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Synthesis, characterization and cytotoxicity of gold(III) complexes with deprotonated pyridyl carboxamide

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Six new gold(III) complexes [Au(bzpam)Cl_2] (1, bzpamH = *N*-benzyl picolinamide), [Au(hetpam)Cl_2] (2, hetpamH = *N*-(2-hydroxyethyl) picolinamide), [Au(pypam)Cl]$AuCl_4$ (3, pypamH = *N*-(pyridin-2-ylmethyl) picolinamide), [Au(dmepam)Cl]$AuCl_4$ (4, dmepamH = *N*-(2-(dimethylamino)ethyl) picolinamide), [Au(bhetpydam)Cl] (5, bhetpydamH_2 = *N*,*N*′-bis(2-hydroxyethyl) pyridine- 2,6-dicarboxamide) and [Au_2(hedam)Cl_4] (6, hedamH_2 = *N*,*N*′-(hexane-1,6-diyl) dipicolinamide) with deprotonated pyridyl carboxamide were synthesized and characterized by elemental analysis, molar conductivity, IR, H^1 NMR and C^{13} NMR techniques. The analytical data showed that deprotonated pyridyl carboxamide coordinated with gold(III) ions through a nitrogen atom. The cytotoxicity against Bel-7402 and HL-60 cell lines was tested by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and SRB (sulforhodamine B) assays. The results indicated that the complexes exerted cytotoxic effects against Bel-7402 and HL-60 cell lines, complex 6 had better cytotoxicity than cisplatin, and complex 3 displayed similar cytotoxicity to cisplatin against Bel-7402 cell line. The results suggested that the characteristics of ligands had an important effect on cytotoxicity of complexes. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: gold(III) complex; deprotonated amide; cytotoxicity

Introduction

Nowadays cisplatin and its derivatives are the most widely used clinical anticancer drugs. However, they have several major drawbacks. Common problems include cumulative toxicities of nephrotoxicity and ototoxicity.[1,2] In addition to the serious side effects, the therapeutic efficacy is also limited by inherent or treatment-induced resistant tumor cells.[3,4] These drawbacks have provided the motivation for alternative chemotherapeutic strategies.

In the search of new therapies avoiding these drawbacks, special attention has been paid to gold(III) complexes because they show chemical features that are very close to those of clinically employed platinum(II) complexes, such as the preference for square-planar coordination and the typical d^8 electronic configuration. However, the high redox potential and relatively poor stability of gold(III) compounds make their use rather problematic under physiological conditions. In recent years, by implementation of appropriate ligand selection strategies, a number of gold(III) compounds have been obtained, exhibiting sufficient stability under physiological-like conditions and manifesting cytotoxic properties *in vitro*. The main types of gold (III) complexes have shown activity as follows: coordination compounds with *N*-polydentate, macrocyclic ligands, bioligands or dithiocarbamato ligands;[5–8] organometallic compounds with an Au-C bond or CNC-pincer backbone.[9–12] Most of the above-mentioned compounds turned out to be highly cytotoxic, with IC_{50} values generally falling in the low micromolar range. Therefore, gold compounds are drawing a great deal of attention within the 'metals in medicine' community owing to their outstanding antiproliferative properties,[13–15] and are being intensely investigated as a rich source of innovative cytotoxic drugs for cancer treatment.[16–18] We previously reported cytotoxic properties of eight gold(III) complexes of 5-aryl-3-(pyridin-2-yl)-4,5-dihydropyrazole-1-carbothioamide (with one Au-S and two Au-N bonds). Five of these complexes have better cytotoxicity than cisplatin against HeLa cell line. The structure and properties of ligands show an important influence on cytotoxicity of complexes.[15]

It has been reported that deprotonated amide as ligands could decrease the redox potential of the gold(III) center and thereby stabilize the gold(III) complexes.[19–22] In order to further explore the structure–activity relationships and discover new metal-based anticancer drugs, the synthesis, characterization and cytotoxicity of six new gold(III) complexes with deprotonated pyridyl carboxamide ligands are described in the present work for the first time. The results of the cytotoxicity experiments

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indicate that the gold(III) complexes with deprotonated pyridyl carboxamide ligands exerted cytotoxic effects against Bel-7402 and HL-60 cell lines, and the structural characteristic of ligands had an influence on cytotoxicity of complexes.

Experimental

Materials

All chemicals and reagents were of analytical grade. RPMI-1640 medium, trypsin and fetal bovine serum were purchased from Gibco. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sulforhodamine B (SRB), benzyl penicillin and streptomycin were from Sigma. Two different human carcinoma cell lines – HL-60 (immature granulocyte leukemia) and Bel-7402 (liver carcinoma) – were obtained from the American Type Culture Collection.

