FULL PAPER

Dalton
www.rsc.org/dalton

Chemical and biological studies of the dichloro(2-phenylpyridine) gold(III) complex and its derivatives †

Daming Fan, Chang-Tong Yang,* John D. Ranford,* Peng Foo Lee and Jagadese J. Vittal
Department of Chemistry, National University of Singapore, Singapore 117543.
E-mail: chmyct@nus.edu.sg; kubalranford@yahoo.co.uk

Received 24th March 2003, Accepted 19th May 2003
First published as an Advance Article on the web 30th May 2003

Four new derivatives of the type [Au(ppy)X_2] (ppy = 2-phenylpyridine) together with [Au(ppy)Cl_2] (**1**) have been synthesized and characterized [X = ac = CH_3COO^- (**2**), bz = $C_6H_5COO^-$ (**3**); mal = $CH_2(COO^-)_2$ (**4**), cbdca = $C_4H_6(COO^-)_2$ (**5**)]. The crystal structures of **2** and **3** are similar in which two carboxylate groups are bound to gold through oxygen, but the two Au–O distances are inequivalent. The crystal structure of **5** shows that gold is bound to *σ-C,N*-phenylpyridine (ppy) and *O,O*-cbdca, forming a five-membered and a six-membered chelate ring, respectively. In all four structures the Au(III) center exhibits a square planar coordination geometry and the *trans* influence of the σ-bonded phenyl group is apparent. The σ-bonded phenyl group contributes to stabilizing these complexes in reducing, biological media. The reaction between **5** and chloride has been investigated by ^{1}H NMR spectroscopy and reveals slow replacement of cbdca by chlorides through an intermediate [Au(ppy)(*O*-cbdca)Cl]. All five complexes have been tested for cytotoxic properties *in vitro* against MOLT-4 (human leukemia) and C2C12 (mouse tumour) cell lines. The results show that these complexes have similar cytotoxicity profile to cisplatin against MOLT-4 but are inactive on C2C12, except for complex **5**.

Introduction

The serendipitous discovery of the antitumour properties of cisplatin in 1969 by Rosenberg *et al.* generated considerable research interest in the area of metal-based antitumour agents.[1] The remarkable activity of cisplatin against testicular cancer suggests that, in principal, it should be possible to synthesize other metal-based anticancer drugs with the potential to treat other specific tumour types.[2,3] Due to Au(III) being isoelectronic with Pt(II) both metal complexes show a square planar configuration, as seen in cisplatin and its homologues. These similarities open the possibilities that Au(III) complexes might have biological activity parallel to cisplatin.[4,5] Compared with Pt(II), Au(III) complexes are relatively unstable, light-sensitive in solution and easily reduced to metallic gold under physiological conditions due to Au(III) having more rapid kinetics, a higher redox potential, and a higher charge.[6,7] Despite the above difficulties, there have been several studies carried out to investigate the properties of Au(III) complexes and their potential antitumour activity. In 1992, Parish reported a series of Au(III) complexes [AuX_2(L–L′)] where X is a mononegative bidentate N,O^- ligands and L–L′ were the pyridine-2-carboxylate groups.[8] In 1997, Orioli and co-workers reported that [Au(Hpm)Cl_3] and [Au(pm)Cl_2] (Hpm = 2-pyridylmethanol) demonstrated good activity on a number of tumour cell lines.[9] However, complexes with mononegative bidentate N–O^- ligands underwent reduction to metallic gold in biological media.[9] Buckley, Parish and their co-workers reported a new series of Au(III) complexes containing N–C^- ligands, involving a σ-bonded phenyl, naphthyl, or similar group with various nitrogen-containing substituents.[10–12] These Au(III) complexes are indeed more stable in reducing, biological media. Some exhibited promising selective cytotoxicity *in vitro* and showed antitumour activity *in vivo* against human carcinoma xenografts.[10–12] Other studies have shown that Au(III) complexes are capable of interacting with DNA but the mechanism of action of these complexes is different from the Pt(II) analogues.[13,14] Recently, Orioli and co-workers reported some crystal structures of Au(III) complexes, their DNA-binding properties and cytotoxicity as potential antitumour agents.[15–17] Au(III) complexes might therefore represent a novel class of metal-containing antitumour agent with a spectrum of activity different from that of cisplatin.

The complex [Au(ppy)Cl_2] contains a N–C^- ligand, in which a five-membered cycloaurated chelated ring was formed, and has been known for more than ten years,[18] but does not seem to have been examined for biological activity. Due to its structural similarity to cisplatin we have been prepared four new complexes containing carboxylate group to improve their aqueous solubility together with [Au(ppy)Cl_2] (**1**). The compounds have been spectroscopically characterized and tested for cytotoxic properties *in vitro* against the MOLT-4 human leukemia and C2Cl2 mouse tumour cell lines. The complexes are all very active against MOLT-4 while are inactive on C2C12 except **5** showing that Au(III) complexes could be useful as potential antitumour drugs on selective tumours.

Experimental

Na[$AuCl_4$] and 2-phenylpyridine were obtained from Aldrich Chemical Co. Milwaukee, WI, USA. All other reagents were purchased from Fluka Chemie AG, Buchs, Switzerland.

