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Synthesis, Structural Characterisation and Quadruplex DNA Binding Studies of a New Gold(III) Pyrazolylpyridine Complex

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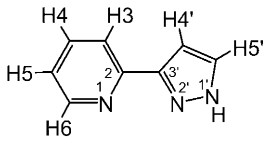
Guanine-rich sequences of DNA can assemble into quadruply stranded structures known as quadruplexes.[1] These structures arise thanks to the formation of guanine tetrads through hydrogen-bonding interactions between the Watson–Crick edge of one guanine and the Hoogsteen edge of its neighbour.[2] Quadruplex DNA structures have attracted recent interest since they have been identified as potential anticancer drug targets.[3] Bioinformatic studies have identified over 350 000 sequences in the human genome with the potential to form quadruplex structures.[4] Many of these sequences are found in the promoter regions of genes and hence it has been proposed that they might play important roles in regulating gene expression, for example, of certain oncogenes such as *c-myc* and c-*kit*.[5] On the other hand, formation of quadruplex DNA structures in the human telomere (*HTelo*) has been shown to inhibit telomerase.[6] The latter is an enzyme over-expressed in approximately 85 % of cancer cells and plays an important role in cancer cell immortalisation.[6a,b] Therefore, molecules that template the formation or stabilise the structure of quadruplex DNA could pave the way for the development of new anticancer drugs.[3] While the majority of quadruplex DNA binders reported to date are based on planar organic heteroaromatic systems,[7] recently, several metal complexes have been shown to be excellent quadruplex DNA binders.[8] Herein we report the synthesis, structural characterisation and DNA binding studies of a new gold(III) complex. This is the first example of a quadruplex DNA binder based on a gold complex.

2-(3′-Pyrazolyl)pyridine[9] (pzpyH), is an excellent chelating ligand for transition-metal ions (Scheme 1).[10] Reaction of pzpyH with $[AuCl_4]^-$ in water (at pH 5), gave $[Au(pzpy)_2]Cl \cdot nH_2O$ (**1**) in good yield. X-ray crystallography of a freshly prepared sample of single crystals of **1** showed n to be 3, while samples dried at 40 °C analysed as the monohydrate ($n=1$). The crystal structure analysis of the title compound (Figure 1) revealed the presence of two anionic, chelating pzpy ligands in mutual *trans* arrangement about the Au^{III} centre.



Scheme 1. 2-(3′-Pyrazolyl)pyridine shown in its 1′H-tautomeric structure and one possible rotamer form.

Coordination is through N1 sites of the pyridyl entities as well as N2′ of the pyrazolate groups. The four coordinating N atoms, which display normal bond lengths between 1.998(4) Å and 2.040(4) Å, form an almost ideal rectangle of size 3.10 Å × 2.60 Å, with N-*N*-N angles of close to 90°. N-Au-N angles expectedly vary, from 79.76(15)° (N31-Au-N42)

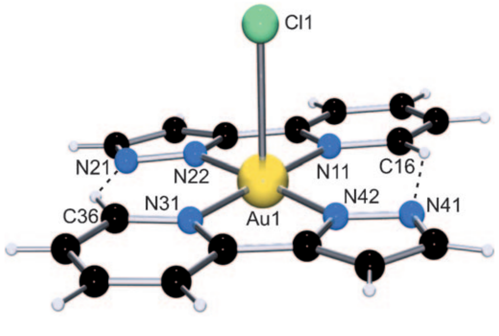


Figure 1. X-ray structure of the title compound **1**. Selected bond lengths [Å]: Au–N11 2.040(4), Au–N21 1.998(4), Au–N31 2.031(4), Au–N41 1.998(4), Au–Cl 3.1326(13).