Analytical and Physical Measurements

Elemental analyses were determined on an Elementar Vario EL III elemental analyzer. Conductivity measurements were made on freshly prepared 1×10^{-3} mol l^{-1} solutions in acetonitrile or DMF at room temperature using a DDS-12DW type conductivity meter. IR spectra were recorded using KBr pellets and a PerkinElmer model 683 spectrophotometer. UV–visible spectra at room temperature were measured in acetonitrile solution using a TU-1901 double-beam UV–visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVIII 600 NMR spectrometer. Optical density (OD) was measured on a microplate spectrophotometer (Bio-Rad Model 680, USA).

Preparation of Ligands and Gold(III) Complexes

Ligands **L1–L6** were prepared as described in the literature.[23–27] Gold(III) complexes were prepared by the reaction of pyridyl carboxamides with K[$AuCl_4$]·H_2O in a mixture of C_2H_5OH/H_2O (v/v, 1:1).

*[Au(bzpam)Cl_2] (**1**)*

To a rapidly stirred solution of K[$AuCl_4$].H_2O (40 mg, 0.1 mmol) in 1.5 ml H_2O, the solution of **L1** (21 mg, 0.1 mmol) in 1.5 ml EtOH was added. The mixture was heated to 70 °C and kept for 6 h, and the resulting red precipitate was filtered. The collected solid was recrystallized in acetonitrile to give **1**. Red solid; yield 77.6%; IR (KBr, cm^{-1}) 3101–3056 (C-H, py), 1639 (C = O), 1633 (C = C), 1604 (C = N), 421 (Au-N); UV (acetonitrile, nm) 200 (C = O), 208 (C = N), 260 (C = C), 310 (LMCT); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.31 (d, J = 6.0 Hz, 1 H, 6-H, py), 8.52 (dd, J = 7.8 Hz, 7.8 Hz, 1 H, 4-H, py), 8.04 (dd, J = 7.8 Hz, 6.0 Hz, 1 H, 5-H, py), 8.01 (d, J = 7.8 Hz, 1 H, 3-H, py), 7.40 (d, J = 7.8 Hz, 2 H, 2′,6′-H, ph), 7.25 (dd, J = 7.8 Hz, 7.8 Hz, 2 H, 3′,5′-H, ph), 7.20 (dd, J = 7.8 Hz, 7.8 Hz, 1 H, 4′-H, ph), 4.74 (s, 2 H, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ 49.5 (CH_2), 126.5 (4′-C, ph), 127.0 (2′,6′-C, ph), 128.0 (3′,5′-C, ph), 128.3 (3-C, py), 130.0 (5-C, py), 139.3 (1′-C, ph), 145.0 (4-C, py), 145.1 (6-C, py), 148.4 (2-C, py), 171.1 (-CON-); Anal. Calcd for $C_{13}H_{11}N_2OAuCl_2$: C, 32.57; H, 2.30; N, 5.84. Found: C, 32.55; H, 2.18; N, 5.64; Λ_m (acetonitrile, S m^2 mol^{-1}) 12.2.

*[Au(hetpam)Cl_2] (**2**)*

To a rapidly stirred solution of K[$AuCl_4$]·H_2O (40 mg, 0.1 mmol) in 1.5 ml H_2O the solution of **L2** (17 mg, 0.1 mmol) in 1.5 ml EtOH was added. The mixture was heated to 70 °C and kept for 6 h, and the resulting red precipitate was filtered. The collected solid was recrystallized in acetonitrile to give **2**. Red solid; yield 77.4%; IR (KBr, cm^{-1}) 3730 (O-H), 3109–3030 (C-H, py), 1639 (C = O), 1633 (C = C), 1604 (C = N), 418 (Au-N); UV (acetonitrile, nm) 205 (C = O), 212 (C = N), 260 (C = C), 310 (LMCT); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.31 (d, J = 6.0 Hz, 1 H, 6-H, py), 8.51 (dd, J = 7.8 Hz, 7.8 Hz, 1 H, 4-H, py), 8.03 (dd, J = 7.8 Hz, 6.0 Hz, 1 H, 5-H, py), 7.99 (d, J = 7.8 Hz, 1 H, 3-H, py), 4.69 (brs, 1 H, OH), 3.57 (t, J = 6.0 Hz, 2 H, NCH_2), 3.52 (t, J = 6.0 Hz, 2 H, CH_2O); ^{13}C NMR (150 MHz, DMSO-d_6) δ 49.0 (-N-CH_2-), 59.9 (-CH_2OH), 128.6 (3-C, py), 130.4 (5-C, py), 145.4 (4-C, py), 145.5 (6-C, py), 149.3 (2-C, py), 171.5 (-CON-); Anal. Calcd for $C_8H_9N_2O_2AuCl_2$: C, 22.19; H, 2.09; N, 6.47. Found: C, 22.11; H, 2.03; N, 6.12; Λ_m (acetonitrile, S m^2 mol^{-1}) 3.1.

