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Razuwika, R., Sookai, S., Aronson, R. et al. (2 more authors) (2025) A Cytotoxic Indazolebased Gold(III) Carboxamide Pincer Complex Targeting DNA through Dual Binding Modes of Groove Binding and Alkylation. Chemistry - A European Journal. e202404345. ISSN 0947-6539 (In Press)

https://doi.org/10.1002/chem.202404345

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Accepted Article

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To be cited as: Chem. Eur. J. 2025, e202404345

Link to VoR: https://doi.org/10.1002/chem.202404345

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A Cytotoxic Indazole-based Gold(III) Carboxamide Pincer Complex Targeting DNA through Dual Binding Modes of Groove Binding and Alkylation

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Abstract: Gold(III) complexes have garnered increasing attention in drug delivery due to their structural and mechanistic similarities to cisplatin. This study investigates an indazole-based gold(III) carboxamide pincer complex, AuL, for its potential as an anticancer agent. Speciation analysis at physiological pH revealed that AuL predominantly exists as a neutral chlorinated species. The complex exhibited strong cytotoxicity against the MCF-7 breast cancer cell line, with an impressive IC₅₀ value of 9 µM, while showing no significant activity against the HT-29 colon cancer cell line. Comprehensive analysis using electrophoresis, viscometry, UV-Vis, CD, LD spectroscopy, and biomolecular simulations demonstrated that AuL binds to DNA via a dual mechanism, specifically minor groove binding and alkylation, with binding constants $K_{a1} = 1.48 \times 10^9 \text{ M}^{-1}$ and $K_{a2} =$ $6.59\times10^5\,M^{-1},$ respectively. Our data indicate that AuL initially binds to the minor groove of DNA, at which point a nucleobase substitutes the CI ion, resulting in AuL binding directly to the DNA bases. In conclusion, the dual binding mode of AuL with DNA underscores its potential as a promising anticancer agent, opening new avenues for drug discovery and the development of metal-based therapeutics.

Introduction

Gold(III) pincer complexes are attracting significant interest as potential anticancer agents due to the gold(III) ion's isoelectronic and isostructural similarity to platinum(II), the central metal ion in cisplatin, the most widely used and successful anticancer metallodrug to date.^{1,2} Recent studies have demonstrated that certain gold(III) complexes exhibit *in vitro* cytotoxicity against specific cancer cell lines, with activity levels that are comparable to or even exceed those of cisplatin.^[1–3] Ongoing research continues to explore the therapeutic potential of gold(III) complexes, aiming to expand the arsenal of effective metal-based anticancer agents.^[4–6]

Despite advancements in the study of gold(III) complexes, further research is necessary, particularly on pincer complexes to better understand their stability, reactivity, and therapeutic potential, given the limited number of available libraries for these molecules. A common strategy to enhance cytotoxicity involves modifying these complexes with moieties known for their anticancer properties. One such group is the indazole moiety, a purine base analogue,^[7,8] which has recently gained prominence as a key building block in pharmaceutical and medicinal chemistry. ^[8-10] Although rare in nature, synthetic indazole derivatives have demonstrated diverse pharmacological properties, including antiinflammatory, analgesic, antiarrhythmic,^[10] antibacterial,^[11] antifungal^[12] anti-HIV^[13-19]. Furthermore, several indazole-based drugs have shown success in cancer treatment, [20-32] leading to the exploration of novel indazole motifs for next-generation therapeutics.^[8] Consequently, the indazole group and its bioisosteres remain a major focus of extensive studies.^[9,33–35]

Several carboxamide pincer complexes have been reported, though only a few feature gold(III) as the central metal ion.^[36–38] These complexes incorporate amide groups, which are prevalent in DNA, cellular proteins,^[39,40] and numerous pharmaceutical drugs.^[39,41] Amide functionalities in these complexes contribute to hydrogen bonding, enhancing their rigidity and stability.^[42,43]

DNA is a primary molecular target in cancer therapy due to its essential role in cellular functions. Disrupting DNA integrity has been shown to impair the activity of cancerous cells, making it a critical focus for treatment strategies.^[44–46] By binding to DNA and disrupting its secondary and tertiary structures, drugs can effectively inhibit uncontrolled cell replication. This inhibition is achieved through mechanisms such as alkylation, base displacement, intercalation, or groove binding.

Herein, we report the synthesis of an indazole-based carboxamide pincer ligand and its corresponding gold(III) complex, **AuL**. We hypothesize that **AuL** facilitates DNA binding

10.1002/chem.202404345

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through π - π stacking interactions between the indazole moiety and nucleic acid bases. This would engender significant, exploitable cytotoxicity for the complexes *in vitro* against the human breast adenocarcinoma cell line, MCF-7 and a human colorectal adenocarcinoma cell line, HT-29.

Results and Discussion

General synthesis and characterization

The ligand H_2L , which has been reported by Razuwika and Munro^[47], was metalated by adapting and modifying a method by Akhmadullina, et al.^[48] This entailed stirring H_2L with KAuCl₄ in a binary mixture of water-acetonitrile (3:1) for 15 h, to yield a novel carboxamide gold(III) pincer complex **AuL** (Figure 1a). The product was characterised using a range of analytical techniques, including NMR and FT-IR spectroscopy, elemental analysis, as well as high-resolution mass spectrometry.

The NMR, FT-IR and electronic spectra of AuL are mainly presented to highlight the salient spectroscopic features of the synthesised compound. In Figure 1b, the ¹H NMR spectrum reflects coordination of the gold(III) ion to the pincer ligand. In the ¹H NMR spectrum of H_2L , the resonance at 12 ppm (peak e) disappears upon the complexation with the gold(III) ion in the spectrum of AuL, signifying successful metallation. This disappearance is attributed to the deprotonation of the amide protons in H₂L, which facilitated metal binding. Metallation of the ligand resulted in a general upfield shift for all protons except proton **b** (at the meta position), which shifts downfield due to increased deshielding. The ¹³C NMR spectrum (Figure S1) supports the ¹H NMR findings, showing the downfield shift of the amide carbonyl peak from 161.98 ppm in H₂L to 170.04 ppm in AuL, which is attributed to the higher electronegativity of Au in comparison to H. [49-51]

The FT-IR spectra (Figure 1c), show that the amide NH stretch (3310-3290 cm⁻¹) and NH bend (1560-1585 cm⁻¹) peaks present in H_2L disappear upon complexation with the gold(III) ion, consistent with metallation. Additionally, the C=O stretch observed at 1690-1700 cm⁻¹ in H_2L shifts to a lower wavenumber (1683-1692 cm⁻¹) presumably from back-donation of metal electron density into the antibonding MO of the amide groups.

Crystallography

The crystal structure of H₂L was previously reported.^[47] Single crystals of AuL were obtained by slow diffusion of tertbutanol (tBuOH) into a DMSO solution of AuL, while single crystals of AuLT (a thymine derivative of AuL) were grown by slow diffusion of a thymine solution in water into a DMSO solution of AuL.

Unlike the carboxamide pincer ligand H_2L , which has been reported to exhibit atropisomerism,^[47] the complex **AuL** does not display this property. The metal ion holds the pyridine and the amide nitrogen atoms, which are initially non-planar, in a relatively planar configuration around the metal centre. While the anchoring site remains planar, the presence of a dihedral angle allows the "pincer arms" to rotate. As a result, the complex crystallizes with C_i symmetry. The rigidity imposed on the anchoring site reduces the flexibility of the compound, leading to the loss of atropisomerism.



Figure 1. **a)** Structure and general synthetic scheme for the indazole based gold(III) carboxamide pincer complex **AuL** at room temperature for 15 h under study.^[48,52] **b**) A comparison between the proton NMR spectra for H₂L (blue) and **AuL** (red) displaying presence and the absence of the amide NH proton at 11.80 ppm. **c)** FT-IR spectra of H₂L and **AuL**, displaying the shift of the carbonyl peaks from 1692 to 1683 cm⁻¹ and the disappearance of the NH stretch and bend peaks at 1583 and 3316 cm⁻¹, respectively upon complexation with gold(III).

Complexes **AuL** and **AuLT** crystallised in the P-1 space group. The coordination environment around the gold(III) ion is planar, however, the "indazole arms" of the pincer ligand deviate from the planarity of the central pyridine and AuN₃ coordination group with inequivalent dihedral angles of 60.1° and 56.5° in **AuL**. This distortion results in the loss of both chirality and C_2 symmetry in **AuL**, in distinct contrast to the precursor ligand H₂L.

AuLT provided important insights into the binding interactions of the thymine base with the gold(III) metal center.

