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Inorganica Chimica Acta 359 (2006) 44–52
Inorganica Chimica Acta
www.elsevier.com/locate/ica

Synthesis, characterization, and aqueous chemistry of cytotoxic Au(III) polypyridyl complexes

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Received 14 April 2005; received in revised form 16 August 2005; accepted 17 August 2005
Available online 18 October 2005

Abstract

This work reports the synthesis, characterization, and aqueous chemistry of a series of cytotoxic [Au(polypyridyl)Cl_2]PF_6 complexes {(where polypyridyl = dipyrido[3,2-f:2′,3′-h] quinoxaline (DPQ), dipyrido[3,2-a:2′,3′-c] phenazine (DPPZ) and dipyrido[3,2-a:2′,3′-c] (6,7,8,9-tetrahydro) phenazine (DPQC))}. The crystal structure of [Au(DPQ)Cl_2]PF_6 was determined as example of the series and exhibits the anticipated square planar geometry common for d^8 coordination complexes. The crystals of the complex belong to the space group $P2_1/n$ with $a = 7.624(2)$ Å, $b = 18.274(5)$ Å, $c = 14.411(14)$ Å, $\beta = 98.03(3)°$, and $Z = 4$. In ^{1}H NMR studies of these compounds in the presence of aqueous buffer, all four complexes rapidly converted to the dihydroxy species [Au(polypyridyl)$(OH)_2$] in a stepwise fashion. However, the [Au(polypyridyl)]$^{3+}$ fragment believed to impart cytotoxicity in human ovarian cancer cell lines (A2780) remained intact and appeared stable for days. It was also noted that these Au(III) complexes were readily reduced in the presence of the common biological reducing agents, reduced glutathione and sodium ascorbate. How solution and redox stability may affect the biological activity of these novel Au(III) complexes is discussed.

Keywords: Gold; Gold(III); Au(III); Anticancer; Antitumor; Polypyridyl; Cytotoxicity; X-ray crystal structure and intercalate; Solution chemistry; A2780; Ovarian cancer

1. Introduction

Over the past few years a renewed interest in Au(III) compounds as potential anticancer agents has developed. Though a number of interesting Au(III) targets have been investigated [1,2], the biological utility of such agents continues to be questioned. This may be due, in part, to the redox instability and poor solubility of common Au(III) coordination complexes under physiologic conditions. Many groups are providing insight into complexes of Au(III) that may possess significant anticancer activity. These ranges from simple Au(III) coordination complexes [3–13], Au(III) complexes containing bioligands [14–27], and organometallic Au(III) species [17,28–37]. Recent studies by Messori et al. [7] were of particular interest in that they have demonstrated that simple bidentate or polydentate ligands containing nitrogen donor atoms may offer sufficient redox stabilization to produce viable Au(III) anticancer drug targets under physiologic conditions. The studies of square planar Au(III) complexes of 1,10-phenanthroline, 2,2′:5′,2-terpyridine, and 2,2′-bipyridine further suggested that the interaction of these Au(III) compounds with DNA by intercalation may also play a role in their anticancer activity.

In this group's search for Au(III) complexes with a unique spectrum of anticancer activity, a series of square planar Au(III) complexes containing chelate polypyridyl ligands were explored. Square planar Au(III) complexes containing the polypyridyl ligands dipyrido[3,2-f:2′,3′-h] quinoxaline (DPQ), dipyrido[3,2-a:2′,3′-c] phenazine (DPPZ) and dipyrido[3,2-a:2′,3′-c](6,7,8,9-tetrahydro)phenazine

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doi:10.1016/j.ica.2005.08.030

(DPQC) were prepared anticipating that the extended ring system would enhance cancer activity by improving DNA binding affinity. These ligand systems have been thoroughly studied in numerous transition metal complexes, but particularly in ruthenium [38–54] and platinum [55–57] coordination chemistry. In order to better understand the relationships between the biological activity and the chemical properties of these new compounds, we have reported the crystal structure of a representative complex from the series and described the solution stability of all synthesized compounds in detail. We also report the antitumor activity of these compounds against the A2780 human ovarian cancer cell line. As these Au(III) polypyridyl compounds posses great structural similarity to the 1,10-phenanthroline complex described by Messori et al., the chemical and biological properties will be compared.

2. Experimental

2.1. Materials and methods

All compounds were synthesized using analytical grade reagents and HPLC quality solvents. The compounds 1,10-phenanthroline, ethylene diamine, *trans*-1,2-diaminocyclohexane, sodium tetrachloroaurate(III) dihydrate and *cis*-dichlorodiammine platinum(II) (Aldrich) and *o*-phenylene diamine (Fluka) were obtained from commercial sources and used as received. Adherent human ovarian adenocarcinoma cells (A2780) used for cytotoxicity studies were obtained from The National Cancer Institute-Frederick Cancer DCI Tumor Repository. The Hank's balanced salt solution, Dulbecco's-phosphate buffer saline, biological grade DMSO, and Triton X-100 were purchased from Sigma and used as received. The RPMI-1640 medium (ATCC), penicillin, streptomycin, fetal bovine serum and trypsin-EDTA (Invitrogen Corporation) were used as received. The fetal bovine serum (FBS) was heat inactivated to avoid complement-mediated cell lysis. RPMI-1640 medium was supplemented with 5% heat inactivated FBS, penicillin and streptomycin. Costor brand sterile, tissue culture treated 96-well black clear bottom plates were purchased from Corning. The live/dead cell viability/cytotoxicity kit was purchased from Molecular Probes.