*[Au(pypam)Cl]$AuCl_4$ (**3**)*

To a rapidly stirred solution of K[$AuCl_4$]·H_2O (80 mg, 0.2 mmol) in 1.5 ml H_2O, the solution of **L3** (21 mg, 0.1 mmol) in 1.5 ml EtOH was added. The mixture was heated to 70 °C and kept for 6 h, and the resulting yellow precipitate was filtered. The collected solid was recrystallized in acetonitrile to give **3**. Yellow solid; yield 79.4%, IR (KBr, cm^{-1}): 3103–2927 (C-H, py), 1665 (C = O), 1635 (C = C), 1607 (C = N), 411 (Au-N); UV (acetonitrile, nm): 198 (C = O), 214 (C = N), 263 (C = C), 320 (LMCT); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.13 (d, J = 6.0 Hz, 1 H, 6-H, pyridyl carboxamide), 9.04 (d, J = 6.0 Hz, 1 H, 6′-H, py), 8.64 (dd, J = 7.8 Hz, 6.0 Hz, 1 H, 4-H, pyridyl carboxamide), 8.50 (dd, J = 7.8 Hz, 6.0 Hz, 1 H, 4′-H, py), 8.15 (td, J = 8.4 Hz, 1.2 Hz, 1 H, 5-H, pyridyl carboxamide), 8.12 (dd, J = 7.8 Hz, 7.8 Hz, 1 H, 5′-H, py), 8.08 (d, J = 7.8 Hz, 1 H, 3-H, pyridyl carboxamide), 8.02 (d, J = 7.8 Hz 1 H, 3′-H, py), 5.40 (s, 2 H, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ 40.5 (CH_2), 123.5 (2 C, 3,3′-C, py), 128.9 (2 C, 5,5′-C, py), 139.2 (2 C, 4,4′-C, py), 148.6 (2 C, 6,6′-C, py), 149.5 (2 C, 2,2′-C, py), 162.2 (-CON-); Anal. Calcd for $C_{12}H_{10}N_3OAu_2Cl_5$: C, 18.40; H, 1.29; N, 5.36. Found: C, 18.48; H, 1.04; N, 4.89; Λ_m (acetonitrile, S m^2 mol^{-1}) 145.4.

*[Au(dmepam)Cl]$AuCl_4$ (**4**)*

To a rapidly stirred solution of K[$AuCl_4$]·H_2O (80 mg, 0.2 mmol) in 1.5 ml H_2O, the solution of **L4** (19 mg, 0.1 mmol) in 1.5 mL EtOH was added. The mixture was heated to 70 °C and kept for 6 h, and the resulting yellow precipitate was filtered. The collected solid was recrystallized in acetonitrile to give **4**. Yellow solid; yield 76.9%; IR (KBr, cm^{-1}): 3116–2928 (C-H, py), 1671 (C = O), 1659 (C = C), 1607 (C = N), 422 (Au-N); UV (acetonitrile, nm): 195 (C = O), 214 (C = N), 265 (C = C), 314 (LMCT); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.00 (d, J = 6.0 Hz, 1 H, 6-H, py), 8.59 (dd, J = 7.8 Hz, 7.8 Hz, 1 H, 4-H, py), 8.06 (dd, J = 7.8 Hz, 6.0 Hz, 1 H, 5-H, py), 8.04 (d, J = 7.8 Hz, 1 H, 3-H, py), 3.66 (t, J = 6.0 Hz, 2 H,-$CONCH_2$-), 3.61 (t, J = 6.0 Hz, 2 H,-CH_2NMe_2), 3.25 (s, 6 H, $2CH_3$); ^{13}C NMR (150 MHz, DMSO-d_6) δ 41.1 (CH_2), 43.4 (CH_3), 57.0 (CH_2), 128.9 (3-C, py), 130.7 (5-C, py), 145.5 (4-C, py), 145.8 (6-C, py), 149.8 (2-C, py), 172.2 (-CON-); Anal. Calcd for $C_{10}H_{14}N_3OAu_2Cl_5$: C, 15.73; H, 1.85; N, 5.50. Found: C, 16.15; H, 1.76; N, 5.30; Λ_m (acetonitrile, S m^2 mol^{-1}) 155.2.

*[Au(bhetpydam)Cl] (**5**)*

To a rapidly stirred solution of K[$AuCl_4$]·H_2O (40 mg, 0.1 mmol) in 1.5 ml H_2O, the solution of **L5** (25 mg, 0.1 mmol) in 1.5 ml EtOH was added. The mixture was heated to 70 °C and kept for 6 h, and the resulting yellow precipitate was filtered. The collected solid was recrystallized in acetonitrile to give **5**. Yellow solid; yield 75.8%; IR (KBr, cm^{-1}): 3340 (O-H), 3074–3051 (C-H, py), 1661 (C = O),

1636 (C = C), 1604 (C = N), 428 (Au-N); ^{1}H NMR (600 MHz, DMSO-d^6) δ 8.58 (dd, J = 7.8 Hz, 7.8 Hz, 1 H, 4-H, py), 8.00 (d, J = 7.8 Hz, 2 H, 3,5-H, py), 4.69 (t, J = 6.0 Hz, 2 H, 2OH), 3.59 (t, J = 6.8 Hz, 4 H, 2CH_2), 3.52 (dd, J = 12.8, 6.8 Hz, 4 H, 2CH_2); ^{13}C NMR (150 MHz, DMSO-d$_6$) δ 49.0 (NCH_2), 60.0 (CH_2OH), 128.2 (2 C, 3,5-C, py), 146.5(4-C,py), 147.1(2 C, 2,6-C, py), 171.0 (-CON-); Anal. Calcd for $C_{11}H_{13}N_3O_4AuCl$: C, 27.32; H, 2.71; N, 8.69. Found: C, 27.01; H, 2.36; N, 8.46; Λ_m (acetonitrile, S m^2 mol^{-1}) 4.2.