Thymine coordinates to the metal in its deprotonated ionic form, resulting in a neutral compound. The introduction of this aromatic group modifies the orientation of the "indazole arms" in **AuLT**, aligning them in the same direction, in contrast to **AuL**, where the arms are oriented oppositely. The dihedral angles between the indazole groups and mean plane passing through the central pyridine ring and AuN₃ coordination group are 86.7° and 89.2° in **AuLT**. While **AuL** exhibits nearly identical bond lengths for both Au–N_{amide} bonds, **AuLT** shows a difference of 0.015 Å between these bonds (see Figure 2). This variation arises from the interactions depicted in Figure 2b, where the thymine group engages in π - π interactions with one of the indazole rings, thereby shortening the corresponding Au–N_{amide} bond.

In general, the Au-N bond lengths are shorter in AuLT compared to those in AuL. The most significant change is observed between the Au-N_{Thymine} (2.014 Å) and Au-CI (2.265 Å) bonds, with a reduction of 0.251 Å upon the substitution of CI⁻ with thymine. This suggests that the Au-N bond is stronger than the Au-CI bond, which is expected given that the anionic nitrogen is more basic than the chloride ion. The ability of nitrogen to donate a pair of electrons to the metal centre stabilises the Au-Nthymine bond. Thymine's bulkier structure compared to Cl⁻ also increases the dihedral angles between the metal pincer core and the two "indazole arms" to ~90° in AuLT, compared to ~65° in AuL as noted above (Figure S5). This suggests that the pincer complex exhibits flexibility, allowing it to accommodate ligands of varying bulkiness. This adaptability could enhance the complex's ability to interact with a diverse range of molecules, potentially influencing its reactivity and stability.

Electronic spectroscopy

Preceding any spectroscopic studies, the gold(III) pincer complex AuL was electronically characterised in DMSO, and the UV-vis absorption spectrum is presented in Figure 3. Three absorption maxima were identified at 267 nm (ε = 9.05 x 10⁴ M⁻¹ cm⁻¹), 296 nm (ε = 8.96 x 10³ M⁻¹ cm⁻¹) and 439 nm (ε = 3.91 x 10³ M⁻¹ cm⁻¹). The peaks at 267 nm and 296 nm are attributed to the indazole rings, in accordance with data in the literature, [53,54] while the peak at 439 nm is characteristic of gold(III) complexes.^[55] The transitions were further assigned using time-dependent density functional theory (TD-DFT) calculations at the HSEH1PBE/SDD level of theory. The DFT calculated electronic spectrum of AuL in DMSO (using a polarizable continuum model) is shown in the inset to Figure 3a. The predicted absorption maxima and the major contributions to each transition are summarised in Figure 3b and Table S1. The calculated peaks correspond well with the experimental data after a wavelength adjustment of +30 nm.

The lower energy transition (378 nm), correlating with the experimental peak at 439 nm, involves excitation from the HOMO to the LUMO or LUMO+3. Higher energy transitions at 267 nm and 296 nm are assigned to excitations from the HOMO-9 to the LUMO+2, and from the HOMO-6 to the LUMO, respectively. The HOMO orbitals are π -bonding and delocalized across the indazole rings, while the LUMO and LUMO+3 are predominantly antibonding (π^*) in nature and localized on the gold metal centre. The LUMO+2 is primarily localized on the pyridine ring. The lowest energy transitions are thus attributed to intraligand charge transfer (ILCT) with a dominant $\pi \rightarrow \pi^*$ character. Due to the significant $5d_{x^2-y^2}$ character in the LUMO, the HOMO-LUMO transition also includes a component of ligand-to-metal charge transfer (LMCT).



Figure 2. Selectively labelled views of the X-ray crystal structures of **a**) AuL and **b**) **AuLT**. Thermal ellipsoids are rendered at the 50% probability level and H atoms are drawn as spheres of an arbitrary radius. Space groups: P1⁻. **AuL** and **AuLT** has C_i symmetry.

Notably, the LMCT band shifts are solvent and pH dependent. In DMSO (Figure 3a), a blue shift of this band is observed, likely due to the solvent's solvation effects that stabilize higher energy states. However, in the multicomponent buffer (Figure 4a), where the pH range overlaps with that of DMSO (5-8), the LMCT band shifts to a lower wavelength, indicating a red shift. This difference may be due to the buffer's ionic interactions and specific solvation effects that stabilize lower energy states. Additionally, increasing the pH causes a shift to a lower wavelength, likely due to protonation effects or changes in the coordination environment, further influencing the electronic transitions and absorption bands.

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Figure 3. a) The Experimental and DFT-calculated absorption spectra of AuL in DMSO, indicating the three Absorption maxima Imax. The absorption envelope for the DFT-calculated spectrum is plotted with full width at half maximum intensity (FWHM) of 3000 cm-1. b) Molecular orbitals involved in the three most intense bands in the DFT-calculated electronic spectrum of AuL in DMSO. The percentage contribution of the electronic transitions to each band is indicated.

Speciation Studies

The identification of distinct chemical species at various pH levels is essential for understanding a compound's behavior under physiological conditions.^[56] In the context of **AuL**, it can be inferred that the number of species corresponds to the number of pK_a values, with each pK_a representing a transition point between two species and indicating an equilibrium shift.^[57] This study aimed to: (i) determine which species of **AuL** is present at physiological pH (pH 7.4), conditions that closely mirror those encountered in biological systems^[56,58] and (ii) accurately delineate the pK_a of **AuL**. We therefore conducted a series of UV-vis spectroscopic measurements on **AuL** across a pH range of

2.433 to 12.915, closely monitoring alterations in the complex's electronic spectrum at 292, 330 and 460 nm, respectively (Figure 4). Even though physiological pH's range from 3.5-7.45, it is important to analyse the behaviour of **AuL** over a wide pH range to accurately delineate the complex's p K_{a} .^[59] The titration data revealed the presence of four distinct transition points, corresponding to five different ionisation states of **AuL** across the pH range, which allowed for the derivation of equations (1) and (2). The equations describe the speciation behaviour of **AuL** and offer valuable insights for further interpretation of the experimental data.

$$[AuLH_2]^{2+} \rightleftharpoons [AuLH]^+ \rightleftharpoons [AuL] \rightleftharpoons [AuLH_2O]^+ \rightleftharpoons [AuLOH]$$
(1)

Equation (1) reflects the dynamic equilibrium involving the different species of **AuL** as a function of pH and enables a quantitative understanding of how the concentration of each species changes with variations in pH. The four steps in the overall equilibrium are described by the equilibrium constants K_1 , K_2 , K_3 , and K_4 for the forward reactions from left to right.

If the respective contributions of the species $[AuLH_2]^{2+}$, $[AuLH]^+$, [AuL], $[AuLH_2O]^+$ and [AuLOH] to the total absorbance (A_T) in a solution at various pH levels are A_A, A_B, A_C, A_D and A_E, then equation for A_T is illustrated in equation (2) below. (The full derivation is given in the ESI.)

 $A_T = \frac{\alpha}{\beta}$

where:

$$\alpha = A_A \times 10^{-4\text{pH}} + K_1 A_B \times 10^{-3\text{pH}} + K_1 K_2 A_C \times 10^{-2\text{pH}} + K_1 K_2 K_3 A_D \times 10^{-\text{pH}} + K_1 K_2 K_3 K_4 A_F$$

 $\beta = 10^{-4pH} + K_1 \times 10^{-3pH} + K_1 K_2 \times 10^{-2pH} + K_1 K_2 K_3 \times 10^{-pH} + K_1 K_2 K_3 K_4$

(2)

To provide a mechanistic understanding of the observed behaviour of the ionisation state of **AuL** at different pH levels, the pK_a values were calculated using equation (2) in OriginPro 2023b.^[60] The calculated pK_a values associated with each ionisation state were found to be 3.2, 5.8, 11.0, and 12.3. Peak maxima at 263 nm and 291 nm are characteristic of the indazole ring,^[53,54] while the maximum at 448 nm is characteristic of the gold(III) metal ion. The pH titration data revealed a red shift in the peak maxima at 263 and 291 nm, between pH's 2 and 5, indicating that the primary changes within this pH range occur on the indazole rings. At more alkaline pH levels, the main spectral changes are centred around 428 nm, which is characteristic of the gold(III) MLCT (metal-to-ligand charge transfer) transitions.

The proposed ionization of **AuL** from pH 2.433 to 12.915 is illustrated in Figure 4b. At acidic pH levels, the indazole rings undergo protonation, and the resulting species carries an overall charge of +2. As the pH increases to 3.2, one of the indazoles is deprotonated, altering the molecule's charge to +1. At pH 5.8, the second indazole ring undergoes deprotonation, yielding a neutral compound. At pH 11.0, an ion exchange occurs between Cl⁻ and H₂O, resulting in the molecule carrying a charge of +1. Finally, at pH 12.3, the water molecule is deprotonated, returning the complex to a neutral state. Based on the collected data, it can be

inferred that under physiological conditions, **AuL** predominantly exists as a neutral species **3** in Figure 4b.