2.2. Physical measurements

Elemental analyses (C, H and N) were carried out by Desert Analytics and reported only for the novel compounds **2**–**4**. Electrical conductance measurements were obtained from 10^{-3} M dimethylformamide solutions of the Au^{III} complexes at room temperature using a Fisher Scientific Conductivity and TDS Model Digital Conductivity Meter with a dip type conductivity cell equipped with automatic temperature compensation. The conductivity meter was calibrated with 0.1 N KCl prior to use. Infrared spectra were obtained for the ligand and its metal ion complexes as KBr discs using a Bruker Vector22 FT-IR spectrophotometer. The ESI-Mass analyses were measured in acetonitrile solutions (ca. 0.001 g in 10 mL) by direct infusion at the heated capillary temperature of 200 °C in the positive ion mode on a Thermo-Finnigan LCQ mass spectrometer. Electronic spectral measurements were recorded on a Beckman DU series 600 spectrophotometer using 100 μM DMSO solutions. All ^{1}H NMR were recorded on a Bruker 400 MHz FTNMR in DMSO-d_6. Fluorescence measurements were obtained using a Spectra Max Gemini XS, Molecular Devices. X-ray diffraction data were collected on a Bruker P4 Diffractometer equipped with a molybdenum tube and a graphite monochromator at 25 °C.

2.3. Synthesis

The polypyridyl ligands dipyrido[3,2-f:2′,3′-h] quinoxaline (DPQ), dipyrido[3,2-a:2′,3′-c] phenazine (DPPZ) and dipyrido[3,2-a:2′,3′-c](6,7,8,9-tetrahydro) phenazine (DPQC) were prepared according to the literature method [58] from 1,10-phenanthroline-5,6-dione [59]. All ligands were purified by recrystallization from methanol. The Au(III) polypyridyl complexes were isolated as their PF_6 salts by a slight modification of a previously described method [60]. To a solution of the ligand (1 mmol) in acetonitrile (5 cm^3) were added an aqueous solution of $NaAuCl_4 \cdot 2H_2O$ (0.3978 g, 1 mmol) (25 cm^3) and solid KPF_6 (0.9210 g, 5 mmol, excess). The resulting yellow suspension was refluxed for 15 h and the colorless hot reaction mixture filtered. The filtrate was washed five times with Nano-pure water to ensure the complete removal of any unreacted $NaAuCl_4$ and air dried. The dried product was dissolved in acetone and the insoluble portion was discarded. Brilliant yellow X-ray quality crystals were obtained by vapor diffusion of diethyl ether into the solution of the compound in acetone. Properties of $[Au(Phen)Cl_2][PF_6]$ were nearly identical to that observed for the previously reported chloride salt [8] and have been included for completeness.

2.4. [Au(Phen)Cl$_2$][PF$_6$] (**1**)

Yield: 80%, M $[Au(Phen)Cl_2]^+$ (448.08), M 449.1; [Au(Phen)Cl] (412.62), M 412.2; Phen (180.20), M 179.4; Λ_M (10^{-3} M, DMF) 35.1 Ω^{-1} cm^2 mol^{-1}; λ_{max} nm (ε_{max}), 259 (19912), 281 (25288); δ_H (400 MHz, DMSO-d_6) 9.76–9.75, d, $J = 6$, 2H; 9.41–9.39, dd, $J = 2$ and 8, 2H; 8.58, s, 2H; 8.51–8.48, dd, $J = 6$ and 8, 2H.

2.5. [Au(DPQ)Cl$_2$][PF$_6$] (**2**)

Yield: 81%, m.p. 256–260 °C (dec), {Found: C, 27.70; H, 1.74; N, 8.68; requires: C, 27.61; H, 1.64; N, 8.31; M $[Au(DPQ)Cl_2]^+$ (500.11), M 501.1, 499.1; [Au(DPQ)Cl] (464.66), M 464.2; DPQ (232.24), M 231.2; Λ_M (10^{-3} M, DMF) 37.6 Ω^{-1} cm^2 mol^{-1}; λ_{max} nm (ε_{max}), 272 (36793), 278 (27648); δ_H(400 MHz, DMSO-d_6) 10.30–10.28, dd, $J = 1$ and 7, 2H; 9.87–9.86, dd, $J = 1$ and 4, 2H; 9.81, s, 2H; 8.85–8.82, dd, $J = 3$ and 5, 2H.