*[Au_2(hedam)Cl_4] (**6**)*

To a rapidly stirred solution of K[$AuCl_4$]·H_2O (80 mg, 0.2 mmol) in 1.5 ml H_2O, the solution of **L6** (26 mg, 0.1 mmol) in 1.5 ml EtOH was added. The mixture was heated to 70 °C and kept for 6 h, and the resulting red precipitate was filtered. The collected solid was recrystallized in acetonitrile to give **6**. Red solid; yield 82.6%; IR (KBr, cm^{-1}): 3108–2932 (C-H, py), 1641 (C = O), 1629 (C = C), 1602 (C = N), 423 (Au-N); UV (acetonitrile, nm): 203 (C = O), 209 (C = N), 268 (C = C), 304 (LMCT); ^{1}H NMR (600 MHz, DMSO-d$_6$) δ 9.29 (d, J = 6.0 Hz, 2 H, 6-H, py), 8.50 (dd, J = 7.8 Hz, 7.8 Hz, 2 H, 4-H, py), 8.01 (dd, J = 7.8 Hz, 6.0 Hz, 2 H, 5-H, py), 7.97 (d, J = 7.8 Hz, 2 H, 3-H, py), 3.44 (t, J = 7.2 Hz, 4 H, 1′-H, 2CH_2), 1.58 (s, 4 H, 2′-H, 2CH_2), 1.33 (s, 4 H, 3′-H, 2CH_2); ^{13}C NMR (150 MHz, DMSO-d$_6$) δ 26.6 (3′-C, CH_2), 30.3 (2′-C, CH_2), 47.1 (1′-C, CH_2), 128.6 (3-C, py), 130.3 (5-C, py), 145.5 (4-C, py), 145.6 (6-H, py), 149.4 (2-C, py), 171.0 (-CON-); Anal. Calcd for $C_{18}H_{20}N_4O_2Au_2Cl_4$: C, 25.14; H, 2.34; N, 6.51. Found: C, 25.47; H, 2.38; N, 6.30; Λ_m (DMF, S m^2 mol^{-1}) 4.5.

Cytotoxic Studies

Cell culture

Two different human carcinoma cell lines – HL-60 and Bel-7402 – were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Solutions

The complexes were dissolved in DMSO at a concentration of 5 mmol l^{-1} as stock solution, and diluted in culture medium at concentrations of 1.0, 10, 100, and 500 μmol l^{-1} as working solution. To avoid DMSO toxicity, the concentration of DMSO was less than 0.1% (v/v) in all experiments.

Cytotoxicity analysis

The cells harvested from exponential phase were seeded equivalently into a 96-well plate, and then the complexes were added to the wells to achieve final concentrations. Control wells were prepared by addition of culture medium. Wells containing culture medium without cells were used as blanks. All experiments were performed in quintuplicate. The MTT assay was performed as previously described for HL-60.[28] Upon completion of the incubation for 44 h, stock MTT dye solution (20 ml, 5 mg ml^{-1}) was added to each well. After 4 h incubation, 2-propanol (100 ml) was added to solubilize the MTT formazan product. The OD of each well was measured on a microplate spectrophotometer at a wavelength of 570 nm. The IC_{50} value was determined from the plot of percent viability against dose of compounds added. The SRB assay was performed as previously described for Bel-7402.[29] Upon completion of the incubation for 44 h, the cells were fixed in 10% trichloroacetic acid (100 ml) for 30 min at 4 °C, washed five times and stained with 0.1% SRB in 1% acetic acid (100 ml) for 15 min. The cells were washed four times in 1% acetic acid and air-dried. The stain was solubilized in 10 mmol l^{-1} unbuffered Tris base (100 ml) and OD was measured at 492 nm as above.[30] The IC_{50} value was determined from plot of percent viability against dose of compounds added.

Results and Discussion

Synthesis and Characterization

The ligands **L1–L6** (as shown in Fig. 1) were prepared following reported procedures.[23–27] Their structure and purity were established by comparing their melting points, IR and NMR data with those reported in the literature.