The NMR spectra were recorded on a Bruker ACF 300 (^{1}H, ^{13}C) spectrometer at 25 °C. Spectra were referenced to the solvent in each case. The ^{1}H–^{1}H shift-correlation (COSY) spectra were recorded on a Bruker ACF 500 spectrometer in order to determine the assignment fully for each proton. The infrared spectra (KBr pellet) were recorded using an FTS165 Bio-Rad FTIR spectrophotometer in the range 4000–450 cm^{-1}. The elemental analyses were performed in the Microanalytical Laboratory, Department of Chemistry, National University of Singapore.

[Au(ppy)Cl_2], 1

1 was synthesized according to a literature method.[18] **2** and **4** were prepared by metathesis following literature methods.[12] Analogous procedures were used to prepare **3** and **5**.

[Au(ppy)$(ac)_2$]·H_2O, 2

1 (0.52 g, 1.2 mmol) was dissolved in acetone (30 mL) and the solution degassed with nitrogen. Silver acetate (0.41 g, 2.4 mmol) was added and the mixture stirred in the dark for 6 h

† Electronic supplementary information (ESI) available: ^{1}H–^{1}H COSY spectrum of **5** in DMSO-d_6. See http://www.rsc.org/suppdata/dt/b3/b303297c/

DOI: 10.1039/b303297c

Table 1 Crystallographic data and structure refinement details for **1**, **2**, **3** and **5**

Complex	**1**	**2**	**3**	**5**
Formula	$C_{11}H_8AuCl_2N$	$C_{15}H_{16}AuNO_5$	$C_{27}H_{23}AuNO_{4.5}$	$C_{17}H_{14}AuNO_4$
Formula weight	422.05	487.25	630.43	493.26
Temp/K	293(2)	293(2)	293(2)	293(2)
Crystal system	Monoclinic	Triclinic	Monoclinic	Monoclinic
Space group	*Cc*	*P*1	$P2_1/n$	$P2_1/c$
a/Å	13.9383(8)	8.9815(1)	11.8963(2)	6.5342(2)
b/Å	10.0796(6)	9.5283(2)	16.8239(4)	21.1840(6)
c/Å	7.84329(4)	11.1538(2)	13.4511(2)	10.7191(3)
α/°	90	65.668(1)	90	90
β/°	94.940(2)	70.962(1)	98.522(1)	101.388(1)
γ/°	90	64.721(1)	90	90
Z	4	2	4	4
μ/mm^{-1}	13.848	9.539	5.557	10.135
Reflns collected	3495	4988	15787	9349
Independent reflns	2274	3553	6429	3486
R_{int}	0.0434	0.0472	0.0434	0.0295
Final R_1 [a] [$I > 2\sigma$]	0.0487	0.0476	0.0639	0.0280
Final wR_2 [b] [$I > 2\sigma$]	0.1045	0.1205	0.1351	0.0515

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

under nitrogen. The resulting mixture was filtered and the colourless filtrate was reduced in volume under reduced pressure until crystallization was observed. Single crystals were obtained from acetone/diethyl ether. Yield: 0.21 g (36%). Anal. for $C_{15}H_{16}AuNO_5$ (calcd in parentheses) C, 36.83(36.97); H, 3.26(3.31); N, 2.75(2.87); Au, 40.01(40.42%). IR (KBr, cm^{-1}): $\nu_{as}(COO^-)$ 1607, $\nu_s(COO^-)$ 1310, $\nu(C–N)_{ppy}$ 1493, 1449, 785, 767, 732.

[Au(ppy)(bz)$_2$]·0.5(C$_2$H$_5$)$_2$O, 3

1 (0.52 g, 1.2 mmol) was dissolved in acetone (30 mL) and silver nitrate (0.40 g, 2.3 mmol) added. The mixture was stirred in the dark for 1 h and the silver chloride was filtered off. The colourless filtrate was treated with sodium benzoate (0.37 g, 2.5 mmol) in water (2 mL). The little precipitate was removed and the filtrate was rotaevaporated to small volume to a crystallize in acetone/diethyl ether. Yield: 0.17 g (23%). Anal. for $C_{27}H_{23}AuNO_{4.50}$ (calcd in parentheses) C, 50.91(51.42); H, 3.81(3.68); N, 2.19(2.22); Au, 31.52(31.24%). IR (KBr, cm^{-1}): $\nu_{as}(COO^-)$ 1585, $\nu_s(COO^-)$ 1358, $\nu(C–N)_{ppy}$ 1492, 1449, 787, 768, 730.

[Au(ppy)(mal)], 4

1 (0.85 g, 2.0 mmol) was dissolved in acetone (50 mL) and the solution purged with nitrogen. Silver malonate (0.64 g, 2.0 mmol) was added, and the reaction mixture was stirred in the dark for 8 h. Mixture was then filtered, and the colourless filtrate was reduced in volume at a reduced pressure. Crystallization of the product was completed by cooling the concentrated filtrate in an ice bath. The white product was collected by filtration and dried *in vacuo*. Yield: 0.26 g (29%). Anal. for $C_{14}H_{10}AuNO_4$ (calcd in parentheses) C, 36.82(37.11); H, 2.19(2.23); N, 3.23(3.09); Au, 43.69(43.46%). IR (KBr, cm^{-1}): $\nu_{as}(COO^-)$ 1621, $\nu_s(COO^-)$ 1375, $\nu(C–N)_{ppy}$ 1492, 1450, 786, 767, 730.