2.6. [Au(DPPZ)Cl$_2$][PF$_6$] (3)

Yield: 78%, m.p. 235–242 °C (dec), {Found: C, 31.66; H, 1.70; N, 8.09; requires: C, 31.10; H, 1.45; N, 8.06; M [Au(DPPZ)Cl$_2$]$^+$ (550.17), M 551.1, 549.1; [Au(DPPZ)Cl] (514.72), M 514.3; DPPZ (282.29), M 281.3; Λ_M (10^{-3} M, DMF) 34.2 Ω^{-1} cm^2 mol^{-1}; λ_{max} nm (ε_{max}), 258 (40256), 272 (47566), 285 (41238), 363 (15442), 381 (14424); δ_H (400 MHz, DMSO-d_6) 9.84–9.82, d, $J = 9$, 2H; 9.36–9.35, d, $J = 5$, 2H; 8.43–8.41, dd, $J = 3$ and 4, 2H; 8.35–8.32, dd, $J = 4$ and 5, 2H; 8.17–8.14, dd, $J = 3$ and 4, 2H.

2.7. [Au(DPQC)Cl$_2$][PF$_6$] (4)

Yield: 85%, m.p. 197–201 °C (dec), {Found: C, 31.52; H, 2.32; N, 8.05; requires: C, 30.92; H, 2.02; N, 8.01; M [Au(DPQC)Cl$_2$]$^+$ (554.20), M 555.1, 553.1; [Au(DPQC)Cl] (518.75), M 518.2; DPQC (286.33), M 287.3, 285.3; Λ_M (10^{-3} M, DMF) 34.6 Ω^{-1} cm^2 mol^{-1}, λ_{max} nm (ε_{max}), 260 (50856), 264 (39354), 349 (10908); δ_H (400 MHz, DMSO-d_6) 10.08–10.06, d, $J = 8$, 2H; 9.91–9.90, dd, $J = 2$ and 6, 2H; 8.68–8.64, dd, $J = 2$ and 6, 2H; 3.49–3.47, m, 4H; 2.41, m, 4H.

The far IR bands for the complexes in the region 390–320 cm^{-1} evidence the presence of Au–Cl bond [61].

2.8. Crystallographic data (CCDC 268700)

The [Au(DPQ)Cl$_2$]PF$_6 \cdot 0.5$Me$_2$CO crystals were isolated by vapor diffusion of ether into a solution of the compound dissolved in acetone. A yellow rectangular prismatic crystal of approximate dimensions $0.10 \times 0.12 \times 0.40$ mm was mounted on a glass fiber using epoxy resin. A total of 4836 reflections were gathered in the 2θ range of 5.3–51° with the data collected having $-9 \leqslant h \leqslant 1$, $-22 \leqslant k \leqslant 1$, $-17 \leqslant l \leqslant 17$ using the XSCANS program [62]. Three standard reflections measured after every 97 reflections exhibited no significant loss of intensity. The data were corrected for Lorentz-polarization effects and absorption. The structure was solved by direct methods and refined by least-squares techniques adapting the full-matrix weighted least-squares scheme, $w^{-1} = \sigma^2 F_o^2 + (0.1121P)^2$, where $P = (F_o^2 + 2F_c^2)/3$, on F^2 using the SHELXTL program [63]. Additional details of the data collection parameters and refinement are collected in Table 1. Appropriate bond lengths and bond angles are provided in Table 2.

2.9. In vitro cytotoxicity studies

Cells from the A 2780 line in supplemented RPMI-1640 medium in a humidified atmosphere of 5% CO_2/95% air, adhesion of cells (~95%) to the culture surface occurred within the first few hours after seeding. Rapid growth was then observed after overnight incubation and allowed to continue to about 70% confluence. The cells were then washed with Hank's balanced salt solution and harvested using standard trypsin-EDTA methods. The harvested cells were then seeded onto sterile tissue culture treated 96-well black clear bottom plates and allowed to grow to about 90% confluence in supplemented RPMI medium. The medium was then aspirated and the cells washed with Dulbecco's-phosphate buffer saline (D-PBS). Following washing, cells were incubated for 2 h in D-PBS solution containing varying concentrations of NaAuCl$_4 \cdot 2$H$_2$O, polypyridyl ligand, Au-ligand complex or the standard drug cisplatin in DMSO. The final volume of DMSO in each well never exceeded 10 μL (5%). Control wells containing similarly grown cells were treated with either a 5% DMSO or a 5% Triton-X100 as marker for cell death. All wells were treated with live/dead viability/cytotoxicity kit and the dye concentration and incubation time optimized. After incubation for 2 h,

Table 1
Crystallographic data for [Au(DPQ)Cl$_2$]PF$_6$0.5(CH$_3$COCH$_3$)

Empirical formula	$C_{15.5}H_{11}AuCl_2F_6N_4PO_{0.5}$
Formula weight	674.12
λ (Å)	0.71073
Space group	$P2_1/n$
a (Å)	7.624(2)
b (Å)	18.274(5)
c (Å)	14.411(14)
α (°)	90
β (°)	98.03(3)
γ (°)	90
V (Å^3)	1988(2)
Z	4
μ (mm^{-1})	7.817
ρ_{calc} (g cm^{-3})	2.252
T (°C)	25
Scan type	ω
Independent reflections (R_{int})	3697 (0.0680)
Observed reflections [$F > 4\sigma(F)$]	2492
Number of parameters	330
S_{gof} on F^2	1.024
R_1[a] [$F > 4\sigma(F)$]	0.0619
wR_2[b]	0.1572

[a] $R_1 = \sum \| F_o | - | F_c \| / \sum | F_o |$.
[b] $wR_2 = [\{w(F_o^2 - F_c^2)^2\} / \{w(F_o^2)^2\}]^{1/2}$.