Gold(III) complexes (**1–6**) with deprotonated pyridyl carboxamide were synthesized as described in the Experimental section (as shown in Scheme 1). Treatment of the free pyridyl carboxamide ligands (**L1–L4**) with K[$AuCl_4$].H_2O in a C_2H_5OH–H_2O mixture affords complexes (**1–4**) in ~80% yield (Scheme 1, Route 1). Reaction of bis(pyridyl) carboxamide (**L5**) with K[$AuCl_4$]·H_2O affords complex **5** in ~83% yield (Scheme 1, Route 2). Using dipyridyl carboxamide (**L6**) as ligand affords complex **6** in ~83% yield (Scheme 1, Route 3). Complexes **1** and **2** contain two Au-N bonds, complexes **3**, **4** and **5** contain three Au-N bonds, whereas complex **6** is a binuclear complex. When the ratio of ligand to $KAuCl_4$ was 2:1, 1:1 or 1:2, the mixture of **L3** or **L4** with K[$AuCl_4$]·H_2O produced the same gold(III) complex (**3** or **4**) with anion [$AuCl_4$]$^-$. The excess ligand in the reaction mixture was found by thin-layer chromatography when the ratio of ligand to $KAuCl_4$ was 2:1 or 1:1. Heating the gold(III) complex **3** (or **4**) with KPF6 in water (or alcohol), or heating **L3** (or **L4**), K[AuCl4]·H_2O with KPF_6 in a C_2H_5OH/H_2O mixture, pure gold(III) complex was not isolated as their PF_6- salt.

The complexes were soluble in DMF, DMSO and acetonitrile, poorly soluble in MeOH, and insoluble in some common organic solvents. Attempts to obtain a single crystal suitable for X-ray determination were unsuccessful. Structural analogies of the title compounds, [Au(Quinpy)Cl]Cl, [Au(Quingly)Cl]Cl and [Au(Quinala)Cl]Cl with three Au-N bonds (where Hquinpy = *N*-(8-quinolyl) pyridine-2-carboxamide; Hquingly = *N*-(8-quinolyl)-glycine-carboxamide; Hquinala = *N*-(8-quinolyl)-L-alanine-carboxamide) were

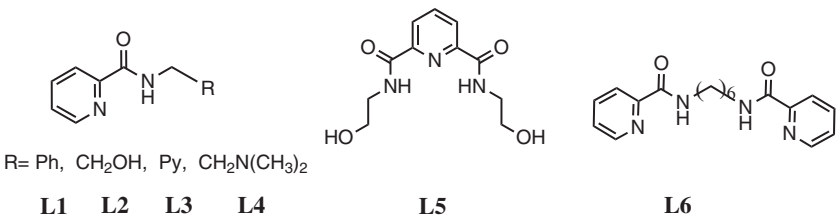
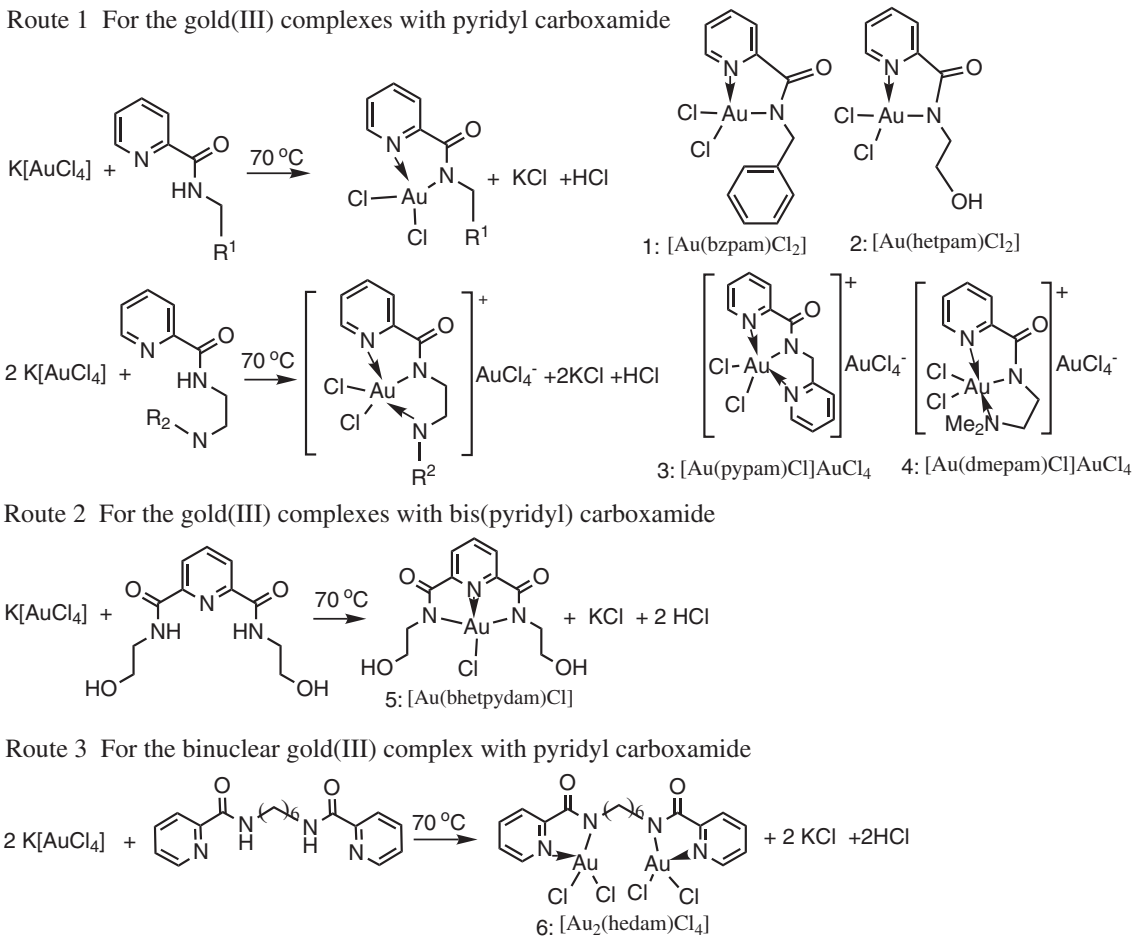