[Au(ppy)(cbdca)], 5

1 (1.06 g, 2.5 mmol), 1,1-cyclobutanedicarboxylic acid (0.43 g, 3.0 mmol) and silver(I) oxide (1.15 g, 5.0 mmol) were dissolved in the mixed solvent methylene chloride/ethanol/water (2/1/1, 40 mL). The reaction mixture was stirred in the dark for 2 days. After filtering, the layers were separated, and the colourless aqueous solution was used to crystallize **5** by diffusing diethyl ether into it. White crystals were obtained. Yield: 0.20 g (17%). Anal. for $C_{17}H_{14}AuNO_4$ (calcd in parentheses) C, 41.07(41.42); H, 2.89(2.86); N, 2.73(2.84); Au, 39.97(39.93%). IR (KBr, cm^{-1}): $\nu_{as}(COO^-)$ 1614, $\nu_s(COO^-)$ 1364, $\nu(C–N)_{ppy}$ 1492, 1450, 787, 768, 729.

X-Ray crystallography

The diffraction experiments were carried out on a Bruker AXS SMART CCD diffractometer. The program SMART[19] was used for collecting the intensity data, for reflections indexing and for the determination of lattice parameters, SAINT[19] was used for integration of the intensity of reflections and scaling, SADABS[20] was used for absorption correction and SHELXTL[21] for space group and structure determination, least-squares refinements on F^2. All non-H atoms were refined with anisotropic thermal parameters. Selected crystallographic data and refinement details are displayed in Table 1.

CCDC reference numbers 206807–206810.

See http://www.rsc.org/suppdata/dt/b3/b303297c/ for crystallographic data in CIF or other electronic format.

Cytotoxicity testing

Comparative cytotoxicity against the MOLT-4 human leukemia and C2C12 mouse tumour cell lines was assessed. A typical assay was set up by dispensing 30000 MOLT-4 cells in 70 μl RPMI 1640 medium supplemented with 10% foetal calf serum in to each well of a 96-well plate. The C2C12 cells were cultured in Dulbecco's modified Eagle's medium with 4 mM *L*-glutamine adjusted to contain 1.5 g L^{-1} sodium bicarbonate, 4.5 g L^{-1} glucose and 1.0 mM sodium pyruvate, 90%; fetal bovine serium, 10%. The test fractions were dissolved in DMSO and added in 10 μl such that the final concentration of DMSO was 1.25% (v/v) in all wells. Controls received DMSO only. Following incubation of cells with test fractions for 14–16 h at 37 °C and 5% CO_2, 20 μl of a 5 mg mL^{-1} solution of 3[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazoliumbromide (MTT) was added into each well (MTT final concentration 1 mg mL^{-1}). Three hours later, 100 μl of lysing solution (20% sodium dodecyl sulfate dissolved in 50% DMF, pH adjusted to 4.7 with acetic acid) was added to each well. Cell lysis was facilitated by mechanical disruption and mixing, after which absorbance was read at 570 nm against the standard mixture of RPMI 1640 medium, MTT, and lysing solution as blank.

Results and discussion

Crystal structures

The crystal structures of **1**, **2**, **3** and **5** were confirmed by X-ray crystallography. Views of the molecular structures are displayed

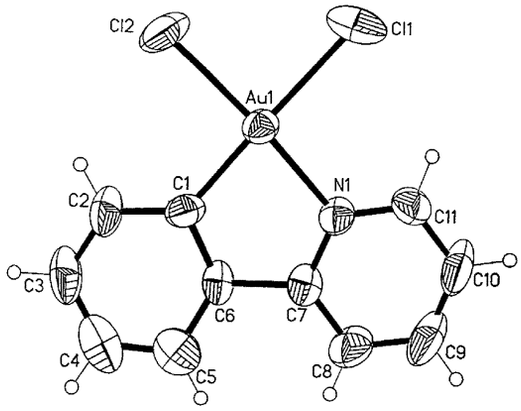


Fig. 1 An ORTEP view of **1** showing the numbering scheme.

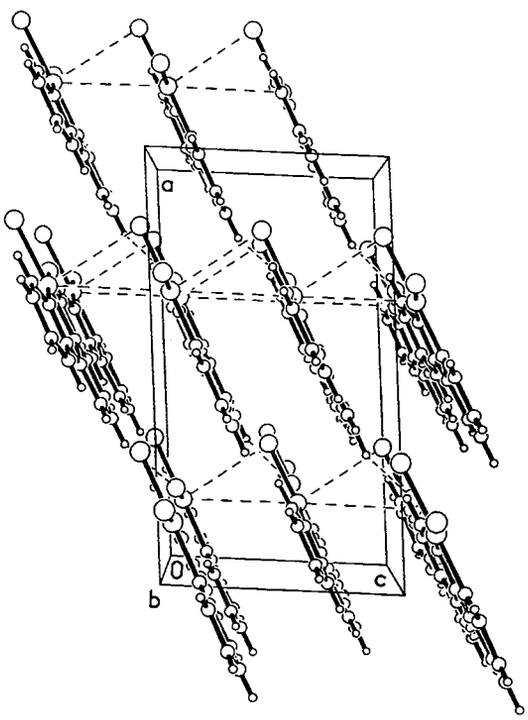


Fig. 2 Packing diagram of **1** showing the intermolecular Au ··· Au interaction.