Table 2
Specified bond lengths and bond angles for [Au(DPQ)Cl$_2$]PF$_6 \cdot 0.5$(CH$_3$COCH$_3$) and comparison to [Au(Phen)Cl$_2$]Cl [8]

Bond length (Å)		
Bonded atoms	[Au(DPQ)Cl$_2$]PF$_6$	[Au(Phen)Cl$_2$]Cl
Au(1)–N(1)	2.018(10)	2.033(8)
Au(1)–N(4)	2.055(11)	2.056(8)
Au(1)–Cl(2)	2.239(4)	2.263(3)
Au(1)–Cl(1)	2.258(4)	2.266(3)
Bond angles (°)		
Cl(1)–Au(1)–Cl(2)	89.76(18)	89.5(1)
Cl(1)–Au(1)–N(1)	94.8(3)	94.2(2)
Cl(1)–Au(1)–N(4)	175.2(3)	174.5(2)
Cl(2)–Au(1)–N(4)	94.4(3)	94.2(2)
Cl(2)–Au(1)–N(1)	175.4(3)	174.6(2)
N(1)–Au(1)–N(4)	81.1(4)	82.0(3)

50 μL of live cell reagent (1 μL calcein AM/1 mL of D-PBS) was added to wells and then incubated for further 2 h. The fluorescence was then measured at excitation and emission wavelengths of 485 and 525 nm, respectively, using a 515 nm cut-off filter. From the fluorescence intensities (obtained from the weighted average of 12 wells from quadruplet trials), the % inhibition was calculated. The inhibiting concentration (IC_{50}) was found out by plotting log (conc) of test compounds versus % inhibition.

3. Results

Initial attempts to synthesize pure [Au(polypyridyl)Cl_2]Cl complexes met with some difficulty. The reaction of equimolar amounts of polypyridyl ligand and either $NaAuCl_4 \cdot 2H_2O$ or $HAuCl_4$ in ethanol consistently yielded a yellow precipitate after a 10 h reflux. The NMR of this precipitate exhibited considerable downfield shift for the protons proximal to the polypyridyl donor nitrogen atoms suggesting ligand coordination. However, the elemental analyses of the resultant solid did not correlate with the expected complex stoichiometry of [Au(polypyridyl)Cl_2]Cl. Further investigation of these complexes led us to believe that the analyzed compound was actually a combination of products with the major contribution being from [Au(polypyridyl)Cl_2][$AuCl_4$]. Isolation of the mononuclear Au(III) polypyridyl complexes as their PF_6 salts offered more consistent results and better corresponding yields. While the PF_6 salts demonstrate decreased aqueous solubility as compared to their chloride counterparts, we believe that the increased hydrophobicity promotes enhanced cellular uptake. This of course assumes that the primary mechanism of uptake would be by passive diffusion and studies are currently underway to determine if this is the case.

3.1. Crystallographic data

The details of the data collection parameters and refinement are collected in Table 1. Key bond lengths and bond angles have been included in Table 2. All non-hydrogen atoms were located in the difference maps during successive cycles of least-squares and refined anisotropically. All of the fluorine atoms in the PF_6 counter ion were disordered; the best model for this disorder was obtained by assigning two sites for each of the fluorine atoms and allowing the values of site occupancy to vary using a free variable. Successive refinements led to 60% and 40% site occupancies for the two sets of fluorine atoms. A highly disordered acetone molecule is also present in the asymmetric center and is situated on a 2-fold symmetry axis. The four atoms of the acetone moiety are disordered over six sites. An assignment of 50% site occupancy to the molecule led to satisfactory refinement of the overall structure. The hydrogen atoms except those of the acetone molecule were placed in calculated positions with the thermal isotropic parameter fixed at 1.2 times U_{eq} of the preceding atom. The final refinement parameters were $R_1 = 0.0619$ and $wR_2 = 0.1572$ for data with $F > 4\sigma(F)$ giving the data to parameter ratio of 12:1. The refinement data for all data were $R_1 = 0.0949$ and $wR_2 = 0.1811$.