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to 100.48(15)° (N22-Au-N31). The size of the complete $[Au(pzpy)_2]^+$ ion is about 9.8 Å × 8.4 Å. There is a slight propeller twist of 7.1° between two chelating ligands. The N1′ sites of the pyrazole groups are deprotonated and act as hydrogen-bond acceptors for the H6 protons of the pyridines, and the distances are N2′−C6, 3.050 Å, and N2′−C6H, 2.240 Å. These interactions are also reflected by the large downfield shift of the H6 resonance in the ^1H NMR spectrum. The Cl^- ion is in contact with the Au^{III} centre (3.1326(13) Å), forming the top of a square pyramid with the four strongly bonded N atoms representing the base. Although relatively long (for comparison the Au−Cl bonds in $[AuCl_4]^-$ are ca. 2.28 Å), this Au⋯Cl contact probably reflects a weak bonding interaction, as this distance is substantially shorter than expected for a non-bonding situation (≥ 3.5 Å), and in agreement with a tendency of Au^{III} to become five-coordinate.[11] It should be pointed out that in solution this weakly coordinated chloride dissociates instantaneously (as demonstrated by immediate precipitation of AgCl upon addition of $AgNO_3$), yielding a square-planar Au^{III} complex.

The packing of the title compound is dominated by two features. First, pairs of cations stack face-to-face at a distance of 3.3 Å. Second, the chloride ions represent anchors for the Au chelates within a 2D mixed chloride, water cluster. This structure is assembled by three different fused mixed water–chloride rings, containing $4(H_2O)\cdot Cl^-$, $2(H_2O)\cdot 2(Cl^-)$ and $8(H_2O)\cdot 2(Cl^-)$ units, respectively (Figure 2). Parallel clusters extend along the *ab* planes and are separated by 11.3 Å.

$[Au(pzpy)_2]Cl\cdot nH_2O$ is moderately soluble in common solvents such as water and methanol and well soluble in DMSO. In its ^1H NMR spectra, the bis(chelate) structure is readily recognised by its characteristically downfield shifted H6-pyridine resonance (d, $^3J = 6.0$ Hz), which occurs between $\delta = 10$ and 11 ppm (D_2O: $\delta = 10.08$ ppm; $[D_6]Me_2SO$: $\delta = 10.49$; CD_3OD: $\delta = 10.74$). These values compare with chemical shifts of this proton in the free ligand pzpyH around $\delta = 8.6$ ppm in D_2O, pD 6.1, or in $[D_6]Me_2SO$. The large downfield shift is attributed to an interaction of this proton with the lone pair of electrons of the pyrazolate-N1′ atom (cf. above and ref. [10b]). The effect of Au^{III} complexation on the other ^1H resonances of pzpy is much less pronounced. The bis(chelate) structure is remarkably robust in Me_2SO. A solution kept for four months at room temperature and in daylight showed very minor signs of decomposition only. In addition, the stability of **1** in aqueous solution containing the buffers employed in the DNA binding studies (vide infra) was investigated by using UV/Vis spectroscopy. Over a period of 8 h, no significant spectroscopic changes were observed, confirming that this complex is stable in solution under the conditions and time frame required to carry out the DNA binding studies.

The geometry, size and propensity of complex **1** to π–π stack, suggested that it could be a good quadruplex DNA binder. To assess this, fluorescent intercalator displacement (FID), surface plasmon resonance (SPR) and circular dichroism (CD) studies were carried out. Two biologically relevant DNA sequences known to form quadruplexes (*HTelo* and *c-myc*) and a duplex DNA sequence were used.