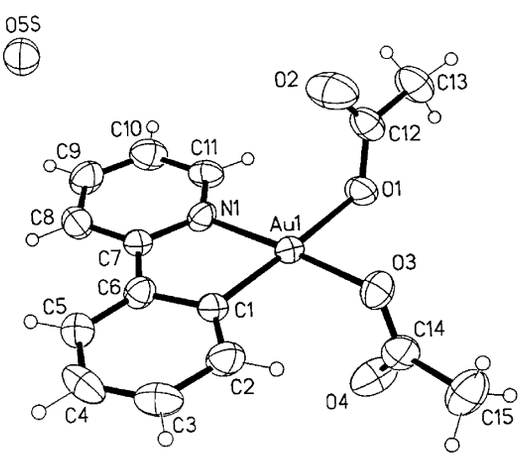


Fig. 3 An ORTEP diagram showing a perspective view of **2**.

in Figs. 1–5. Selected bond distances and angles are given in Table 2. The Au–C and Au–N bond distances are shorter than those reported for other Au(III) complexes.[6,22–24]

1 has the expected structure in which Au(III) is coordinated to one chelated ppy ligand and two chlorides (Fig. 1). The square planar coordination geometry in this complex deviates slightly

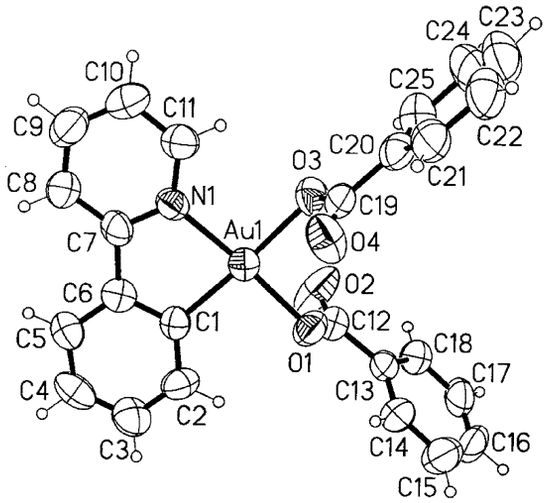


Fig. 4 An ORTEP diagram showing the coordination environment of **3**. The diethyl ether molecule has been omitted for clarity.

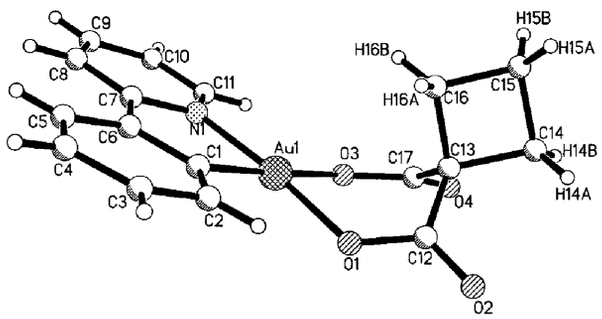


Fig. 5 A perspective view of **5**.

from perfect 90° bond angles owing to the constraints of the chelating 2-phenylpyridine ligand, with a C(1)–Au–N(1) angle of 83.0(6)° and a consequent N(1)–Au–Cl(1) angle of 95.4(4)°. The Cl(1)–Au–Cl(2) angle is nearly ideal at 90.0(3)°. The overall planarity of the molecule favours stacking in the solid state (Fig. 2). The molecules stack in a zig-zag arrangement with Au ··· Au distances of 3.943(2) Å. The Au ··· Au distances are longer than that found in the $[Au(dpp)Cl]^+$ cations (3.6 Å),[25] but are much shorter than the nearest intermolecular Au ··· Au interaction in systems such as $[Au(bpy)Cl_2]BF_4$ (6.85 Å).[26] The most important structural feature of **1** is the difference in Au–Cl bond lengths. The Au–Cl(1) distance (2.361(8) Å), which is *trans* to the Au–C bond, is longer than the Au–Cl(2) distance (2.282(5) Å) *trans* to the pyridyl nitrogen by *ca.* 0.1 Å as a result of a structural *trans* influence.

Crystal structures of **2** (Fig. 3) and **3** (Fig. 4) have features common with **1**. Both exhibit square planar coordination geometries and the two Au–O bond lengths are appreciably different, with that *trans* to C being the longer as expected on *trans* influence grounds. This phenomenon is similar to that observed for other complexes.[10,11,27] To our knowledge, these are the first two crystal structures in which two oxygen donors of carboxylate groups are coordinated to Au(III). The Au–C, Au–N and Au–O bond lengths in **2** and **3** are similar.