Figure 4. a) Electronic spectra of AuL at selected pH values in a multicomponent buffer maintained at constant ionic strength. b) The proposed scheme for changes in the structure of AuL with the increase in pH from 2-13. The pKa values for the speciation of AuL calculated using Equation 2 (illustrated in Figure S6), are indicated in the scheme.

Stability of AuL to reduction

Glutathione (GSH) serves a crucial role as the major component of the intracellular antioxidant system.^[61] One of its main functions is the detoxification of heavy metal complexes within cells. With GSH concentrations ranging between 1 and 10 mM in cells, it is imperative to assess the stability of **AuL** in the presence of the intracellular reductant GSH. In this study, the reaction between GSH and **AuL** was investigated to estimate the stability of **AuL** under physiological GSH concentrations.^[62,63]

The reaction of **AuL** and GSH was monitored over time by electronic spectroscopy with spectral acquisition from 250–600 nm using a 1:1 mole ratio (**AuL**:GSH) of the two reactants and is presented in Figure 5. The change in the spectrum of **AuL** observed was the appearance of two isosbestic points at 315 and 439 nm, respectively. The highest-energy band at 258 nm redshifts by only 3 nm to 261 nm, while the 354 nm band only redshifts by 2 nm to 356 nm. Inspection of the region around 354 nm indicates that two separate isosbestic points are established close to one another over the time course of the reaction,

suggesting a biphasic reaction overall. The UV-vis spectra for the reaction of **AuL** with GSH reveal that the Au(III) metal ion is not reduced (i.e., from Au(III) to Au(I)/Au(0)). Instead, the formation of the isosbestic points and minor redshifted wavelengths are likely due to the binding of GSH to **AuL**, forming an **AuL**{GSH} adduct. The main reaction of GSH with **AuL** is described in eq. 3.

 $AuL + GSH \rightleftharpoons AuL - GSH + CI^{-}$ (3) 0.90 1.0 0.8 $\tau_1 = 42.61 (4.4) s$ $k_1 = 0.10(0.005) \text{ s}^{-1}$ 9.0 Absorbance 4.0 Absorbance $\tau_2 = 388.9(27) \text{ s}$ k₂ = 0.079(0.005) s⁻¹ 0.70 ò 500 1000 1500 2000 2500 Time (s) 0.2 0.0 300 400 500 550 250 350 450 600 Wavelength (nm)

Figure 5. Electronic absorption spectra of 40 µM AuL in a water (10% V/V) and DMSO mixture containing GSH at a molar ratio of 1 :1 [AuL]:[GSH]. The inset shows the change in absorbance at 258 nm as a function of time. The data are fitted to a standard double exponential kinetic function, $A = K_1 e^{-x/t_1} + K_2 e^{-x/t_2} + A_{\infty}$, where k_1 and k_2 are adjustable pre-exponential factors, t_1 and t_2 are the time constants, and A_{∞} the limiting absorbance.

The kinetic trace of **AuL** in the presence of GSH was recorded from the time-dependent spectra at 258 nm and confirms the biphasic nature of the reaction with two distinct rate constants: $k_1 = 0.100(0.005) \text{ s}^{-1}$ and $k_2 = 0.079(0.005) \text{ s}^{-1}$. The mean lifetimes for these steps are $\tau_1 = 42.61(4.4) \text{ s}$ and $\tau_2 = 388.9$ (27) s. A comprehensive kinetic study of the reaction mechanism would be valuable and feasible for future work, although it is beyond the scope of the current article. Of particular significance is the observation that GSH does not reductively demetallate **AuL**; instead, a spectroscopically distinct Au^{III}-GSH adduct is formed and remains stable in the presence of excess GSH. Given that many Au^{III} complexes are reduced by GSH *in vivo* to either Au^I species or even completely to Au(0),^[64] this study emphasizes the exceptional stability of Au^{III} conferred by the NNN pincer ligands.

Cytotoxicity of AuL in vitro

Following confirmation that **AuL** is sufficiently stable at physiological pH, we proceeded to assess its cytotoxicity *in vitro*. This was tested against two invasive adenocarcinoma cell lines namely MCF-7 (hormone responsive breast cancer) and HT-29 (colorectal cancer). Cell viability was investigated using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and early stages of apoptosis were detected using an APOPercentage assay. This section validates the effectiveness of **AuL** as a potential anti-cancer agent. 40 µM Plumbagin (PL) was utilised as a positive control for cell death. Plumbagin was

used as the positive control in the MTT experiment because it is a well-characterized cytotoxic agent with a rapid and strong effect on cell viability, ensuring the assay's technical reliability. It exhibits broad-spectrum cytotoxicity and is commonly used in *in vitro* cytotoxicity studies. Unlike Auranofin or Cisplatin, Plumbagin exerts cytotoxic effects by inducing reactive oxygen species (ROS), triggering cell cycle arrest and apoptosis cell death primarily through ROS generation, providing a distinct mechanism from the test compound **AuL**. Plumbagin's consistent and broadspectrum cytotoxicity makes it a widely accepted standard in *in vitro* assays.^[65]

An MTT assay assesses the effect of a drug or compound on the cytotoxicity of cells, by measuring changes in cell viability.^[66,67] Both cancer cell lines were exposed to a range of AuL concentrations $(0-100 \mu M)$, over a period of 24 and 48 hours. AuL did not appear to have a cytotoxic effect in the HT-29 and MCF-7 cell line after 24 hours (Figure 6a). This is likely due to the slow uptake of AuL by the cells. Following 48 hours of exposure, there was not a significant reduction in cell viability in the HT-29 cells, in both the low (0.1-5 µM) and high (10-100 µM) concentration ranges, where IC_{50} >100 µM, compared to the negative control (Figures 6b and c). Low toxicity of AuL in the HT-29 cells was confirmed with the APOPercentage assay. The red APOPercentage dye is selectively taken up by apoptotic cells, during the membrane flip of phosphatidylserine in the early stages of apoptosis.[68,69] Subsequently, cytotoxicity can be detected at 550 nm and visualised via microscopy. The micrographs indicate APOPercentage dye uptake following 48 h exposure to AuL (Figure 6).

AuL did not induce significant apoptosis in both the low (5 μ M) and high (100 μ M) concentration range, at 6.3% and 8.3%, respectively, relative to the untreated control (Figure 6e). In the MCF-7 cell line, **AuL** was not shown to be cytotoxic in the low concentration range at 48 hours. However, at high concentrations, viability was significantly reduced, showing an IC₅₀ = 9 μ M (Figures 6b and c). This IC₅₀ is comparable to that of cisplatin which has been recorded in literature to be within the range of 0.5

- 10 μ M.^[70–72] This shows that the cytotoxicity of **AuL** is comparable to cisplatin in breast cancer cells *in vitro*. Our data indicate that **AuL** could potentially be an effective anti-cancer agent in breast cancer.

This was validated following analysis of apoptosis. The apoptosis-inducing properties of **AuL** in MCF-7 cells was visualised at low concentrations of 5 and 10 μ M (Figure 6f). **AuL** induced 33.9% and a significant 54.8% apoptosis in MCF-7 cells, at 5 and 10 μ M (Figure 6g), which is comparable to the cell viability data. The sensitivity of **AuL** in MCF-7 cells and not in HT-29 cells indicates that this compound may act as an anti-cancer agent in selective cancer types, likely targeting breast cancer (oestrogen receptor positive, progesterone receptor positive). Further analysis is required to understand solution stability under physiological conditions, cellular uptake and the mechanism of action of **AuL** *in vitro*.

Although direct comparison is not possible due to various variables, such as different incubation times, methodology for the cytotoxicity assay, and cell lines, our limited data for **AuL** compares favourably with the cytotoxicity reported for other gold(III) complexes, of which IC₅₀ values range from 0.1 to 35 μ M against several different cell lines.^[73-79] Importantly, **AuL** IC₅₀ values compared favourably to the closest set of Au^{III} pincer ligands we could find, IC₅₀ = 20 μ M, for a class of [Au(N–N'– N)Cl]Cl₂^[77] pincer complexes.

In contrast to the notable cytotoxicity of **AuL** against the MCF-7 cells, **H**₂**L** exhibited no significant cytotoxicity against either the MCF-7 or HT-9 cell lines at concentration below 100 μ M (Figure S15). These findings emphasize that the gold(III) ion, and its unique chemical properties, are essential for the efficacy of the gold(III) complex synthesized in this study. These collective findings are highly encouraging, as they demonstrate that the gold(III) chelates successfully reach their cellular targets before reduction or decomposition. Furthermore, the results highlight the critical role of the gold(III) ion in the efficacy of gold(III) chelates as chemotherapeutic agents, currently *in vitro* and potentially also *in vivo* going forward.



