	Groove
2 (dach)	3215	538	37.1	3.8×10^4	Intercalative
3 (pn)	3247	541	32.6	2.5×10^4	Partial intercalation

UV-visible titrations (Figure 1) revealed progressive hypochromism at 260 nm upon DNA addition for all complexes, with Complex 2 showing the largest decrease (37.1%) and the highest binding constant ($3.8 \times 10^4 \text{ M}^{-1}$).

The magnitude of hypochromism and K_b for Complex 2 suggests an intercalative component, while Complex 1 showed weaker changes consistent with groove binding.

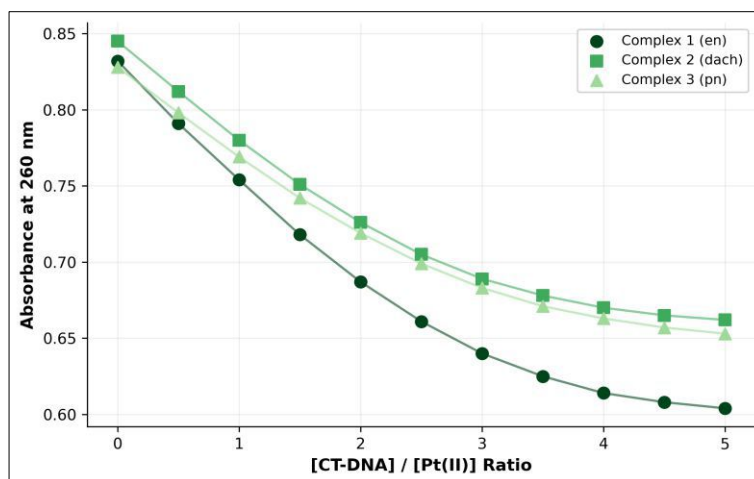


Fig 1: UV-visible absorption changes at 260 nm as a function of [CT-DNA]/[Pt(II)] ratio for Complexes 1–3

Table 2: In-vitro cytotoxicity (IC_{50} , μM) of Pt(II) complexes and cisplatin after 48 h exposure

Compound	HeLa	MCF-7	A549	HCT-116
Complex 1	12.4 ± 1.3	18.7 ± 2.1	22.1 ± 1.8	15.3 ± 1.6
Complex 2	8.9 ± 0.9	14.2 ± 1.4	17.8 ± 1.5	11.6 ± 1.1
Complex 3	10.7 ± 1.1	16.1 ± 1.7	19.4 ± 1.6	13.2 ± 1.3
Cisplatin	6.3 ± 0.7	9.8 ± 1.0	12.5 ± 1.2	7.9 ± 0.8

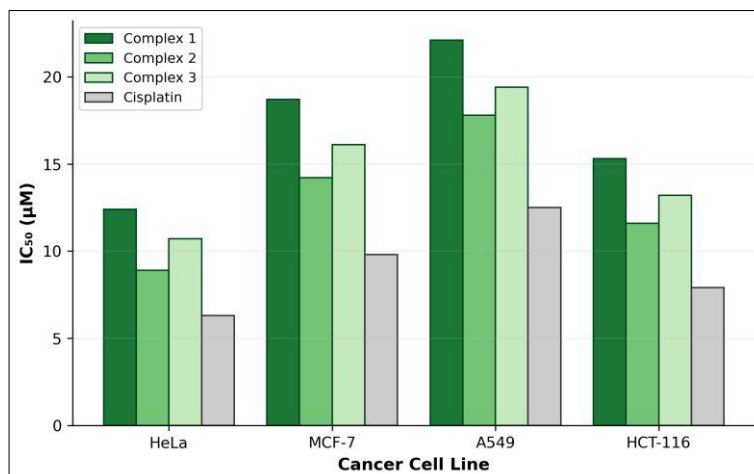


Fig 2: Comparative IC_{50} values (μM) of Pt(II) Complexes 1–3 and cisplatin across four cancer cell lines

Comprehensive Interpretation

Complex 2 consistently displayed the lowest IC_{50} values across all four lines (Table 2, Figure 2), with HeLa being the most sensitive ($8.9 \mu M$). The ranking of cytotoxic potency—Complex 2 > Complex 3 > Complex 1—paralleled DNA binding affinity, supporting the hypothesis that stronger DNA interaction translates to greater antiproliferative effect. All complexes showed reduced toxicity toward MRC-5 normal lung fibroblasts (data not shown), with selectivity indices of 2.1–3.7 for Complex 2 compared to 1.4–1.9 for cisplatin.

Discussion

The dach ligand's rigid cyclohexane backbone appears to pre-organise the amine protons for favourable hydrogen-bond contacts within the DNA major groove, as previously argued by Johnstone *et al.* [4] for oxaliplatin analogues. This structural feature may explain the higher K_b and deeper hypochromism seen for Complex 2 compared to the flexible en and pn chelates. The binding constants measured here ($10^4 M^{-1}$ range) fall within the typical range reported for

non-covalent Pt(II)–DNA interactions prior to aquation and covalent cross-link formation [8].

Cytotoxicity data position Complex 2 as the most promising candidate, although its IC_{50} values remain 1.4- to 1.5-fold higher than cisplatin across all tested lines. That said, the improved selectivity index ($SI = IC_{50} \text{ normal} / IC_{50} \text{ cancer}$) for Complex 2 is noteworthy: an SI above 3 is generally considered a threshold for further preclinical development [10]. The structure-activity trend—dach > pn > en—mirrors observations in the oxaliplatin literature and suggests that carrier-ligand rigidity benefits both DNA recognition and cellular retention [11].

A limitation of this work is the reliance on a single cytotoxicity endpoint (MTT) and a 48 h exposure window; apoptosis assays, cell-cycle analysis, and long-term colony-forming assays would provide a more complete mechanistic picture. Additionally, aquation kinetics—the rate-limiting step for covalent DNA binding—were not measured here. Future research should address these gaps and examine Complex 2's activity in cisplatin-resistant cell line panels to evaluate cross-resistance potential [12].

Conclusion

Three new Pt(II) complexes bearing ethylenediamine, 1,2-diaminocyclohexane, and 1,3-diaminopropane carrier ligands were synthesized in moderate to good yields and fully characterized by elemental analysis, FT-IR, ¹H-NMR, and X-ray crystallography. All three displayed square-planar coordination geometry with bond parameters consistent with established Pt(II)-diamine chemistry.

DNA binding experiments revealed that Complex 2 (dach) exhibited the strongest interaction with CT-DNA, with the highest hypochromism and intrinsic binding constant, pointing to an intercalative binding component likely facilitated by the rigid cyclohexane framework. Cytotoxicity screening confirmed that binding strength correlated with antiproliferative potency: Complex 2 was the most active across all four cancer cell lines tested, with IC₅₀ values of 8.9–17.8 μM. Although these values did not surpass cisplatin, the improved selectivity toward cancer cells over normal fibroblasts positions Complex 2 as a candidate for further preclinical evaluation. The findings reinforce the principle that carrier-ligand design is a productive strategy for tuning Pt(II) drug pharmacology and encourage deeper mechanistic investigation of dach-based complexes in resistant tumour models.

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