The planar DPQ ligand exhibits a bidentate chelate mode of binding and non-involvement of the quinoxaline nitrogen in metal–ligand interactions. This planarity of the molecule is an essential criterion for π-stacking interactions and intercalation. The structure of the complex consists of a discrete monomeric species with the Au(III) in a square planar coordination geometry (Fig. 1). The donor atoms in the basal plane are the two ring nitrogen and chloride atoms. The crystal packing consists of layers which are formed through C–H···X intermolecular contacts. The C–H···halogen intermolecular contacts controls

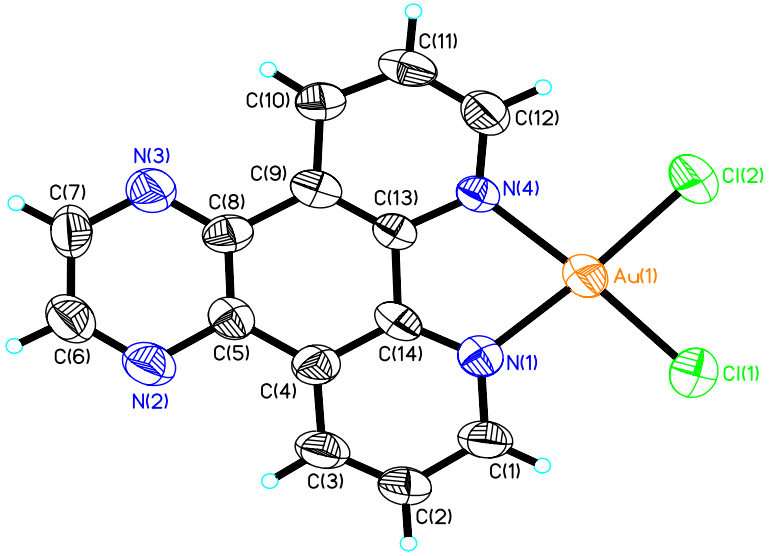


Fig. 1. Ortep view of [Au(DPQ)Cl_2]PF_6.

the packing. It is interesting to note that the key structural features of the $[Au(DPQ)Cl_2]^+$ complex are quite similar to those observed previously for $[Au(Phen)Cl_2]^+$ [7]. Specifically, bond lengths for Au–Cl and Au–N as well as Cl–Au–Cl and N–Au–N bond angles were quite comparable between the two complexes. Such great similarity between ligand environments about the gold center for both the $[Au(DPQ)Cl_2]^+$ and $[Au(Phen)Cl_2]^+$ is suggestive of comparable Au(III) centered activity imparted by these σ-donor ligands. These expectations were also supported by aqueous stability studies.

3.2. Aqueous stability of Au-polypyridyl complexes

Compounds **1**–**4** show very modest solubility in water and in aqueous buffered solutions. As described in Section 2, some studies required dissolving the complexes in a small amount of DMSO followed by dilution with a large excess of buffer.

3.3. UV–Vis

Complexes **1**–**4** demonstrated no observable change in their UV–Vis spectra in water, aqueous 50 mM phosphate buffer, or RPMI-1640 medium after 24 h at room temperature. Though this did demonstrate the stability of these complexes in aqueous and physiological conditions it was in contrast to the exchange behavior observed by ^{1}H NMR (see below). It was concluded that the UV–Vis spectra of the mono and dihydroxy versions of complexes **1**–**4** must be sufficiently similar to the starting complexes as to be indistinguishable. It was also concluded that the $[Au(ligand)]^{3+}$ fragment was sufficiently stabile in aqueous solution for 24 h at room temperature. Addition of sodium ascorbate to an aqueous buffered solution of **1**–**4** showed only minor changes in the UV spectra at the λ_{max} for each complex. Sodium ascorbate produced a significant signal in this area of the spectrum and it was decided that NMR studies may be more suitable for in-depth analysis of Au(III) complexes in the presence of a biologically relevant reducing agent.

3.4. ^{1}H NMR

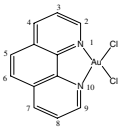
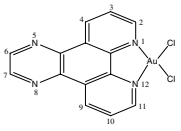
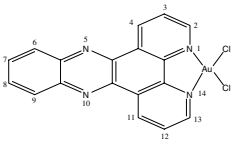
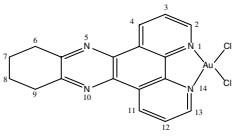
The protons in proximity to coordinating nitrogen atoms of the polypyridyl ligands all experience a downfield shift in DMSO-d_6 by about 0.3–0.9 δ in complexes **1**–**4** indicating the involvement of the pyridyl nitrogens in coordination. The polypyridyl aromatic proton signals were also shifted downfield (~0.2 δ) in DMSO-d_6 indicating that a degree of electron reorganization in the aromatic ring current was also evident on coordination. Additional NMR studies of aqueous complex stability gave a further insight for the progressive detachment and formation of Au–Cl and Au–OH bonds, respectively.