Figure 6 Cytotoxic effect of **AuL** in HT-29 and MCF-7 cells. Dose-response curves of **AuL** at **a**) 24 and **b**) 48 hours in HT-29 and MCF-7 cells. **c**) HT-29 and MCF-7 cells. **c**) HT-29 and MCF-7 cell viability following exposure to 0.1, 0.2, 0.5, 1, 5, 10, 20, 50 and 100 μ M **AuL**. 40 μ M plumbagin (PL) acted as the positive control. Visual representation of the effect of **AuL** on apoptosis in **d**) HT-29 and **e**) MCF-7 cells. **f**) 5 μ M and 100 μ M **AuL** had no significant effect on HT-29 apoptosis, relative to the untreated group. **g**) 5 μ M and 10 μ M **AuL** induced 33.9% and 54.8% apoptosis in MCF-7 cells, relative to the untreated group. The scale bar represents 100 μ m. Data represents the mean ± standard deviation. A one-way ANOVA was performed for statistical analysis, with Bonferroni's multiple comparisons test used for pairwise analyses, where * P < 0.05, ** P < 0.01, *** P < 0.001.

MOA of AuL with ctDNA - spectroscopic titrations

Based on the design of **AuL** and reported literature of gold(III) pincer complexes binding to DNA,^[77] we investigated if **AuL** binding to DNA is a potential mechanism of action (MOA) of its cytotoxicity. The interaction between **AuL** and calf thymus DNA (ctDNA) was investigated by spectroscopic titrations (Figure 7), which indicates the change in the electronic spectrum of **AuL** for a single titration (of triplicate results). In Figure 7a, the red spectral line (before adding ctDNA to **AuL**) and the blue spectral line (after adding 37 μ M of ctDNA) marks the end point, which is reached due to partial precipitation and turbidity of the solutions after incremental additions of ctDNA. The electronic spectra (Figure 7a) indicate a non-linear decrease in absorbance as a function of increasing ctDNA concentrations.

The hypochromic spectral changes of **AuL** were monitored at 342 nm (Figure 7b) and the non-linear data set was fitted to the modified Hill-Langmuir model,^[80,81] with a sigmoidal function using OriginPro2023.^[60] This fit quantified the affinity of **AuL** to DNA as binding constants K_{a1} = 1.48 x10⁹ M⁻¹ and K_{a2} = 6.59 x10⁵ with an R² value of 0.997, which indicated a relatively good fit (Figure 7b, Table 1). Furthermore, the 342 nm band exhibited minor bathochromic shifts in peak position (~2 nm), while isosbestic points were only observable at concentrations of ctDNA below 90 nM (insert in Figure 6a). This hypochromicity is typical of most NNN type gold(III) pincer complexes in literature, however, the K_{a} values are several orders of magnitude greater than reported binding constants of other known gold(III) terpyridines $10^{3} - 10^{5} \text{ M}^{-1}$.^[82–87]

Interestingly, the resultant spectral profile of the interaction of **AuL** with ctDNA was partially similar to that of a cationic Ru(II)polypyridine complex (order of binding constant of 10⁶)^[88] and an anionic DNA-binding probe (order of binding constant of 10², where the low value was ascribed to the negative charge),^[89] as they all exhibit a significant drop in absorbance between 300-500 nm.

The DNA binding constants may be suggestive of the mode of binding of **AuL** to DNA. Alkylators typically exhibit moderately high binding constants, as they bind to DNA primarily through covalent bonding, with this interaction often being irreversible. In contrast, other types of DNA binding mainly depend on electrostatic, hydrophobic interactions, and π - π stacking (intercalation).^[84,90,91] Notably, groove binders tend to have even higher binding constants, as they interact more deeply within the DNA grooves facilitating stronger and more stable binding.^[92,93]

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Figure 7 **a**) A representative of the triplicates for the UV-vis absorption spectra of 117 μ M **AuL** in 0.10 M TRIS-HCI buffer 10% DMSO (red spectrum) after sequential additions of ctDNA from 0 to 0.1 μ M (purple spectrum) and to a final concentration of 37 μ M ctDNA before precipitation (blue spectrum). The insert shows the isosbestic points at low concentrations of DNA. **b**) Illustration of the UV-vis titration spectra monitored at 342 nm as a function of the concentration ctDNA at fixed **AuL** concentration.

Most intercalators in the literature have binding constants between $10^3 - 10^6 M^{-1}$, examples of which include ethidium bromide $(2.5 \times 10^6 - 8.2 \times 10^4 M^{-1})^{[94]}$ psoralen $(9.75 \times 10^3 M^{-1})^{[95]}$ and acridine $(1.74 \times 10^4 - 1.0 \times 10^6 M^{-1})^{[96]}$ Groove binders however generally have higher affinities with binding constants between 10^5 and $10^8 M^{-1}^{.[92,93]}$ It is difficult to distinguish alkylators based on the binding constant, but some base-binding gold(III) pincer complexes have been reported to have binding constants of 4.5×10^5 and $5.4 \times 10^5 M^{-1}$. Although these ranges often overlap, they provide an insight on the binding mechanism of the binders.

The potential of **AuL** base-binding to ctDNA was investigated by electronic spectroscopy titration between the metal complex and DNA bases, nucleosides, and nucleotides (Figure 8a). This was done to delineate the binding behaviour of **AuL** with DNA components, and ATP, and to evaluate the impact of steric bulk (from ribose and phosphate groups) on **AuL**'s binding to DNA bases, as well as identify atoms involved in the base-binding of **AuL**. The interaction between **AuL** and thymine (Figure 8), as well as other DNA components, was investigated through spectroscopic titrations within the 300–700 nm range. Hypochromic spectral changes of **AuL** were monitored between 400-450 nm for the various DNA components, and the non-linear data were fitted to the Hill model (Figures 8 and S7 -S9).^[80,81] This analysis quantified the affinity of **AuL** for the native DNA bases yielding K_a values ranging from 1.26×10^3 to 1.21×10^4 M⁻¹ (Table 1). Unlike the titrations with ctDNA, minor bathochromic shifts (~4 nm) were observed along with distinct isosbestic points throughout the titration with all bases, nucleosides and nucleotides, suggesting a permanent interaction (covalent bonding) between **AuL** and the DNA components.

The moderate binding affinities of the DNA components to **AuL** indicate that the gold(III) complex has the ability to interact directly with these DNA residues. Notably, we successfully obtained a crystal structure of the **AuL** complex with thymine (**AuLT**; Figures 2b and 8b). Despite many attempts with all four native DNA bases, only the complex with thymine was successfully crystallized.

Table 1: The binding parameters of DNA and its components with ctDNA.

		Binding constant (<i>K</i> _a) / M ⁻¹		
DNA	K _{a1}	1.48×10^9		
	K _{a2}	6.59×10^{5}		
		Base	Nucleoside	Nucleotide
Adenine		1.21×10^4	$1.75 imes 10^3$	3.72×10^3
Thymine		$1.83 imes 10^3$	2.19×10^3	NA
Cytosine		$2.20 imes 10^3$	1.74×10^3	3.60×10^3
Guanine		NA	$1.65 imes 10^3$	$1.26 imes 10^3$

NA refers to the component not being available for testing.

The binding of AuL with thymine in Figures 2b and 8b shows a preference for the N3 position over N1, likely due to the polarized N3-H bond. This polarization facilitates deprotonation, and making N3 more reactive than N1.[97] Studies indicate that alkylating agents like cisplatin preferentially bind at the N7 position in guanine, suggesting that N1 in pyrimidines and N9 in purines are not central to alkylation.[98] Consequently, it is proposed that AuL, as a "DNA alkylator", will similarly favor binding at N3 in pyrimidines and N7 in purines. The N7 position in purines is more accessible, as it is not involved in hydrogen bonding between bases or in the base-sugar linkage, which may explain the binding preference of metal complexes like cisplatin for guanine.^[99] Conversely, the involvement of N3 in purines in hydrogen bonding with adenine in double helix DNA limits its availability for metal complex binding, unless conformational displacement of thymine from the base stack occurs during the reaction.

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Figure 8. a) A representative of the titration triplicates for the UVvis absorption spectra of 117 μ M AuL in 0.10 M TRIS-HCI buffer 10% DMSO after sequential additions of thymine until the final concentration before precipitation. The insert graph illustrates the UV-vis titration spectra at 406 nm as a function of the increasing ratio (concentration) of thymine to AuL. b) An illustration showing the atom numbering on the thymine residue in AuLT along with the numbering of the DNA bases: guanine (G), adenine (A), cytosine (C) and thymine (T). The bases are linked to ribose through N1 for pyrimidines and N9 purines.