The crystal structure of **5** (Fig. 5) shows Au(III) in a square planar coordination environment (O(1)–Au–C(1) 93.3°, O(1)–Au–O(3) 90.4°, C(1)–Au–N(1) 81.4°, O(3)–Au–N(1) 95.02°) bound to ppy and chelated *O*,*O*-cbdca, forming five- and six-membered chelate rings, respectively. The ppy is co-planar with the gold coordination sphere whereas the cyclobutane ring in cbdca is perpendicular to it. Like [*cis*-$Pt(NH_3)_2$CBDCA][28] and [Pd(*O*,*O*′-cbdca)$(NH_3)_2$],[29] the six-membered chelate ring has a boat conformation. The Au–O bond lengths (2.074(3) and 1.989(3) Å) in **5** are shorter than that in the structure of **2** (2.108(6) and 2.031(6) Å) due to the formation

Table 2 Selected bond lengths (Å) and angles (°) for **1**, **2**, **3** and **5**

1		**2**		**3**		**5**	
Au–C(1)	1.950(2)	Au–C(1)	2.005(8)	Au–C(1)	2.001(9)	Au–C(1)	1.998(4)
Au–N(1)	2.034(1)	Au–N(1)	2.028(6)	Au–N(1)	2.007(8)	Au–N(1)	2.002(3)
Au–Cl(1)	2.361(8)	Au–O(1)	2.018(6)	Au–O(1)	2.063(7)	Au–O(1)	1.989(3)
Au–Cl(2)	2.282(5)	Au–O(3)	2.031(6)	Au–O(3)	2.014(6)	Au–O(3)	2.074(3)
C(1)–Au–N(1)	83.0(6)	C(1)–Au–N(1)	81.6(3)	C(1)–Au–N(1)	81.8(3)	C(1)–Au–N(1)	81.4(2)
C(1)–Au–Cl(2)	178.0(6)	C(1)–Au–O(1)	177.8(2)	C(1)–Au–O(3)	176.5(3)	C(1)–Au–O(3)	176.4(1)
C(1)–Au–Cl(2)	91.6(6)	C(1)–Au–O(3)	93.1(3)	C(1)–Au–O(1)	94.3(3)	C(1)–Au–O(1)	93.3(2)
N(1)–Au–Cl(2)	174.6(4)	N(1)–Au–O(3)	174.6(2)	N(1)–Au–O(1)	175.7(3)	N(1)–Au–O(1)	173.6(1)
N(1)–Au–Cl(1)	95.4(4)	N(1)–Au–O(1)	96.3(3)	N(1)–Au–O(3)	95.1(3)	N(1)–Au–O(3)	95.0(1)
Cl(2)–Au–Cl(1)	90.0(3)	O(3)–Au–O(1)	89.0(3)	O(1)–Au–O(3)	88.9(3)	O(1)–Au–O(3)	90.4(1)

Table 3 ^{1}H NMR data for complexes (δ, DMSO-d_6 solutions)[a]

Complex	H^{11}	H^{9}	H^{8}	H^{5}	H^{2}	H^{10}	H^{4}	H^{3}	R
1	9.53	8.41	7.97	7.83	7.78	7.48	7.40	7.40	
2	8.71	8.42	7.94	7.77	7.48	7.36	7.23	7.22	2.90, 2.73
3	8.60	8.44	7.95	7.73	7.51	7.35	7.32	7.30	7.99, 7.57, 7.09
4	8.72	8.42	7.96	7.78	7.52	7.39	7.33	7.31	3.62
5	8.73	8.42	7.96	7.76	7.51	7.42	7.35	7.42	2.65, 2.56, 1.74
Isomer 1	9.28	8.43	8.40	7.97	7.36	7.78	7.52	7.42	2.65, 1.76
Isomer 2	8.72	8.43	8.40	7.97	7.62	7.78	7.52	7.42	2.64, 1.75

[a] See Fig. 5 for ^{1}H numbering scheme.

Table 4 ^{13}C NMR data for complexes (δ, DMSO-d_6 solutions unless otherwise stated)

Complex	2-phenylpyridine	R
1	122.0, 125.2, 126.6, 129.2, 129.9, 131.5, 142.8, 143.8, 147.9, 152.1, 163.8	
2[a]	123.9, 127.1, 127.5, 129.3, 130.1, 132.3, 143.4, 145.7, 146.2, 149.5, 163.2	21.6, 22.3, 177.2, 179.5
3[b]	123.7, 127.5, 127.6, 129.5, 130.0, 132.2, 142.2, 143.4, 145.5, 149.2, 161.6	123.8, 127.2, 129.6, 130.6, 132.5, 145.3, 174.9, 176.6
4	121.9, 125.0, 125.9, 127.7, 129.5, 131.1, 142.7, 144.4, 147.6, 154.8, 164.3	58.6, 171.6, 173.8
5	121.5, 124.5, 125.6, 128.1, 129.0, 131.3, 142.2, 144.3, 146.2, 151.7, 162.6	15.2, 29.1, 30.3, 48.5, 176.5, 178.6

[a] In DMF-d_6. [b] Peaks between 123 and 145 for aromatic carbon atoms from the both ligands in **3** are overlap.

of a six-membered chelate ring in **5**. The difference of the two Au–O bond distances in **5** is due to the *trans* influence of σ-bonded phenyl group. The Au–O(3) bond length (2.074(3) Å, *trans* to C), is longer than the Au–O(1) distance (1.989(3) Å, *trans* to pyridyl nitrogen) by *ca.* 0.1 Å.