The addition of a slight molar excess of physiological buffer (50 mM phosphate, 4 mM NaCl, pH 7.4) to $[Au(polypyridyl)Cl_2]PF_6$ dissolved in DMSO-d_6 immediately generated several new signals in the proton NMR spectrum. The increase in new signal intensities with time suggests the formation of both a mono- and di-hydroxy species. The variation in chemical shifts upon addition of physiological buffer is given in Table 3. After equilibration of the complexes in the buffer/DMSO-d_6 solution for 24 h, the complete conversion to the dihydroxy complex was evident from simplification of the ^{1}H NMR spectrum (Fig. 2). From these studies, we can conclude (a) the stability of the $[Au(polypyridyl)]^{3+}$ fragment under physiological conditions and (b) that formation of the dihydroxy species of $[Au(polypyridyl)]^{3+}$ complexes occurs much more readily than observed for the 2,2′-bipyridyl analogs which required stringent conditions for formation [5,64]. Analysis of the ^{1}H NMR spectra recorded 5 min after mixing the buffer with complexes gave some clue regarding the reactivity towards hydrolysis. A considerable change in the position of signals corresponding to the protons adjacent to donor atoms was observed in the case of complexes **2** and **3**, a marginal change in the case of complex **4** and no change in the case of complex **1**. The disappearance of the proton signal corresponding to the dichloro-analog in the case of complex **2** after adding buffer indicates that the equilibrium favors the mono-, di-hydroxyl products under these conditions. The interaction of buffer with complex **3** also leads to a decreased intensity of signals corresponding to the dichloro-analog and still there was some residual signal which led us to believe that the reactivity towards hydrolysis was less compared to complex **2**. Based on the time taken for the disappearance of the proton signals corresponding to the dichloro-analog after adding buffer to the complexes, the qualitative rate of hydrolysis for the complexes is **2** > **3** > **4** > **1**. The ^{1}H NMR studies also indicate that a higher concentration of phosphate buffer influences the rate of hydrolysis by increasing the conversion to mono-/di-analog.

3.5. Interaction of Au(III) polypyridyl complexes with biologically relevant reducing agents

Clinical application of cisplatin to a wider array of cancers has been hampered by cellular resistance to the agent. It is presumed that a primary mode of drug resistance was due to the scavenging behavior of reduced glutathione (GSH). Although attack of cisplatin on DNA is the accepted cause for antitumor activity [65], cisplatin has the potential to interact with other biomolecules, particularly sulfur-containing compounds. The normal intracellular concentration of the tripeptide GSH ranges from 5 to 10 mM [66]. At this concentration, the direct coordination of platinum with GSH is a contributor to cellular drug resistance. It is well established that the A2780 cell line will exhibit resistance to cisplatin after several passages of continued exposure, presumably due to enhanced production of GSH. Being that our square planar Au(III) complexes are both isoelectronic and isostructural with Pt(II), we

Table 3
Change in chemical shift values [δ (ppm)] upon the interaction of gold(III) polypyridyls with physiological buffer

Compound	Protons	Dichloro species	Monohydroxy species	Dihydroxy species
	H_2 and H_9	9.76	9.52	9.33
	H_4 and H_7	9.41	9.28	9.13
	H_5 and H_6	8.58	8.50	8.40
	H_3 and H_8	8.51	8.37	8.25
	H_2 and H_{11}	10.30	9.85	9.62
	H_4 and H_9	9.87	9.50	9.24
	H_6 and H_7	9.81	9.05	9.20
	H_3 and H_{10}	8.85	8.40	8.26
	H_2 and H_{13}	9.83	9.72	9.65
	H_4 and H_{11}	9.36	9.25	9.18
	H_6 and H_9	8.42	8.37	8.30
	H_7 and H_8	8.34	8.28	8.20
	H_3 and H_{12}	8.16	8.10	8.06
	H_2 and H_{13}	10.08	9.89	9.68
	H_4 and H_{11}	9.91	9.63	9.42
	H_3 and H_{12}	8.68	8.63	8.58
	$H_{6(A,B)}$ and $H_{9(A,B)}$	3.49	3.45	3.42
	$H_{7(A,B)}$ and $H_{8(A,B)}$	2.41	2.38	2.36