Our data for the titration of **AuL** with the four native DNA components (base, nucleoside, nucleotide) suggest that the interactions involve the same donor atom across these targets, and presumably also double-stranded DNA. Notably, the increase in molecular size from bases to nucleotides did not significantly impact the binding affinity of A, C, G, and T components to **AuL** (Table 1). While smaller DNA components might be expected to bind more efficiently to **AuL** than the full DNA structure, steric hindrance did not substantially affect binding among bases, nucleosides, and nucleotides. This observation suggests that steric bulk from ribose and phosphate groups does not obstruct the complexation process. Rather, electronic factors appear to predominantly govern the reactivity of these components.

Adenine exhibited a relatively higher binding affinity to **AuL** compared to guanine, cytosine, and thymine, though no consistent trend was observed among nucleotides and nucleosides, indicating no clear preference. The binding constants for bases, nucleosides, and nucleotides were moderately high, ranging from $1.26 \times 10^3 - 1.21 \times 10^4$ M⁻¹, suggesting that alkylation may be a primary interaction mode for **AuL** with DNA.

Linear dichroism (LD) and circular dichroism (CD) of DNA

The LD spectra of ctDNA and ctDNA with AuL (ctDNA-AuL) are depicted in Figure 9a, while ctDNA with Hoechst 33258 (ctDNA-HS) and with ethidium bromide (ctDNA-EB) served as comparison controls (Figure S10c). The ctDNA-AuL system has a negative signal throughout the $\pi - \pi^*$ region of the LD spectra (230-290 nm), with a minimum at 260 nm, as a result of the nitrogenous bases within ctDNA.[100] This is indicative that the transition moments are oriented perpendicular to the helix axis. Figure 9a highlights that the 260 nm minimum decreases in a dose-dependent manner (i.e., with increasing AuL concentration). This is due to AuL binding within the nucleobases of ctDNA, suggesting that its ability to orient along the perpendicular flow lines is significantly impaired as function of AuL concentration. The observation that the induced LD signal from the $\pi-\pi^*$ bands of AuL (~400 nm) becomes increasingly positive in the LD spectrum at higher [AuL] is consistent with the spectroscopic signature of ctDNA-HS, a known minor groove binder Figure S10d.^[101]

Regarding the induced dichroic signal observed between 300-540 nm, this is specifically in a spectral region where no contribution from the ctDNA nucleobases can be detected and only AuL absorbs. AuL itself is isotropic and cannot be orientated in the flow field. Therefore, the dichroic signal peaking is indicative of an induced LD signal^[102] and its occurrence suggests that AuL forms a molecular complex with ctDNA, resulting in the bound complex becoming oriented in the flow field. The positive dichroic signal signifies that the π - π * transition dipoles of AuL are in a plane tilted almost 45° in relation to the helix axis, consistent with minor groove binders. Furthermore, the data is consistent with that of HS binding to ctDNA; a strong positive induced LD signal was observed with a maximum at 347 nm (Figure S10). This is consistent with previously reported induced LD spectra of HS.^[103] From Figure S10d, we see HS is orientated close to 45° to the base pair plane and helical axis, indicating it is a grove binder.^[104]

Electrophoresis

To corroborate the data from LD spectroscopy, we conducted electrophoretic mobility shift assays (EMSA)^[105] to examine alterations in the tertiary and secondary structure of pUC57 plasmid DNA. EMSA was performed using plasmid DNA equilibrated with varying concentrations of **AuL**, **HS**, and **EB**. As shown in Figure 9b, the reference lane contained only native DNA, representing the mobility of untreated pUC57 plasmid DNA. **EB** served as an intercalator control, while **HS** acted as a control for a minor groove binder. The well-defined binding modes of both **EB** and **HS** to pUC57 plasmid DNA made them suitable controls, with their migration patterns in the EMSA gel helping to evaluate the binding mode of **AuL** to pUC57 plasmid DNA.

In the EMSA gel, linear pUC57 plasmid DNA is marginally affected by the binding of either the intercalator or the minor groove binder. The binding of **EB** reduces the mobility of supercoiled (SC) and nicked-open circular (NOC) pUC57 plasmid DNA on the agarose gel.^[106] This reduction occurs because intercalation partially unwinds the SC DNA, increasing steric bulkiness in the NOC form, thereby reducing mobility due to increased hydrodynamic drag.^[106] Conversely, the minor groove binder **HS** increases DNA mobility along the gel, as the DNA molecules become more compactly packed.^[92] Although the change is less pronounced than with **EB**, an increase in **HS**

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esulting in Figure 8b, dependent bases (alkylation), it increases the bulkiness of the pUC57 plasmid DNA, causing kinking and resulting in higher hydrodynamic drag and reduced mobility on the gel. This reduction in pUC57 plasmid DNA mobility is apparent even at low concentrations, starting from 10 μ M, highlighting **AuL** as a DNA alkylator. This is consistent with findings in literature where cisplatin, a prototypical alkylating agent, exhibits comparably high hydrodynamic drag due to its interaction with DNA and its subsequent structural modifications.^[107–110] To reinforce these EMSA findings, additional experiments were conducted. **Thermal melt analysis:**

The thermal melt temperature (T_m) of ctDNA was analysed in the presence of **AuL** to elucidate the two potential binding mechanisms of the complex to DNA (Figure 9c). At the highest concentration of **AuL** (25 µM), the observed Δ T_m was +2.7 °C. While DNA intercalators typically increase the T_m by at least 5°C, minor groove binders tend to slightly increase the T_m (<5 °C).^[111] Minor groove binders stabilise the DNA double helix structure through hydrogen bonding and van der Waals interaction within the groove, which enhances the overall structural integrity of the DNA.^[112–114] Conversely, alkylators disrupt base pair interactions, leading to local denaturing, and a decrease in T_m of ctDNA.^[115–117] This effect is characteristic of monofunctional alkylation, while bifunctional alkylation (interstrand crosslinking) stabilizes the DNA, thereby increasing the energy required for denaturation and elevating T_m.

As shown in Figure 9c, at **AuL**:ctDNA concentration ratios of below 0.05, a slight decrease in the T_m was observed, indicative of alkylation. At higher ratios (above 0.05), the data suggest that minor groove binding also contributes to the observed interactions.

The modest ΔT_m of +2.7 °C, along with the two binding constants (K_{a1} and K_{a2}) and the LD and EMSA data, supports a mechanism involving dual binding modes of **AuL** to DNA: specifically, minor groove binding and alkylation. This conclusion is further substantiated by viscosity data Figure S11.

Biomolecular simulations:

To further corroborate the binding mechanism of AuL to DNA, we incorporated molecular dynamics (MD) simulations. This was achieved by first docking AuL into two oligonucleotides using Glide XP. The two DNA oligonucleotide X-ray structures used were PDB 425D (2.80 Å) [118] and PDB 4E1U (0.92 Å)[119] for flexible ligand docking. Docking data are presented in Figure S12. MD simulations may aid in drawing conclusions based on the interactions between the oligonucleotide and AuL over the simulated time, capturing the system's dynamic behaviour and offering a more accurate reflection of physiological conditions.^[120] As shown in Figure 10, the root-mean-square deviation (RMSD) of AuL indicates that it remains stably bound within the minor grove of an AT cluster until 79 ns, thereafter a large deviation is observed for about 2 ns before an equilibrium is reached, with AuL now occupying a new conformation within DNA. The MD simulation to a degree corroborates the experimental data (vide supra), indicating a dual binding mechanism: (i) initially minor groove binding and (ii) alkylation. Due to the limitations of Desmond with regards to metal complexes, alkylation would not be accurately portrayed.

Based on comprehensive experimental and theoretical data, we propose a probable mechanism by which **AuL** interacts with DNA (Figure 10b). Initially, **AuL** binds to the minor groove of DNA,

concentration enhances the supercoiling of SC DNA, resulting in increased mobility across the agarose gel. As shown in Figure 8b, **EB** binding reduces DNA mobility in a concentration-dependent manner, whereas **HS** exhibits the opposite effect.



Figure 9. a) The LD spectrum of AuL with 100 µM of ctDNA at increasing AuL concentration in KH₂PO₄ (50 mM, pH 7.5) at 37 °C. The intense minimum at 260 nm represents ctDNA and the 300-540 nm band represents an induced gold(III) complex LD spectrum. b) EMSA agarose gel for AuL with pUC57 (2.5 ng/well, 1x TAE buffer, 5% v/v DMSO). The DNA bands formed are for supercoiled (SC), nicked-open circular (NOC), and linear (Lin). Lane 1 is the control lane containing ctDNA only. Lanes 2-14 have increasing concentrations of EB (2-4), AuL (5-11), and Hoechst 33258 (HS, 12-14), and they compare the behaviour of a DNA intercalator, EB, the gold(III) complex, and a DNA minor groove binder, HS respectively. Experiments were done in triplicates and a representative assay is shown. c) The melt curves for ctDNA with different concentrations of AuL to calculate T_m from the Boltzmann sigmoidal fit. The inset is the change in T_m with the change in [AuL], fitted to a Hill sigmoidal function ($T_m = +2.7$ °C).