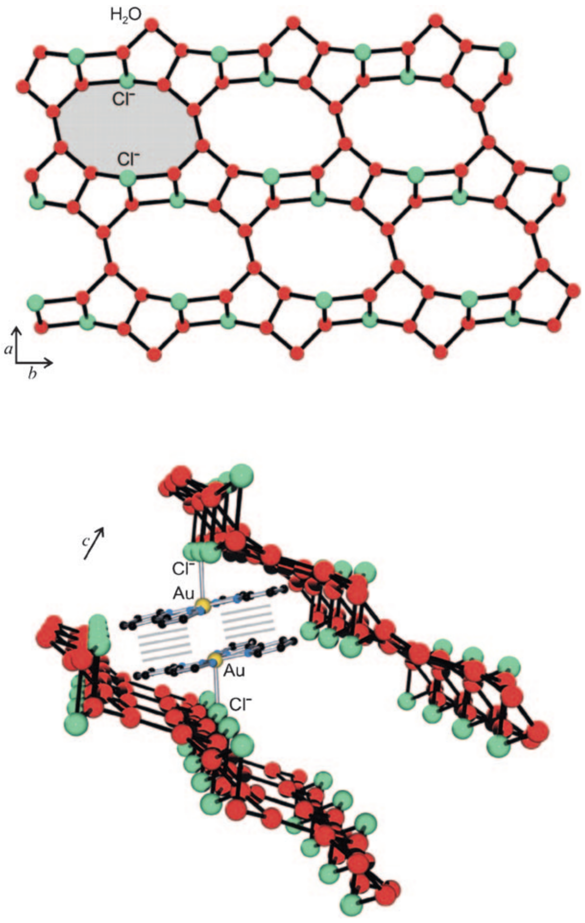

Figure 2. Schematic representation of the 2D mixed chloride–water cluster with chloride ions acting as anchor for the cations (top). View of the anchored gold cations (**1**), which stack pairwise (bottom).

FID[12] showed complex **1** to interact strongly with quadruplex DNA with DC_{50} values of 0.34 μM (*c-myc*) and 1.03 μM (*HTelo*). These low micromolar DC_{50} values are comparable to those previously found for a range of good quadruplex DNA binders.[12] In addition, **1** shows good selectivity for *c-myc* quadruplex versus duplex DNA (for duplex DNA, a DC_{50} of 3.05 μM was determined, giving a selectivity just below the "selectivity threshold" of 10 previously proposed for successful quadruplex DNA binders[12]).

To obtain quantitative data for the interaction between the gold complex and DNA, SPR[13] studies were carried out. Three different 5′-biotin-labelled DNA sequences (*HTelo*, *c-myc* and duplex) were immobilised on a sensor chip. Binding experiments were carried out under salt/buffer conditions suitable for DNA quadruplex formation (i.e. HBS-EP buffer supplemented with 0.2 M KCl). A range of concentrations of complex **1** (0.1 to 0.7 μM) were investigated by injecting the sample simultaneously over the three different sequences of immobilised DNA and the blank reference. Sensorgrams were obtained for the concentration-dependent binding of **1** to the three different sequences of

DNA and binding constants were calculated (see Supporting Information). For both quadruplex DNA sequences, the best fit was obtained by using a two inequivalent-binding-sites model, while for duplex DNA the best fit was obtained with a four-equivalent-site model. This gave the following K_a values: 1.8×10^6 for *HTelo* DNA, 1.1×10^6 *c-myc* DNA and 3.4×10^5 for duplex DNA. These values indicate that **1** interacts strongly with quadruplex DNA, and a degree of selectivity over duplex DNA is observed. Overall this is consistent with the FID results.

To assess the ability of the molecule to template the formation of quadruplex DNA, circular dichroism studies were carried out.[14] For this, we compared the CD spectrum of *HTelo* DNA (in the absence of K^+) before and after addition of **1**. As expected, the CD spectrum of *HTelo* in the absence of **1** gave two bands at about 290 nm (indicative of an anti-parallel quadruplex structure) and at about 250 nm (associated to singly stranded DNA). Upon addition of two equivalents of complex **1**, the band corresponding to the singly stranded DNA (250 nm) disappeared completely and a new band at 260 nm with negative ellipticity appeared (Figure 3). Furthermore an increase in the 290 nm band (associated with the anti-parallel quadruplex structure) was observed. These results clearly indicate that **1** templates the folding of singly stranded *HTelo* DNA into the anti-parallel quadruplex conformation.

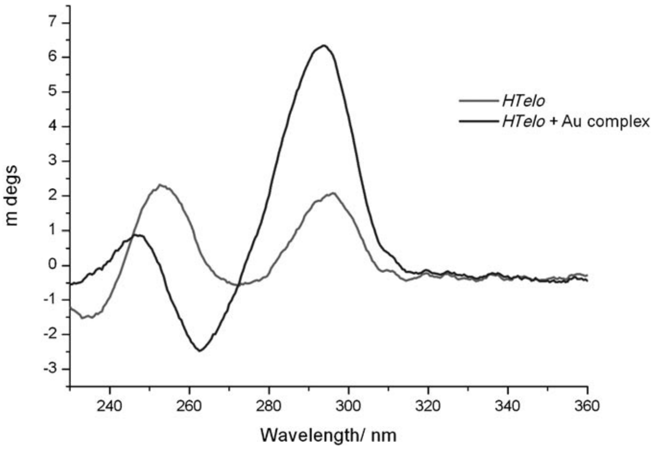

Figure 3. CD spectra of *HTelo* DNA (10 μM) in Tris-HCl buffer in the absence and presence of two equivalents of complex **1** (20 μM).