Figure 1. Structures of the ligands



Scheme 1. Synthetic routes for the gold(III) complexes with deprotonated amide

synthesized by Yang *et al.*[19] The crystal structures of complexes [Au(Quingly)Cl]Cl and [Au(Quinala)Cl]Cl revealed that gold(III) is coordinated by three nitrogen atoms from the ligand, one of which is deprotonated amide nitrogen. Hill *et al.*[31] synthesized a dichloro(pyridine-2-carboxamido-*N*1,*N*2) gold(III) complex. Its X-ray crystal structure showed that the ligand had been deprotonated after complexation.

The structures of the synthesized gold(III) complexes were established with the help of elemental analyses data, and IR, UV and NMR spectra.

As shown in Table 1, elemental analysis data of the complexes are in good agreement with the calculated values. The molar conductance values in acetonitrile or DMF for the complexes **1**, **2**, **5** and **6** correspond to non-electrolyte, while complexes **3** and **4** correspond to 1:1 electrolyte type.[32] This can be accounted for the coordination of gold (III) with different nitrogen atoms..

Comparison of the IR spectra of the ligands and complexes provided information about the mode of bonding of the ligands in gold(III) complexes. The significant data are given in Table 2. The bands of $u_{C\,O}$ (amide) in 1677–1657 cm^{-1} shifted to 1671–1639 cm^{-1} after complexation. The stretching frequency u_{NH} of amide which appeared at 3382–3300 cm^{-1} in the spectra of free ligands disappeared in the spectra of all gold complexes. In addition, the complexes exhibited new bands assigned to $u_{Au\text{-}N}$ appeared at 424–411 cm^{-1}, indicating that the ligands had been deprotonated after complexation. The bands of $u_{C\,N}$ (pyridyl) at 1640–1617 cm^{-1} shifted to a lower frequency by 15–36 cm^{-1}, and the strong and sharp bands of $u_{C\text{-}H}$ (pyridyl) at 3082–2853 cm^{-1} shifted to 3116–

Table 1. Analytical and physical data of the complexes

Complex	Formula	Color	Found (calculated) (%)			Molar conductance ($S\,cm^2\,mol^{-1}$)
			C	H	N	
1	$C_{13}H_{11}AuCl_2N_2O$	Red	32.55 (32.59)	2.18 (2.31)	5.64 (5.85)	Acetonitrile, 12.2
2	$C_8H_9AuCl_2N_2O_2$	Red	22.11 (22.19)	2.03 (2.09)	6.12 (6.47)	Acetonitrile, 3.1
3	$C_{12}H_{10}Au_2Cl_5N_3O$	Yellow	18.48 (18.40)	1.04 (1.29)	4.89 (5.36)	Acetonitrile, 145.4
4	$C_{10}H_{14}Au_2Cl_5N_3O$	Yellow	16.15 (15.73)	1.76 (1.85)	5.30 (5.50)	Acetonitrile, 155.2
5	$C_{11}H_{13}AuClN_3O_4$	Yellow	27.01 (27.32)	2.36 (2.71)	8.46 (8.69)	Acetonitrile, 4.2
6	$C_{18}H_{20}Au_2Cl_4N_4O_2$	Red	25.47 (25.14)	2.38 (2.34)	6.30 (6.51)	Dimethyllformamide, 4.5

Table 2. Infrared spectral data of ligand and its complexes (cm^{-1})

Compound	ν(N-H)	ν(C-H, Py)	ν(C O)	ν(C N, Py)	ν(Au-N)	ν(O-H)
L1	3300	3082–3026	1667	1640		
L2	3366	3093–3015	1657	1628		3730
L3	3369	3058–2853	1677	1620		
L4	3382	3045–2853	1677	1634		
L5	3282	2968–2856	1665	1617		3407
L6	3372	3071–2920	1657	1617		
1		3101–3056	1639	1604	421	
2		3109–3030	1639	1604	418	3730
3		3103–2927	1665	1607	411	
4		3116–2928	1671	1607	422	
5		3074–3051	1661	1604	428	3340
6		3108–2932	1641	1602	423	