The **AuL** complex exhibited a pronounced reduction in the mobility of SC and NOC pUC57 plasmid DNA, suggesting a strong interaction between **AuL** and pUC57 plasmid DNA, and likely involving alkylation. When **AuL** covalently binds to the DNA

specifically within an AT-rich cluster, with an affinity constant (K_a) of $1.48\times10^9\,M^{-1}$, which is consistent with typical values for groove binders. Following this, a second binding event occurs wherein the coordinated chloride ion is displaced by the amide of a thymine residue through nucleophilic substitution. This secondary interaction, referred to as alkylation, proceeds with an affinity constant of $6.59\times10^5\,M^{-1}.$



Figure 10. **a)** Molecular dynamics (MD) simulation over 100 ns of the best-docked GLIDE XP structure of **AuL** binding within the minor groove of a DNA oligonucleotide (PDB $425D^{[118]}$, 5'-D(ACCGACGTCGGT)-3'; ligand removed). A large target grid was generated for ligand docking at nucleic acid sites close to the centre of the DNA (with all solvents removed), spanning 40 x 40 x 40 Å³, thereby facilitating a search of alternative binding pockets radiating throughout the oligonucleotide. The RMSD indicates that there is minimal fluctuation of complex **AuL** throughout the MD trajectory. **b)** Proposed reaction mechanism of **AuL** binding to an AT cluster and alkylating via N3 on the thymine residue.

Conclusion

A cytotoxic indazole-based gold(III) complex, AuL, was successfully synthesized. AuL demonstrated significant cytotoxicity against the MCF-7 breast cancer cell line, with activity comparable to that of cisplatin, despite showing limited effects against the HT-29 colon cancer cell line. The interaction of AuL with DNA was investigated using various biophysical techniques, which revealed dual binding modes: at lower concentrations, AuL predominantly binds within the minor groove, while at higher concentrations, alkylation (covalent binding) becomes significant. Biomolecular simulations further supported minor groove binding; however, confirmation of alkylation was limited by the constraints of the Desmond software.

A proposed interaction scheme suggests that **AuL** initially binds to the minor groove of the DNA double helix, followed by interactions with DNA bases as concentration increases. This dual binding mechanism highlights the potential of **AuL** as an effective anticancer agent, warranting further investigation. Importantly, the dual binding behaviour of **AuL** suggests broader implications for drug design, particularly in developing novel metal-based therapeutics that can target DNA through multiple modes of interaction. While the interaction of the complex with DNA may play a crucial role in its cytotoxicity, it remains unclear whether this is the primary *in vitro* cellular target as studies to confirm this are still ongoing. Additional pathways, including potential interactions with cellular proteins, remain to be explored, opening avenues for future studies and optimization in therapeutic applications.

Supporting Information

Crystallographic data: Deposition numbers 2403562 and 2403563 contain the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service.

Acknowledgements

This work is based on research supported by the South African Research Chairs Initiative of the Department of Science and Innovation (DSI) and National Research Foundation (NRF) of South Africa (Grant No 64799, OQM). The authors thank WITS University and the NRF for funding to purchase a JASCO J-1500 MCD spectrometer (Grant No 116177, OQM) and a dualwavelength Bruker D8 Venture X-ray diffractometer (Grant No 129920, OQM). We also thank the Centre for High Performance Computing (Project CHEM1633, CHPC, Cape Town) for both the CPU time and resources needed for the DFT and MD simulations. The authors thank Prof Manuel Fernandes for refining and depositing the crystal structures.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Pincer ligand • metal chelate • DNA • cytotoxicity • DFT simulations

References

- [1] C. Roder, M. J. Thomson, *Drugs R D* **2015**, *15*, 13–20.
- P. Calamai, S. Carotti, A. Guerri, T. Mazzei, L. Messori,
 E. Mini, P. Orioli, G. P. Speroni, *Anti-cancer drug design* 1998, 13, 67–80.
- [3] C.-M. Che, R. W.-Y. Sun, W.-Y. Yu, C.-B. Ko, N. Zhu, H. Sun, Chemical Communications 2003, 1718–1719.
- [4] Y. Lu, X. Ma, X. Chang, Z. Liang, L. Lv, M. Shan, Q. Lu, Z. Wen, R. Gust, W. Liu, *Chemical Society Reviews* 2022, 51, 5518–5556.
- [5] Y. Sun, Y. Lu, M. Bian, Z. Yang, X. Ma, W. Liu, European journal of medicinal chemistry 2021, 211, 113098.
- [6] M. Bian, X. Wang, Y. Sun, W. Liu, European Journal of Medicinal Chemistry 2020, 193, 112234.
- [7] G. Sági, K. Szűcs, G. Vereb, L. Otvös, J Med Chem 1992, 35, 4549–4556.
- [8] S. Mal, U. Malik, M. Mahapatra, A. Mishra, D. Pal, S. K. Paidesetty, *Drug Development Research* 2022, 83, 1469–1504.
- [9] N. A. S. Ali, B. A. Dar, V. Pradhan, M. Farooqui, *Mini Rev Med Chem* 2013, *13*, 1792–1800.
- L. Mosti, G. Menozzi, P. Fossa, W. Filippelli, S. Gessi, B. Rinaldi, G. Falcone, *Arzneimittelforschung* 2000, *50*, 963–972.
- [11] V. K. Tandon, D. B. Yadav, A. K. Chaturvedi, P. K. Shukla, *Bioorganic & Medicinal Chemistry Letters* 2005, 15, 3288–3291.

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- [12] D. Raffa, G. Daidone, B. Maggio, D. Schillaci, F. Plescia, Archiv der Pharmazie 1999, 332, 317-320.
- [13] J.-H. Sun, C. A. Teleha, J.-S. Yan, J. D. Rodgers, D. A. Nugiel, J. Org. Chem. 1997, 62, 5627-5629.
- M. Patel, R. F. Kaltenbach, D. A. Nugiel, R. J. McHugh, [14] P. K. Jadhav, L. T. Bacheler, B. C. Cordova, R. M. Klabe, S. Erickson-Viitanen, S. Garber, C. Reid, S. P. Seitz, Bioorg Med Chem Lett 1998, 8, 1077-1082.
- W. Han, J. C. Pelletier, C. N. Hodge, Bioorganic & [15] Medicinal Chemistry Letters 1998, 8, 3615-3620.
- M. Boulouard, P. Schumann-Bard, S. Butt-Gueulle, E. [16] Lohou, S. Stiebing, V. Collot, S. Rault, Bioorganic & Medicinal Chemistry Letters 2007, 17, 3177-3180.
- [17] V. J. Arán, C. Ochoa, L. Boiani, P. Buccino, H. Cerecetto, A. Gerpe, M. González, D. Montero, J. J. Nogal, A. Gómez-Barrio, A. Azqueta, A. López de Ceráin, O. E. Piro, E. E. Castellano, Bioorganic & Medicinal Chemistry 2005, 13, 3197-3207.
- [18] J. D. Rodgers, B. L. Johnson, H. Wang, R. A. Greenberg, S. Erickson-Viitanen, R. M. Klabe, B. C. Cordova, M. M. Rayner, G. N. Lam, C.-H. Chang, Bioorganic & Medicinal Chemistry Letters 1996, 6, 2919-2924.
- [19] S. Bräse, C. Gil, K. Knepper, Bioorganic & Medicinal Chemistry 2002, 10, 2415-2437.
- [20] M. K. Brawer, Rev Urol 2005, 7, S21-S26.
- C. M. Carapella, M. G. Paggi, F. Calvosa, F. Cattani, B. [21] Jandolo, R. Mastrostefano, L. Raus, A. Riccio, J Neurosurg Sci 1990, 34, 261-264.
- P. Feyer, M. H. Seegenschmiedt, M. Steingraeber, [22] Support Care Cancer 2005, 13, 671-678.
- M. Tan, Expert Opin Pharmacother 2003, 4, 1563-1571. [23]
- T. J. Gan, CNS Drugs 2005, 19, 225-238. [24]
- M. Gross-Goupil, L. François, A. Quivy, A. Ravaud, Clin [25] *Med Insights Oncol* **2013**, 7, 269–277. E. D. Deeks, *Drugs* **2012**, 72, 2129–2140.
- [26]
- [27] Y. Deng, C. Sychterz, A. B. Suttle, M. M. Dar, D. Bershas, K. Negash, Y. Qian, E. P. Chen, P. D. Gorycki, M. Y. K. Ho, Xenobiotica 2013, 43, 443-453.
- G. Sonpavde, T. E. Hutson, C. N. Sternberg, Expert [28] Opin Investig Drugs 2008, 17, 253-261.
- [29] J. Verweij, S. Sleijfer, Expert Opin Pharmacother 2013, 14. 929-935.
- Y.-A. Heo, S. T. Duggan, Targ Oncol 2018, 13, 533-[30] 539.
- G. Ison, L. J. Howie, L. Amiri-Kordestani, L. Zhang, S. [31] Tang, R. Sridhara, V. Pierre, R. Charlab, A. Ramamoorthy, P. Song, F. Li, J. Yu, W. Manheng, T. R. Palmby, S. Ghosh, H. N. Horne, E. Y. Lee, R. Philip, K. Dave, X. H. Chen, S. L. Kelly, K. G. Janoria, A. Banerjee, O. Eradiri, J. Dinin, K. B. Goldberg, W. F. Pierce, A. Ibrahim, P. G. Kluetz, G. M. Blumenthal, J. A. Beaver, R. Pazdur, Clin Cancer Res 2018, 24, 4066-4071.
- [32] A. Chen, Chin J Cancer 2011, 30, 463-471.
- [33] H. Cerecetto, A. Gerpe, M. González, V. J. Arán, C. O. de Ocáriz, Mini Rev Med Chem 2005, 5, 869-878.
- M. A. Jakupec, E. Reisner, A. Eichinger, M. Pongratz, V. B. Arion, M. S. Galanski, C. G. Hartinger, B. K. Keppler, [34] J. Med. Chem. 2005, 48, 2831-2837.
- H. H. Showalter, M. M. Angelo, E. M. Berman, G. D. [35] Kanter, D. F. Ortwine, S. G. Ross-Kesten, A. D. Sercel, W. R. Turner, L. M. Werbel, Journal of medicinal chemistry 1988, 31, 1527-1539.
- M. W. Johnson, A. G. DiPasquale, R. G. Bergman, F. D. [36] Toste, Organometallics 2014, 33, 4169-4172
- [37] W.-P. To, G. S.-M. Tong, W. Lu, C. Ma, J. Liu, A. L.-F. Chow, C.-M. Che, Angewandte Chemie International Edition 2012, 51, 2654-2657.