To confirm the strong interaction of **1** with *c-myc* DNA (as observed by FID and SPR), a variable-temperature CD study was carried out. We first determined the melting temperature (T_m) of *c-myc* in the absence of **1** by monitoring the decrease in the band at about 260 nm. Plotting the intensity of this band versus temperature (Figure 4) gave a T_m of 53 °C for *c-myc* DNA. Upon addition of two equivalents of **1**, the T_m increased to 79 °C giving a ΔT_m of 26 °C. This indicates a very strong interaction between complex **1** and *c-myc* quadruplex DNA. For comparison, the ΔT_m of TMPyP4 (a porphyrin known to interact strongly with *c-myc* DNA[15]) was also determined, giving a value of 11 °C.

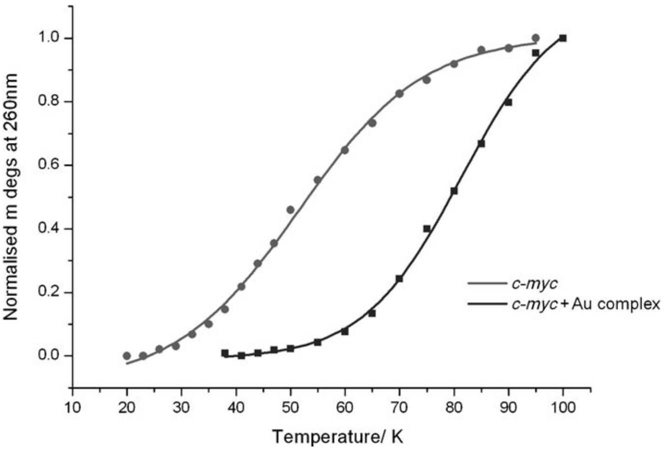

Figure 4. Melting curves of *c-myc* quadruplex DNA as determined by variable-temperature CD spectroscopy.

In summary, a new gold(III) complex has been reported which is an excellent quadruplex DNA binder. This complex is able to template the formation of quadruplex DNA from a single-stranded unfolded structure, in addition to stabilising preformed quadruplexes. In contrast to most previously reported quadruplex DNA binders, this compound does not feature substituents that could aid the interaction by electrostatic interactions with the grooves and loops of DNA. It is hence likely that the strong binding is largely driven by π–π interactions with the guanine quartet. Future work will focus on modifying the metal complex to improve its selectivity for specific quadruplex DNA sequences.

Experimental Section

Synthesis: Complex **1** was prepared by reacting $HAuCl_4 \cdot 3H_2O$ (520 mg, 1.32 mmol) and pzpyH (364 mg, 2.5 mmol) in aqueous suspension (150 mL, pH adjusted to 5.1 by means of NaOH and HNO_3) at 40 °C for three days. Following filtration of a first fraction of the product, the filtrate was kept at room temperature overnight, and then filtered from a second fraction. The combined precipitates, which proved identical according to IR and ^1H NMR spectroscopy, were dried in an oven at 40 °C, yielding *trans*-$[Au(pzpy)_2]Cl \cdot H_2O$ (464 mg; 65 %). ^1H NMR (D_2O, 1H each): $\delta = 10.08$ (d, $J = 6.3$ Hz), 8.34 (ddd, $J = 7.8$ Hz, 7.8 Hz, 1.2 Hz), 8.05 (dd, $J = 7.9$ Hz, 1.2 Hz), 7.62 (d, $J = 2.1$ Hz), 7.59 (ddd, $J = 7.5$ Hz, 6.2 Hz, 1.5 Hz), 6.86 ppm (d, $J = 2.1$ Hz). Elemental analysis calcd (%) for $C_{16}H_{14}N_6AuCl \cdot H_2O$ (540.76): C 35.5, H 3.0, N 15.5; found C 35.1, H 2.9, N 15.6. Single crystals of the corresponding trihydrate, *trans*-$[Au(pzpy)_2]Cl \cdot 3H_2O$, suitable for X-ray analysis, were recovered from a small sample of the initial first filtrate kept at 4 °C for a day.