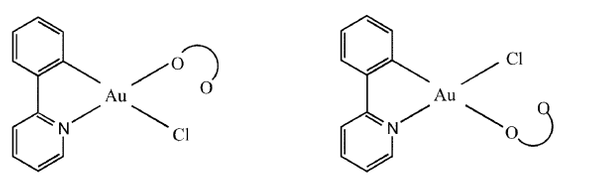
Spectroscopic studies

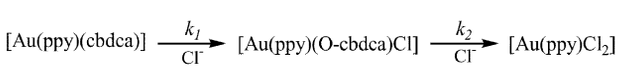
The infrared spectra of the four new complexes are consistent with the presence of coordinated carboxylate. The band in the region 1585–1621 cm^{-1} is due to the asymmetric vibration of coordinated carboxylate group [$\nu_{as}(CO_2^-)$] and the band in the region 1348–1449 cm^{-1} may be attributed to the symmetric stretching vibration of carboxylate group [$\nu_s(CO_2^-)$]. The difference between the $\nu_{as}(COO^-)$ and $\nu_{sym}(COO^-)$ stretching frequencies is > 200 cm^{-1}, consistent with a terminal coordination mode for the carboxylate group,[30–32] as seen in the crystal structures of **2**, **3**, and **5**. The IR spectra of the four complexes exhibit strong ppy ligand modes at *ca.* 1493, 1449, 787, 768, and 730 cm^{-1}.[33–35]

^{1}H (Table 3) and ^{13}C (Table 4) NMR spectra of complexes were acquired in DMSO-d_6 and DMF. The ^{1}H NMR spectra of **2**–**5** indicate there is a significant upfield chemical shift of H^6 and H^9 resonances of ppy ligand after the two chloride donors were replaced by carboxylate groups. It was assumed that the higher electron density of the carboxylate group shields H^6 and H^9 nuclei and shifts them to higher field. The chemical shift at δ 2.65 in **5** could be assigned to H14A and H16B, with H14B and H16A at 2.56. The assignment for each proton in **5** were made based on ^{1}H–^{1}H COSY (supplementary material†). For all the complexes studied, C^9 shows a strong downfield coordination shift as it is directly bound to gold. The order of chemical shifts of other carbon atoms in the ppy ligand was the same for each derivative.

Unlike [*cis*-Pt(NH$_3$)$_2$CBDCA][28] and [Pd(*O*,*O*′-cbdca)-(NH$_3$)$_2$],[29] the asymmetry of the five-membered ring in **5** leads to different magnetic environments for H14A, H16B and H14B, H16A (see Fig. 5 for numbering). The former two complexes have crystallographically-imposed mirror symmetries with the metal and all four cyclobutane carbons in the mirror plane so that the corresponding protons on cbdca in these two complexes are magnetically equivalent. However, in **5**, H14B and H16A are on the N side whereas H14A and H16B are on the C anion side as there is rapid ring-flipping through O(1)–O(3) in solution.

The reaction between **5** and chloride ions was studied by ^{1}H NMR spectroscopy in DMSO-d_6 (one drop of D$_2$O) containing 5 mM of **5** and 20 mM NaCl for 1.5 h at 300 K (Scheme 1 and Fig. 6). After 4 min, the doublets from H^{11} (δ 8.72) and H^2 (δ 7.36) of **5** decreased in intensity and new doublets appeared





Scheme 1

Table 5 IC_{50} values (μM) against MOLT-4 and C2C12 cell lines

Complex	**1**	**2**	**3**	**4**	**5**	Cisplatin
MOLT-4	4.2	4.8	4.1	5.1	3.3	6.8
C2C12	83	73	75	27	9	14.7

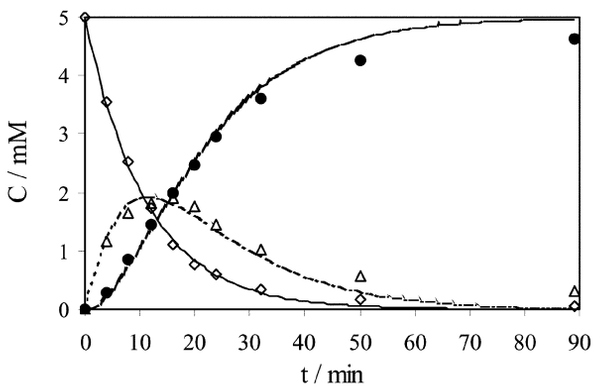


Fig. 6 Plots of the concentrations of species detected during the reaction of **5** with Cl^- {◇ = [Au(ppy)(cbdca)]; Δ = [Au(ppy)(*O*-cbdca).-Cl]; ● = [Au(ppy)Cl_2]}