- [38] K. M.-C. Wong, L.-L. Hung, W. H. Lam, N. Zhu, V. W.-W. Yam, Journal of the American Chemical Society 2007, 129, 4350-4365
- [39] R. M. de Figueiredo, J.-S. Suppo, J.-M. Campagne, Chemical Reviews 2016, 116, 12029-12122
- [40] V. R. Pattabiraman, J. W. Bode, Nature 2011, 480, 471.
- [41] J. S. Carey, D. Laffan, C. Thomson, M. T. Williams, Organic & Biomolecular Chemistry 2006, 4, 2337-2347.
- K. T. Neumann, A. S. Donslund, T. L. Andersen, D. U. [42] Nielsen, T. Skrydstrup, Chemistry - A European Journal 2018, 24, 14946-14949.
- R. M. Lanigan, T. D. Sheppard, European Journal of [43] Organic Chemistry 2013, 2013, 7453-7465.
- [44] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, in Molecular Biology of the Cell. 4th Edition, Garland Science, 2002.
- K. Collins, T. Jacks, N. P. Pavletich, Proceedings of the [45] National Academy of Sciences 1997, 94, 2776-2778.
- [46] G. M. Cooper, R. E. Hausman, The Cell: A Molecular Approach, ASM Press; Sinauer Associates, Washington, D.C.: Sunderland, Mass, 2007.
- R. Razuwika, O. Q. Munro, New J. Chem. 2024, 48, [47] 4229-4244.
- N. S. Akhmadullina, A. V. Churakov, V. M. Retivov, R. [48] A. Sandu, O. N. Shishilov, Russian Journal of Coordination Chemistry 2012, 38, 589-595.
- M. Hanif, S. Moon, M. P. Sullivan, S. Movassaghi, M. [49] Kubanik, D. C. Goldstone, T. Söhnel, S. M. F. Jamieson, C. G. Hartinger, J Inorg Biochem 2016, 165, 100-107.
- [50] R. Graeser, N. Esser, H. Unger, I. Fichtner, A. Zhu, C. Unger, F. Kratz, Invest New Drugs 2010, 28, 14-19.
- [51] W. H. Ang, E. Daldini, L. Juillerat-Jeanneret, P. J. Dyson, Inorg Chem 2007, 46, 9048-9050.
- [52] A. Casini, M. C. Diawara, R. Scopelliti, S. M. Zakeeruddin, M. Grätzel, P. J. Dyson, Dalton Trans. 2010, 39, 2239-2245.
- V. Rousseau, H. G. Lindwall, Journal of the American [53] Chemical Society 1950, 72, 3047-3051.
- [54] A. Roniboss, K. Chanda, M. Motilal Balamurali, ChemistrySelect 2020, 5, 7505-7516.
- Y. Zhu, F. Liao, G. Suning, A. Gao, S. Lu, M. Shao, [55] Journal of the Electrochemical Society August 8, 161, B265-B268
- [56] Munessar. Sankar, R. G. Bates, Anal. Chem. 1978, 50, 1922-1924.
- [57] L. V. Salgado, C. Vargas-Hernández, American Journal of Analytical Chemistry 2014, 5, 1290.
- [58] T. M, "Buffers for the physiological pH range: acidic dissociation constants of zwitterionic compounds in various hydroorganic media," DOI 10.1002/adic.200590001can be found under https://pubmed.ncbi.nlm.nih.gov/15801182/, 2005.
- [59] N. Karimova, O. Alija, S. L. M. García, V. H. Grassian, R. B. Gerber, J. G. Navea, Physical Chemistry Chemical Physics 2023, 25, 17306-17319.
- [60] OriginLab Corporation, n.d.
- J. P. Richie, S. Nichenametla, W. Neidig, A. Ī61Ī Calcagnotto, J. S. Haley, T. D. Schell, J. E. Muscat, Eur J Nutr 2015, 54, 251-263.
- [62] "Reduced Glutathione | Pure | Doctors and Patients Access," can be found under https://www.pureencapsulationspro.com/reducedglutathione.html, n.d.
- Ă. Krezel, W. Bal, Acta Biochim Pol 1999, 46, 567-580. [63]
- [64] T. Zou, C. Tung Lum, C.-N. Lok, J.-J. Zhang, C.-M. Che, Chemical Society Reviews 2015, 44, 8786-8801.
- [65] S. Sagar, L. Esau, B. Moosa, N. M. Khashab, V. B. Bajic, M. Kaur, Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 2014, 14, 170-180.

15213765, ja, Downloaded from https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/ch