X-ray crystallography: Crystal data for *trans*-[Au(2-(pyrazol-3-yl)pyridine)$_2$]Cl · $3H_2O$ (**1**): $[C_{16}H_{18}Au_1Cl_1N_6O_3]$, triclinic, $P\bar{1}$, $a = 9.2155(4)$, $b = 9.8589(5)$, $c = 11.3273(4)$ Å, $\alpha = 75.769(4)$, $\beta = 80.122(3)$, $\gamma = 67.531(4)°$, $Z = 2$, fw = 574.78 $g\,mol^{-1}$, $V = 918.37(7)$ Å^3, $\rho_{calcd} = 2.079\,Mg\,m^{-3}$, $\lambda(Mo_{K\alpha}) = 0.71073$ Å, $\mu = 8.186\,mm^{-1}$, 9150 reflections collected, 4233 unique ($R_{int} = 0.0382$), $R1(F_o) = 0.0296$ $[I > 2\sigma(I)]$, $wR2$ $(F_o^2) = 0.0469$ (all data), GOF = 0.927.

FID assay procedure:[12] To a mixture of the DNA sequence (0.25 μM) and TO (0.50 μM) in 10 mM potassium cacodylate buffer (pH 7.4) supplemented with 50 mM KCl, increasing amounts of **1** were added (from 0.25

to 5 μM). After an equilibration time of 3 min, the emission spectrum was recorded between 510–750 nm with an excitation wavelength of 501 nm. The fluorescence area was calculated by using the "trapezium rule" method and converted into %TO displacement using: %TO displacement = 100 − [(fluorescence area of sample)/(fluorescence area of standard) × 100]. The standard fluorescence spectrum was obtained in the absence of the gold complex %TO displacement was then plotted against the concentration of **1** to give the respective FID curves.

SPR procedure: The SPR experiments were carried out on the four-channel BIACore 3000 optical biosensor instrument as previously reported.[13] The 22AG strand (5′-biotin-AGG-GTT-AGG-GTT-AGG-GTT-AGG-G-3′) and 19AG strand (5′-biotin-AGG-GTG-GGG-AGG-GTG-GGG-A-3′) were used for the *HTelo* and *c-myc* studies, respectively. For the duplex studies, the 22-base-pair strand (5′-biotin-GGC-ATA-GTG-CGT-GGG-CGT-TAG-C-3′) with its complementary strand (5′-GCT-AAC-GCC-CAC-GCA-CTA-TGC-C-3′) was used. The oligonucleotides were loaded onto streptavidin-coated four flow cell sensor chips purchased from GE Healthcare UK Ltd. The sensorchip was first conditioned with injections of 1 M NaCl in 50 mM NaOH followed by prolonged washings with buffer. Three of the four flow cells were loaded with the respective labelled DNA strands by manual injection of the 10 nM oligonucleotide solutions at a flow rate of 2 $\mu L\,min^{-1}$ for 7 min. This was sufficient to achieve RU levels of about 600RU. The last flow cell was left blank as a reference. Once the oligonucleotides were immobilised onto the sensor chip, the SPR experiments were conducted in BS-EP/KCl buffer by multiple injections of a range of gold complex concentrations (0.1 to 0.7 μM). Each analyte concentration was passed over the four flow cells for 5 min at a flow rate of 20 $\mu L\,min^{-1}$. Between injections the sensor chip surface was regenerated by running buffer.

CD procedure: The CD spectra were recorded on an Applied Photophysics Ltd Chirascan spectrometer using a strain-free 10 mm × 2 mm rectangular cuvette. The CD spectra were measured between 700 and 180 nm with the following parameters: bandwidth, 1 nm; spectral range, 230–360 nm; step-size, 0.5 nm; time-pep-point, 1.5 s. Two different CD experiments were conducted: a) CD spectra for the *HTelo* sequence (10 μM) with and without the presence of **1** (20 μM, 2 equiv) in Tris-HCl buffer; b) variable temperature CD study with *c-myc* sequence (5 μM) without and with **1** (10 μM, 2 equiv) in Tris-HCl buffer.

CCDC-758909 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Keywords: bioinorganic chemistry · coordination chemistry · gold · quadruplex DNA · telomere

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