at δ 9.28 and 7.62. At the same time the cbdca signal of **5** decreased in intensity and the free cbdca peaks at δ 2.36 and 1.84 appeared and a ring-opened intermediate, [Au(ppy)-(*O*-cbdca)Cl], formed during the reaction. The intermediate reached a maximum concentration of *ca.* 1.90 mM at 16 min and then decreased in intensity. There are two possible isomers for the intermediate due to the asymmetry of the ppy ligand. Isomer 1 has the monodentate *O*-cbdca *trans* to the nitrogen whereas isomer 2 has *O*-cbdca *trans* to carbon. The ^{1}H chemical shifts for these two isomers are given in Table 3. Of the two, isomer 1 is expected to predominate based on the greater *trans* influence of C, and indeed, the maximum ratio for the intensity of the isomers was 1 : 0.6 at 8 min. The monodentate *O*-cbdca ligand was gradually replaced by chloride ions to yield the product [Au(ppy)Cl_2]. After 1.5 h, [Au(ppy)Cl_2] is the dominant species with [Au(ppy)(cbdca)] and [Au(ppy)(*O*-cbdca)Cl] now being minor ones. Reasonable fits to the time dependences of the concentrations of **5**, the intermediate [Au(ppy)(*O*-cbdca)Cl] and product [Au(ppy)Cl_2] were obtained by assuming two consecutive first-order reactions and using the standard equations[36] (Scheme 1, Fig. 6). This gave the two similar rates of $k_1 = (9.02 \pm 0.12) \times 10^{-2}$ s^{-1} and $k_2 = (8.19 \pm 0.12) \times 10^{-2}$ s^{-1}.

The ^{13}C NMR spectrum of the hydrolysis reaction of **2** in DMF and D_2O shows a significant broadening of one set of acetate signals. The broadening could be explained on the assumption that one acetate ligand is undergoing exchange with a water molecule rapidly on the NMR time-scale. On the basis of the *trans* effect, this would be that *trans* to the Au–C bond, since a carbanion has a stronger *trans* influence effect than nitrogen. A DMSO-d_6 solution of 3 mM of **5** with one drop D_2O added was also monitored by ^{1}H NMR for two hours. However, there was no chemical shift change observed for the chelated cbdca. **5** is stable towards hydrolysis although it is attacked by chloride ions in DMSO.

Cytotoxicity studies

In vitro cytotoxic activity of complexes including cisplatin as a comparison, as determined against the human tumour cell line MOLT-4 and the mouse tumour cell line C2C12. Cytotoxicity data are given as IC_{50} values, the concentration of complex required to inhibit the growth of cells by 50% compared with that of a control, in Table 5. The results show that the cytotoxicity profile is similar for all five gold(III) complexes, with IC_{50} values for MOLT-4 in the low-micromolar range. The data can be interpreted that these complexes possess high antitumour activity on MOLT-4 and are slightly more cytotoxic compared with cisplatin. As compounds with IC_{50} values > 20 μM was considered as inactive,[37,38] the results obtained from the C2C12 cells surprisingly show that these complexes are inactive on C2C12 with the exception of complex **5**. It is possible that ligands herewith may play partial role in the biological activity. Compared with the result for cisplatin, complex **5** showed greater toxicity (lower IC_{50} values) against both MOLT-4 and C2C12 cell lines. Such differential cytotoxicity has been used as an indicator of antitumour activity.[9,12,39] It is suggested that complex **5** deserves further biological study against a wide range of tumour cell lines.

Conclusion

A range of Au(III) complexes has been synthesized and characterized. The results indicate that [Au(ppy)Cl_2] has a rich and interesting substitution chemistry. Its O-donor ligand derivatives have better aqueous solubility than [Au(ppy)Cl_2] due to the presence of carboxylate groups. **2**–**5** are relatively stable due to greater σ-donation of the ppy ligand and the displacement of ppy ligand in substitution reactions is not observed.

The Au(III) complexes structurally resemble cisplatin and indeed exhibit cytotoxic properties. This finding leads us to believe that Au(III) complexes have potential antitumour properties for some human tumours and full evaluation and exploitation is now needed to investigate the possibility of clinical use for these Au(III) complexes.

Acknowledgements

We are grateful for grant 970614 from the National University of Singapore.