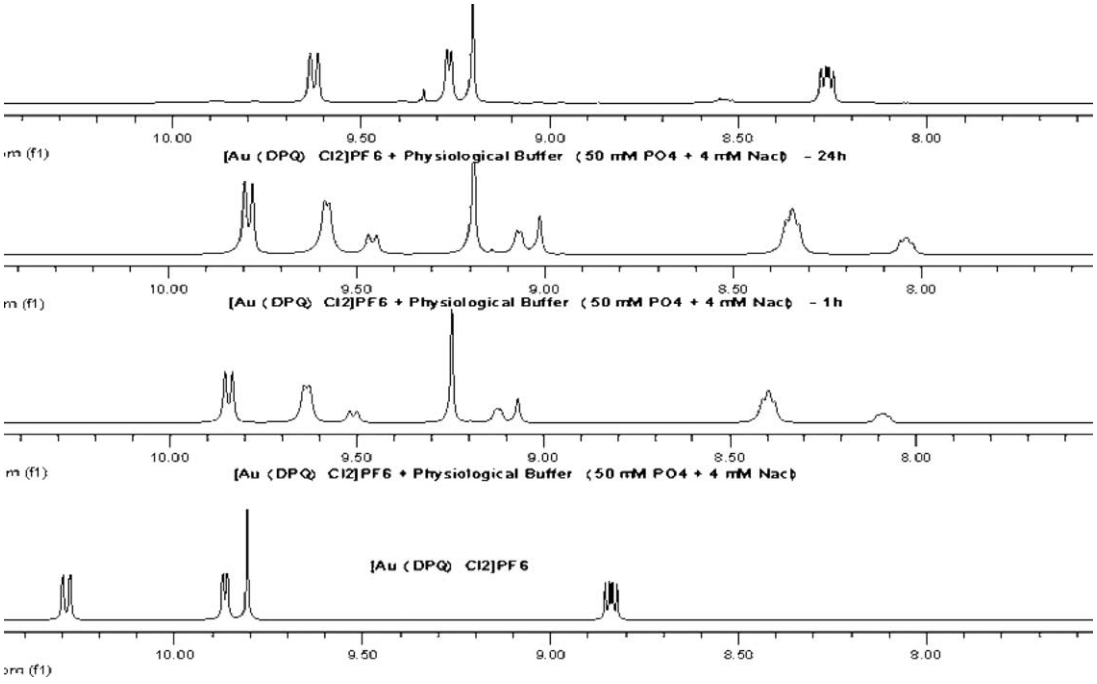


Fig. 2. NMR spectrum depicting the interaction of $[Au(DPQ)Cl_2]PF_6$ in DMSO with physiological buffer.

examined the stability of these compounds toward the common biological reducing agents; glutathione and sodium ascorbate.

Any binding of Au(III) to GSH through the thiol group was followed by shifts in the protons bound to the carbon atom attached to the sulfhydryl group of the cysteinyl residue (Table 4). NMR spectra for the equimolar mixture of **2** and GSH/GSSG were obtained in DMSO-d_6. The difference in the NMR spectra between GSH and GSSG is the methylene group (β-CH_2) bonded to the sulfhydryl group of the cysteinyl residue. The adjacent carbon atom, the α-C atom, and its methine proton were also shifted

Table 4
^{1}H NMR chemical shift values for the interaction of $[Au(DPQ)Cl_2]PF_6$ with glutathione

Protons	GSH	AuDPQ-GSH	GSSG	AuDPQ-GSSG
Cys β-CH_2	2.95–2.93	3.33–3.29 3.13–3.06 3.03–2.93 2.83–2.77	2.95–2.93 3.14–3.01	2.84–2.78 3.14–3.10
Cys α-CH	3.83	3.83	3.41–3.38	3.96–3.93

but to a reduced extent. The two protons β-CH_2 protons of GSH appeared as closely spaced multiplets at δ 2.95–2.93 ppm. One of the two β-CH_2 protons of GSSG appeared as multiplet in an identical position, δ 2.95–2.93, while the other gave a multiplet at δ 3.14–3.01 ppm.

Upon addition of **2** to GSH, the multiplet corresponding to the β-CH_2 protons split into four multiplets and two of them experience an upfield shift, whereas the α-CH signal remains the same. This observation is suggestive of the interaction of sulfhydryl groups with Au(III). While adding **2** to GSSG, a reverse type of effect was observed when compared to the GSH. The position of β-CH_2 protons remain almost the same, whereas the α-CH signal experience an upfield shift by about 0.6 δ. These studies indicate that **2** interacts in a differing fashion with the reduced and oxidized forms of the tripeptide based on the appropriate chemical shift values for the cysteine α and β carbon protons.

Studies were also conducted to monitor the reaction of complexes **1**–**4** in the presence of the reducing agent, sodium ascorbate. Addition of sodium ascorbate to complexes **1**–**4** dissolved in a small amount d^6-DMSO and diluted with D_2O produced visible precipitation of metallic gold almost immediately. The ^{1}H NMR displayed only peaks for that of the free ligand in all cases signifying the complete removal of Au(III) from the coordination sphere upon addition of sodium ascorbate. While the bidentate nature of these ligands appears to stabilize the $[Au(ligand)]^{3+}$ in aqueous solution, it appears that it does little to afford enhanced redox stability toward common biological reductants. Interestingly, this had little negative effect on the biological activity of these compounds in the A2780 human ovarian cancer cell line.

3.6. Cytotoxicity studies

The cytotoxicity of **1**–**4** were evaluated in an adherent ovarian cancer cell line (A2780) using cisplatin (alkylating agent standard), sodium tetrachloroaurate dihydrate (Au^{III} standard), and polypyridyl ligands as standards. The results from these experiments are summarized as bar chart and presented in Fig. 3 and show that **1**–**4** exhibit a measurable cytotoxic effect when compared to the standards. It should be mentioned that some inherent issues resulted from the use of the live/dead cell assay. The dead cell reagent, ethidium homodimer, gave values co-relatable to the live cell reagent in the case of positive and negative controls but not for the test compounds. This observation can be explained clearly by considering the mechanism of fluorescence emission by the dye. The live cell reagent Calcein AM is an electrically neutral, non-fluorescent, esterase substrate that diffuses into the live cells and becomes enzymatically cleaved by ubiquitous cytoplasmic esterases. This releases the free calcein fluorophore that is retained inside live cells. In contrast, ethidium homodimer is a polar nucleic acid stain that can penetrate dead, but not live cell membranes. Once intercalated into nucleic acid, it