2927 cm^{-1}, suggesting that pyridyl coordinated with gold through nitrogen atom.[33] The band of $u_{Au\text{-}Cl}$ is not observed in far-infrared region.[11] Hill *et al.* reported that the bands of $u_{C\,O}$ (amide) in 1678 cm^{-1} shifted to 1650.8 cm^{-1} after complexation.[31]

The UV–visible spectra of ligands and gold(III) complexes in acetonitrile were measured. At a concentration of 1×10^{-4} mol l^{-1}, three main absorption peaks at about 196, 220 and 262 nm for ligands are assigned to C O, C N and C C, respectively. The peak at 220 nm blue shifts by about 8 nm, indicating that py coordinates with gold(III) ion through a nitrogen atom. Gold(III) complexes give high-intensity bands at about 310 nm which can be assigned to ligand–metal charge transfer.

The ^{1}H NMR spectra of ligands and gold(III) complexes were recorded in DMSO-d_6. All the protons were found to be in their expected regions and numbers of protons calculated from the integration curves agreed with those obtained from the values of C, H, and N element analyses. In comparison ^{1}H NMR of the gold(III) complexes with that of the free ligands, a broad signal of-CONH- (at 8.05–8.77 ppm) disappeared in the complexes, indicating that the ligands had been deprotonated due to coordination with gold ion. For example, the proton signal at 8.77 ppm attributed to-CONH- (1 H) of **L1** disappeared in complex **1**.

The chemical shift of the protons which are near the coordinated pyridine nitrogen shifted 0.07–0.54 ppm down-field compared to free ligands.

The ^{13}C NMR (DMSO-d_6) spectra further provide support for the structure of the complexes, e.g. chemical shifts of complex **1** differing from **L1** (Fig. 2). The chemical shift of the carbon of-C O (at 159.1 ppm in pyridyl carboxamide) moves down-field by 12.0 ppm; the chemical shift of the carbon of C2 (py, at 144.9 ppm in pyridyl carboxamide) moves down-field by 3.5 ppm; and the chemical shift of the carbon of-NH**C**H_2Ph (at 38.8 ppm in pyridyl carboxamide) moves down-field by 10.7 ppm after complexation. A similar result was obtained by Hill *et al.*[31] They reported that the chemical shift of the carbon of-CONH- (at 166.89 ppm in picolinamide) shifted 4.38 ppm down-field, and the chemical shift of the carbon of C2 (py, at 150.81 ppm picolinamide) shifted 11.66 ppm down-field after complexation. The conclusions drawn from these studies lend further support to the mode of bonding discussed in IR spectra.

Based on all the above physical and spectral studies, we propose a tentative structure for the complexes as shown in Fig. 3.

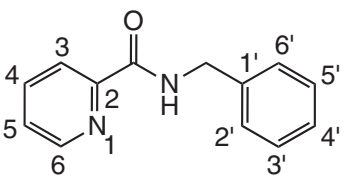


Figure 2. Numbering of carbons of **L1**

Cytotoxic Studies

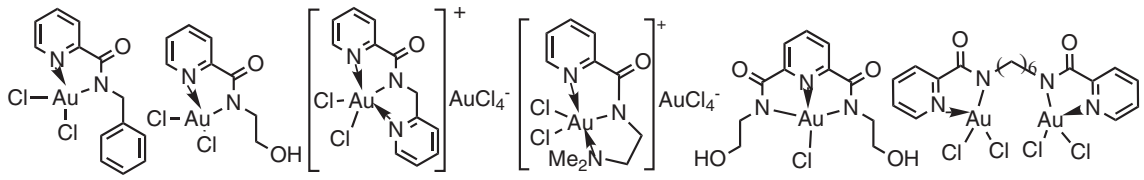
Previous investigations had already revealed favorable cytotoxic properties for some gold(III) complexes. However, the data present in the literature about gold(III) complexes with deprotonated amide ligands are still very scarce. Hill *et al.* synthesized and characterized an *N,N′*-bis-coordinated gold(III) aurocycle.[31] However, they did not evaluate the cytotoxic activity of the gold(III) complex. Yang *et al.*[19] reported three gold(III) complexes ([Au (Quinpy)Cl]Cl, [Au(Quingly)Cl]Cl and [Au(Quinala)Cl]Cl) with deprotonated amide ligands, which were tested against B16-BL6, P388, HL-60, A-549 and BEL-7402 cell lines. The cytotoxicity of [Au(Quinpy)Cl]Cl against A549 cells is about three times higher than that of cisplatin. [Au(Quinala)Cl]Cl is active against B16-BL6 with an inhibition rate of 67.52% at a concentration of 10^{-7} mol L^{-1}.