References

1 B. Rosenberg, L. V. Camp, T. E. Trosko and V. H. Mansour, *Nature (London)*, 1969, **222**, 385.
2 N. Farrell, in *Transition Metal Complexes as Drugs and Chemotherapeutic Agents*, Kluwer Academic Press, Boston, 1989.
3 D. C. H. McBrien and T. F. Slater, in *Biochemical Mechanisms of Platinum Antitumor Drugs*, IRL Press, Oxford, 1986.
4 B. K. Keppler, in *Metal Complexes in Cancer Chemotherapy*, VCH, Weinheim, New York, 1993.
5 P. J. Sadler and R. E. Sue, *Metal-Based Drugs*, 1994, **1**, 107.
6 L. Cattalini, A. Orio and M. L. Tobe, *J. Am. Chem. Soc.*, 1967, **89**, 3130.
7 J. J. Pesek and W. R. Mason, *Inorg. Chem.*, 1983, **22**, 2958.
8 M. A. Dar, K. Moss, S. M. Cottrill, R. V. Parish, C. A. McAuliffe, R. G. Pritchard, B. Beagley and J. Sandbank, *J. Chem. Soc., Dalton Trans.*, 1992, 1907.
9 P. Calamai, S. Carotti, A. Guerri, L. Messori, E. Mini, P. Orioli and G. Paolo, *J. Inorg. Biochem.*, 1997, **66**, 103.
10 R. V. Parish, B. P. Howe, J. P. Wright, J. Mack, R. G. Pritchard, R. G. Buckley, A. M. Elsome and S. P. Fricker, *Inorg. Chem.*, 1996, **35**, 1659.
11 R. V. Parish, J. Mack, L. Hargreaves, J. P. Wright, R. G. Buckley, A. M. Elsome, S. P. Fricker and B. R. C. Theobald, *J. Chem. Soc., Dalton Trans.*, 1996, 69.
12 R. G. Buckley, A. M. Elsome, S. P. Fricker, G. R. Henderson, B. R. C. Theobald, R. V. Parish, B. P. Howe and L. R. Kelland, *J. Med. Chem.*, 1996, **39**, 5208.
13 C. K. Mirabelli, C. M. Sung, J. P. Zimmerman, D. T. Hill, S. Mong and S. T. Crooke, *Biochem. Phamacol.*, 1986, **35**, 1427.
14 C. K. Mirabelli, J. P. Zimmerman, H. R. Bartus, C. M. Sung and S. T. Crooke, *Biochem. Pharmacol.*, 1986, **35**, 1435.
15 P. Calamai, A. Guerri, L. Messori, P. Orioli and G. P. Speroni, *Inorg. Chim. Acta*, 1999, **285**, 309.
16 F. Abbate, P. Orioli, B. Bruni, G. Marcon and L. Messori, *Inorg. Chim. Acta*, 2000, **311**, 1.
17 S. Carotti, A. Guerri, T. Mazzei, L. Messori, E. Mini and P. Orioli, *Inorg. Chim. Acta*, 1998, **281**, 90.
18 E. C. Constable and T. A. Leese, *J. Organomet. Chem.*, 1989, **363**, 419.

19 SMART & SAINT, Version 6.22, Bruker AXS Analytic X-Ray Systems, Inc., Madison, WI, 2000.
20 G. M. Sheldrick, SADABS, Software for Empirical Absorption Correction, University of Göttingen, Germany, 2000.
21 SHELXTL Reverence Manual, Version 5.1, Bruker AXS Analytic X-Ray Systems, Inc., Madison, WI, 1997.
22 P. A. Bonnardel, R. V. Parish and R. G. Pritchard, *J. Chem. Soc., Dalton Trans.*, 1996, 3185.
23 J. Vicente, M. D. Bermúdez, M. T. Chicote and M. J. Sánchez-Santato, *J. Organomet. Chem.*, 1989, **371**, 129.
24 J. Vicente, M. T. Chicote, M. D. Bermúdez, P. G. Jones, C. Fittschen and G. M. Sheldrick, *J. Chem. Soc., Dalton Trans.*, 1986, 2361.
25 C. W. Chan, W. T. Wong and C. M. Che, *Inorg. Chem.*, 1994, **33**, 1266.
26 J. L. McInnes, A. J. Welch and L. J. Yellowlees, *Acta Crystallogr., Sect. C*, 1995, **51**, 2023.
27 M. A. Mansour, R. J. Lachicotte, H. J. Gysling and R. Eisenberg, *Inorg. Chem.*, 1998, **37**, 4625.
28 B. Beagley, D. W. J. Cruickshank, C. A. McAuliffe, R. G. Pritchard, A. M. Zaki, R. L. Beddoes, R. J. Cernik and O. S. Mills, *J. Mol. Struct.*, 1985, **130**, 97.
29 K. J. Barnham, M. I. Djuran, U. Frey, M. A. Mazid and P. J. Sadler, *J. Chem. Soc., Chem. Commun.*, 1994, 65.
30 C. Djordjevic, M. Lee and E. Sinn, *Inorg. Chem.*, 1989, **28**, 719.
31 G. B. Deacon and R. Philips, *J. Coord. Chem. Rev.*, 1980, **33**, 227.
32 M. Tsaramyrsi, M. Kaliva, A. Salifoglou, C. P. Raptopoulou, A. Terzis, V. Tangorlis and J. Giapintzakis, *Inorg. Chem.*, 2001, **40**, 5772.
33 E. C. Constable and J. M. homes, *J. Organomet. Chem.*, 1986, **310**, 203.
34 E. C. Constable, *J. Chem. Soc., Dalton Trans.*, 1985, 1719.
35 E. C. Constable, R. G. Henney and T. A. Leese, *J. Organomet. Chem.*, 1990, **365**, 293.
36 P. W. Atkins, *Physical Chemistry*, OUP, Oxford, 2nd edn., 1982, p. 940.
37 E. W. Aniscough, A. M. Brodie, W. A. Denny, G. J. Finlay, S. A. Gothe and J. D. Ranford, *J. Inorg. Biochem.*, 1998, **70**, 175.
38 E. W. Aniscough, A. M. Brodie, W. A. Denny, G. J. Finlay, S. A. Gothe and J. D. Ranford, *J. Inorg. Biochem.*, 1999, **77**, 125.
39 L. Messori, F. Abbate, G. Marcon, P. Orioli, M. Fontani, E. Mini, T. Mazzei, S. Carotti, T. O'Connell and P. Zanallo, *J. Med. Chem.*, 2000, **43**, 3541.