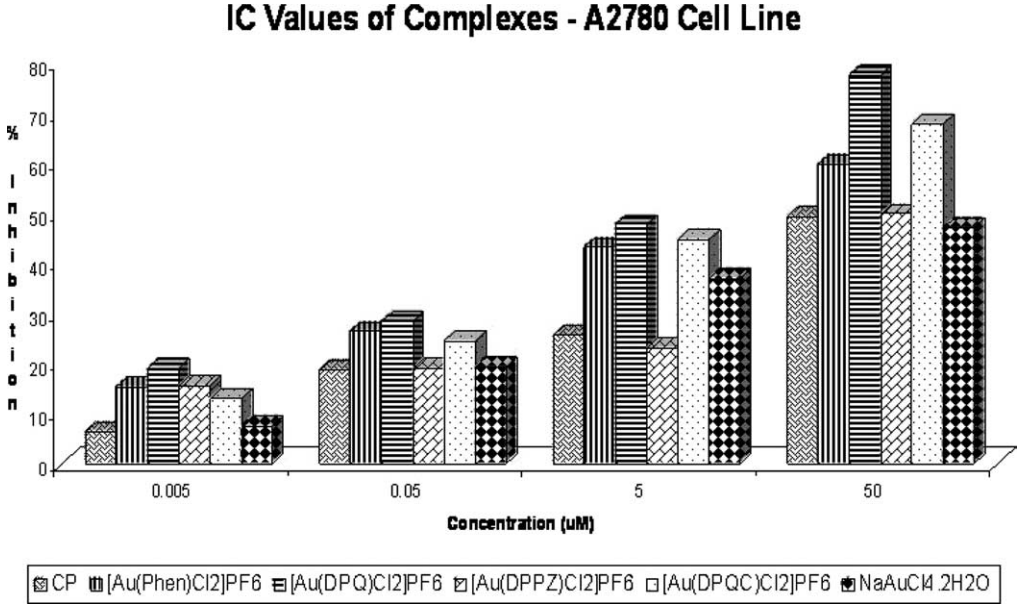


Fig. 3. Percent inhibition of compounds tested against A-2780 cell line.

produces 40-fold increase in red fluorescence. Since these test compounds contain the ligand species DPQ [67,48,68–70], DPPZ [71–80], and DPQC [68,70,81–83] that are well known to intercalate, the interference by them is expected. Accordingly, the dead cell reagent use was terminated due to competition with test compounds. The observed IC_{50} values suggest that changes in the extended aromatic array did not appear to substantially increase complex cytotoxicity. Our initial hypothesis is that these complexes may demonstrate differing degrees of cytotoxicity based on two primary factors: (a) their ability to effectively intercalate into cellular DNA and (b) the effect of the ligand on cellular uptake of the Au(III) complex. To attempt to better understand the role of each of these factors, DNA binding affinity and intracellular concentration dependence studies for **1**–**4** are underway. Until thorough work is completed, we can only assume that the mechanism of cytotoxic action of these agents is due to some combination of DNA intercalation and redox activity of the Au(III) center. Since limited cell kill rates were observed with $NaAuCl_4$ or the ligands alone, we further conclude that the $[Au(ligand)]^{3+}$ species plays an important role in the antitumor activity of this series.

4. Summary

These novel Au(III) complexes are isoelectronic and isostructural with clinically important Pt(II) complexes. This provides motivation for investigation of these compounds as potential candidates through which Pt(II) drug resistance may be overcome. If the compound undergoes rapid transformation within the physiological environment, it may be inferred that their cytotoxicity is not the direct influence of the Au(III) complexes but is probably mediated by some active metabolite, possibly in a reduced form. On the other hand, extensive stabilization of Au(III) center by a polydentate ligand such as cyclam leads to reduction or even loss of the biological activity. Our future study of Au(III) as a potential anticancer agent must address these fundamental issues in order to further understand how it is that these complexes exert their cytotoxic activity.

Single crystal XRD studies demonstrate that the isolated Au(III) complexes have square planar symmetry and the polypyridyl ligands form a six-membered chelate ring in which nitrogen atoms are bonded to the metal. Solution studies have shown that this ligand configuration appears to impart suitable stability of the $[Au(polypyridyl)]^{3+}$ fragment under aqueous physiological conditions. Complexes **1**–**4** demonstrate significant cytotoxicity against a common ovarian cancer cell line with IC_{50} values comparable to that observed for cisplatin. Though we have improved our understanding of the synthesis, aqueous stability, and cytoxicity of these compounds, many new questions have arisen. Our current hypothesis is that the cytotoxic activity imparted by complexes **1**–**4** may be due to the intercalation of the compounds with DNA and/or reactivity with proteins through a Au(III) → Au(I) mediated redox process. Studies are currently underway to investigate the manner by which these complexes kill cells (i.e., apoptosis versus necrosis) and the extent that they bind to DNA. We are also examining how cellular uptake of these and other Au(III) compounds directly correlates with cytotoxicity.

Acknowledgments

The authors thank The University of Wyoming Faculty Grant-in-aid and NIH/NCRR BRIN Grant #RR-16474 for financial support. We thank Dr. Nair Sreejayan for generous assistance with the A2780 cytotoxicity studies.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ica.2005.08.030.

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