In the present work, in the search for novel gold compounds as potential anticancer treatments, we synthesized two gold(III) complexes (**1** and **2**) containing two Au-N bonds, three complexes (**3**, **4** and **5**) containing three Au-N bonds, and a binuclear complex (**6**). The cytotoxicity of ligands and their metal complexes was tested by MTT and SRB assays, and the results obtained with gold(III) complexes are presented in Table 3. The corresponding ligands had no cytotoxicity against Bel-7402 and HL-60 cell lines ($IC_{50} > 200$ μmol l^{-1}), whereas the gold(III) complexes exerted cytotoxic effects against Bel-7402 and HL-60 cell lines. Most complexes showed lower IC_{50} values (<20 μmol l^{-1}); complex **6** had better cytotoxicity than cisplatin; complex **3** displayed similar cytotoxicity to cisplatin against Bel-7402 cell line; complex **3** also showed the highest cytotoxic activity against HL-60 cell line among the six complexes. Complexes **1**, **3** and **6** exhibited comparable cytotoxicity to cisplatin, and they demonstrated similar or better cytotoxicity than that of gold(III) compounds containing bis(pyridyl) carboxamide ligands (IC_{50} = 10–30 μM)[34] and [PtL_2Cl] (where HL = *N*-(4-methylphenyl)-2-pyridine-carboxamide). In HL-60, Bel-7402, P388 and A-549 cell lines, the growth inhibitory rates of [PtL_2Cl] were 81.7%, 47.7%, 99.5% and 87.5%, respectively, at a concentration of 10^{-5} mol l^{-1}.[35]

The experimental results indicated that the characteristics of ligands had an important effect on cytotoxicity of gold(III) complexes. In general, the cytotoxicty of these gold(III) complexes with deprotonated pyridyl carboxamide decreased in sequences as follows: binuclear complex > mononuclear complex; complex wherein R is an aromatic group on picolinamide > complex wherein R is an aliphatic group.

The binuclear complex **6** with two Au-N bonds per unit showed higher cytotoxicity than mononuclear complexes (**1** and **2**) with two Au-N bonds.

For mononuclear complexes with two Au-N bonds, complex **1**, wherein R is an aromatic group on picolinamide, exhibited higher cytotoxicity than complex **2**, wherein R is an aliphatic group. For mononuclear complexes with three Au-N bonds, a similar result is obtained: complex **3**, wherein R is an aromatic group on picolinamide, showed higher cytotoxicity than complexes (**4** and **5**) wherein R is an aliphatic group. A similar result was obtained by Yang *et al.*[19] They reported that [Au(Quinpy)Cl]Cl with



[Au(bzpam)Cl_2] [Au(hetpam)Cl_2] [Au(pypam)Cl]$AuCl_4$ [Au(dmepam)Cl]$AuCl_4$ [Au(bhetpydam)Cl] [Au_2(hedam)Cl_4]

1 **2** **3** **4** **5** **6**

Figure 3. Suggested structures of gold(III) complexes

Table 3. The cytotoxicity of the complexes against HL-60 and Bel-7402 cell lines ($n = 3$)

Complexes	$IC_{50} \pm SD$ ($\mu mol\ l^{-1}$)	
	HL-60	Bel-7402
1	11.47 ± 0.17	11.77 ± 0.23
2	39.93 ± 1.44	17.37 ± 0.24
3	9.45 ± 0.18	8.55 ± 0.18
4	30.39 ± 1.21	14.81 ± 0.22
5	35.43 ± 0.22	13.43 ± 0.18
6	10.47 ± 0.24	6.03 ± 0.14
Cisplatin	2.89 ± 0.18	8.12 ± 0.20

deprotonated pyridyl carboxamide ligand showed better cytotoxicity against the A-549 cell line than [Au(Quingly)Cl]Cl and [Au(Quinala)Cl]Cl) with deprotonated glycine-carboxamide and L-alanine-carboxamide ligands. For cytotoxicity effects mediated largely through the lipophilicity of the ligands, we hypothesize that the hydrophobic nature of aromatic groups in ligands may increase the lipophilicity of the complexes, which will enhance the absorption of the molecule and may enhance bioactivity. Other substituents on the aromatic ring are needed to explore the structure–activity relationship.

Conclusion

In this work we have synthesized six novel Gold(III) complexes with deprotonated pyridyl carboxamide ligands. The formation of complex is dependent on the structure of the ligand. Preliminary cytotoxicity data showed that binuclear complex **6** demonstrated better cytotoxicity than cisplatin; complex **3** displayed similar cytotoxicity to cisplatin against Bel-7402 cell line; and complex **3** showed the highest cytotoxic activity against HL-60 cell line; a complex using an aromatic group on picolinamide as ligand exhibited better cytotoxicity than that using an aliphatic group on picolinamide as ligand. The results indicated that gold (III) complexes with deprotonated amide might be a promising source of metal-based antitumor agents. Further, more binuclear complexes or complexes with aromatic groups are needed to reveal the relationship between chemical structure and biological activity of gold(III) complexes, which may be helpful for the design of new metal-based antitumor agents.

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