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RESEARCH ARTICLE

- [66] M. V. Berridge, P. M. Herst, A. S. Tan, in *Biotechnology Annual Review*, Elsevier, **2005**, pp. 127–152.
- [67] M. Ghasemi, T. Turnbull, S. Sebastian, I. Kempson, *Int J Mol Sci* **2021**, *22*, 12827.
- [68] M. Oancea, S. Mazumder, M. E. Crosby, A. Almasan, *Methods Mol Med* 2006, 129, 279–290.
- [69] O. Kepp, L. Galluzzi, M. Lipinski, J. Yuan, G. Kroemer, *Nat Rev Drug Discov* **2011**, *10*, 221–237.
- [70] T. Kashkoulinejad-Kouhi, S. Safarian, B. Arnaiz, L. Saa, Oncol Rep **2021**, *45*, 665–679.
- [71] J. Suberu, I. Romero-Canelón, N. Sulluvan, A. Lapkin, G. Barker, *ChemMedChem* 2014, DOI 10.1002/cmdc.201402285.
- J. Zou, L. Zhu, X. Jiang, Y. Wang, Y. Wang, X. Wang, B. Chen, *Oncotarget* 2018, *9*, 11268–11278.
- [73] A. Casini, R. W.-Y. Sun, I. Ott, in *Metal lons in Life Sciences* (Eds.: A. Sigel, H. Sigel, E. Freisinger, R.K.O. Sigel), De Gruyter, Berlin, Boston, **2018**, pp. 199–217.
- [74] S. Sookai, M. P. Akerman, O. Q. Munro, *Dalton Transactions* 2024, 53, 5089–5104.
- [75] D. Van der Westhuizen, C. A. Slabber, M. A.
 Fernandes, D. F. Joubert, G. Kleinhans, C. J. van der Westhuizen, A. Stander, O. Q. Munro, D. I.
 Bezuidenhout, *Chemistry–A European Journal* 2021, 27, 8295–8307.
- [76] K. J. Akerman, A. M. Fagenson, V. Cyril, M. Taylor, M. T. Muller, M. P. Akerman, O. Q. Munro, *J. Am. Chem. Soc.* 2014, 136, 5670–5682.
- [77] S. Radisavljević, I. Bratsos, A. Scheurer, J. Korzekwa, R. Masnikosa, A. Tot, N. Gligorijević, S. Radulović, A. R. Simović, *Dalton Trans.* 2018, 47, 13696–13712.
 [78] B. Bertrand, M. A. O'Connell, Z. A. E. Waller, M.
- [78] B. Bertrand, M. A. O'Connell, Z. A. E. Waller, M. Bochmann, *Chemistry – A European Journal* 2018, 24, 3613–3622.
- [79] S. Sookai, M. Akerman, M. Færch, Y. Sayed, O. Q. Munro, *European Journal of Medicinal Chemistry* 2025, 117330.
- [80] S. Goutelle, M. Maurin, F. Rougier, X. Barbaut, L. Bourguignon, M. Ducher, P. Maire, *Fundam Clin Pharmacol* 2008, 22, 633–648.
- [81] R. Gesztelyi, J. Zsuga, A. Kemeny-Beke, B. Varga, B. Juhasz, A. Tosaki, Arch. Hist. Exact Sci. 2012, 66, 427– 438.
- [82] "Pincer Compounds 1st Edition," can be found under https://www.elsevier.com/books/pincercompounds/morales-morales/978-0-12-812931-9, n.d.
- [83] J. J. Yan, A. L.-F. Chow, C.-H. Leung, R. W.-Y. Sun, D.-L. Ma, C.-M. Che, *Chem. Commun.* 2010, *46*, 3893–3895.
- [84] D. van der Westhuizen, C. A. Slabber, M. A. Fernandes,
 D. F. Joubert, G. Kleinhans, C. J. van der Westhuizen,
 A. Stander, O. Q. Munro, D. I. Bezuidenhout, *Chemistry* A European Journal 2021, 27, 8295–8307.
- [85] B. Bertrand, M. R. M. Williams, M. Bochmann, Chemistry – A European Journal 2018, 24, 11840– 11851.
- [86] S. Radisavljević, B. Petrović, Front Chem 2020, 8, 379.
- [87] S. Radisavljević, I. Bratsos, A. Scheurer, J. Korzekwa, R. Masnikosa, A. Tot, N. Gligorijević, S. Radulović, A. R. Simović, *Dalton Trans.* **2018**, *47*, 13696–13712.
- [88] B. C. Poulsen, S. Estalayo-Adrián, S. Blasco, S. A. Bright, J. M. Kelly, D. C. Williams, T. Gunnlaugsson, *Dalton Trans.* 2016, 45, 18208–18220.
- [89] S. Ghosh, P. Kundu, B. K. Paul, N. Chattopadhyay, RSC Adv. 2014, 4, 63549–63558.
- [90] S. Li, V. R. Cooper, T. Thonhauser, B. I. Lundqvist, D. C. Langreth, *J. Phys. Chem. B* 2009, *113*, 11166–11172.
- [91] I. Ahmad, M. Ahmad, *International Journal of Biological Macromolecules* **2015**, *79*, 193–200.

- [92] P. E. Pjura, K. Grzeskowiak, R. E. Dickerson, Journal of Molecular Biology 1987, 197, 257–271.
- [93] B. H. Geierstanger, D. E. Wemmer, Annual Review of Biophysics and Biomolecular Structure 1995, 24, 463– 493.
- [94] J.-B. Lepecq, C. Paoletti, Journal of Molecular Biology 1967, 27, 87–106.
- [95] X. Zhou, G. Zhang, L. Wang, International Journal of Biological Macromolecules 2014, 67, 228–237.
- [96] S. M. V. De Almeida, E. A. Lafayette, L. P. B. G. Da Silva, C. A. da C. Amorim, T. B. De Oliveira, A. L. T. G. Ruiz, J. E. De Carvalho, R. O. De Moura, E. I. C. Beltrão, M. D. C. A. De Lima, L. B. de C. Júnior, *International Journal of Molecular Sciences* **2015**, *16*, 13023–13042.
- [97] S. Perreault, F. Arjmand, J. Chandrasekhar, J. Hao, K. S. Keegan, D. Koditek, E.-I. Lepist, C. K. Matson, M. E. McGrath, L. Patel, K. Sedillo, J. Therrien, N. A. Till, A. Tomkinson, J. Treiberg, Y. Zherebina, G. Phillips, ACS Med. Chem. Lett. 2020, 11, 1236–1243.
- [98] W. Zeng, Y. Zhang, W. Zheng, Q. Luo, J. Han, J. Liu, Y. Zhao, F. Jia, K. Wu, F. Wang, *Molecules* 2019, 24, 1852.
- [99] S. Ghosh, *Bioorganic Chemistry* **2019**, *88*, 102925.
- [100] B. Nordén, T. Kurucsev, *J. Mol. Recognit.* **1994**, 7, 141– 155.
- [101] B. Chazotte, *Cold Spring Harb Protoc* **2011**, *2011*, pdb.prot5557.
- [102] S. Sookai, M. L. Bracken, M. Nowakowska, *Molecules* **2023**, *28*, 7466.
- [103] M. R. Hicks, J. Kowa\lski, A. Rodger, *Chemical Society Reviews* **2010**, *39*, 3380–3393.
- [104] P. E. Pjura, K. Grzeskowiak, R. E. Dickerson, Journal of Molecular Biology 1987, 197, 257–271.
- [105] H. R. Reese, *Biopolymers* **1994**, *34*, 1349–1358.
- [106] J. Sigmon, L. L. Larcom, *ELECTROPHORESIS* **1996**, 17, 1524–1527.
- [107] B. Zhang, S. Seki, K. Akiyama, K. Tsutsui, T. Li, K. Nagao, *Acta Medica Okayama* **1992**, *46*, 427–434.
- [108] S. Šeki, A. Hongo, B. Zhang, K. Akiyama, A. H. Sarker, T. Kudo, *Japanese Journal of Cancer Research* 1993, 84, 462–467.
- [109] B. W. Johnson, M. W. Burgess, V. Murray, J. R. Aldrich-Wright, M. D. Temple, *BMC Cancer* **2018**, *18*, 1284.
- [110] H. Åtsushi, S. Shuji, A. Kosuke, K. Takafumi, International Journal of Biochemistry 1994, 26, 1009– 1016.
- [111] S. U. Rehman, T. Sarwar, M. A. Husain, H. M. Ishqi, M. Tabish, Archives of Biochemistry and Biophysics 2015, 576, 49–60.
- Y.-X. Mi, S. Wang, X.-X. Xu, H.-Q. Zhao, Z.-B. Zheng,
 X.-L. Zhao, Y.-X. Mi, S. Wang, X.-X. Xu, H.-Q. Zhao, Z.-B. Zheng, X.-L. Zhao, *Journal of the Chilean Chemical* Society 2019, 64, 4392–4398.
- [113] C. Bailly, J. B. Chaires, *Bioconjugate Chem.* **1998**, 9, 513–538.
- [114] T.-P. Chiu, S. Rao, R. S. Mann, B. Honig, R. Rohs, *Nucleic Acids Res* 2017, *45*, 12565–12576.
 [115] D. Y. Lando, E. N. Galyuk, C.-L. Chang, C.-K. Hu,
- [115] D. Y. Lando, E. N. Galyuk, C.-L. Chang, C.-K. Hu, Journal of Inorganic Biochemistry 2012, 117, 164–170.
 [116] D. Y. Landa, C. L. Chang, A. S. Fridman, J. F.
- [116] D. Y. Lando, C.-L. Chang, A. S. Fridman, I. E. Grigoryan, E. N. Galyuk, Y.-W. Hsueh, C.-K. Hu, *Journal of Inorganic Biochemistry* **2014**, *137*, 85–93.
- [117] A. S. Fridman, V. Brabec, S. G. Haroutiunian, R. M. Wartell, D. Y. Lando, *Journal of Biomolecular Structure* and Dynamics **2003**, 20, 533–545.
- [118] H. Rozenberg, D. Rabinovich, F. Frolow, R. S. Hegde,
 Z. Shakked, *Proc. Natl. Acad. Sci. U.S.A.* **1998**, 95,
 15194–15199.
- [119] H. Song, J. T. Kaiser, J. K. Barton, Nat. Chem. 2012, 4, 615–620.

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RESEARCH ARTICLE

[120] S. A. Hollingsworth, R. O. Dror, Neuron 2018, 99, 1129– 1143. 14

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Towards new gold(III) anticancer agents. Herein, we report on an indazole-based gold(III) carboxamide pincer complex exhibiting significant cytotoxicity, likely mediated through dual interactions with DNA. Mechanistic investigations suggest that the complex binds to the DNA and forms specific interactions with nucleotide bases. Structural studies with thymine reveal its DNA base recognition mechanism, offering insights into its anticancer activity.