European Journal of Medicinal Chemistry 46 (2011) 1914–1918

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis, characterization and cytotoxicity of the gold(III) complexes of 4,5-dihydropyrazole-1-carbothioamide derivatives

Shuxiang Wang [a,*], Wenqing Shao [a], Hongdong Li [a], Cui Liu [a], Ke Wang [b], Jinchao Zhang [a,*]

[a] Chemical Biology Key Laboratory of Hebei Province, College of Chemistry and Environmental Science, Hebei University, Baoding 071002, Hebei, PR China
[b] Shijiazhuang Center for Disease Control and Prevention, Shijiazhuang 050011, Hebei, PR China

ARTICLE INFO

Article history:
Received 8 November 2010
Received in revised form
6 February 2011
Accepted 13 February 2011
Available online 22 February 2011

Keywords:
Gold(III) complex
Dihydropyrazole derivative
Cytotoxicity

ABSTRACT

Eight new gold(III) complexes (**1**–**8**) of 5-aryl-3-(pyridin-2-yl)-4,5-dihydropyrazole-1-carbothioamide have been synthesized and characterized by elemental analysis, molar conductivity, IR, UV, ^{1}H NMR, ^{13}C NMR, MS, and thermal analysis techniques. The cytotoxicity was tested by MTT assay. The results indicate that the complexes **1**–**8** exert cytotoxic effects against HeLa and A549 cell lines. Moreover, the complexes **1**, **4**, **5**, **7** and **8** have higher cytotoxicity than cisplatin against HeLa cell line. It suggests that the substituent groups on benzene have important effect on cytotoxicity.

1. Introduction

The landmark discovery of cisplatin by Rosenberg in 1965 heralded a new area of anticancer drug research based on metallopharmaceuticals [1]. To date, cisplatin and its analogues are some of the most effective chemotherapeutic agents in clinical use as the first line of treatment in testicular and ovarian cancers. Unfortunately, they have several major drawbacks. Common problems include cumulative toxicities of nephrotoxicity and ototoxicity [2–5]. In addition to the serious side effects, the therapeutic efficacy is also limited by inherent or treatment-induced resistant tumor cells. These drawbacks have provided the motivation for alternative chemotherapeutic strategies.

Metals, in particularly, transition metals offer potential advantages over the more common organic-based drugs. For example, a wide range of coordination numbers and geometries, accessible redox states, 'tune-ability' of the thermodynamics and kinetics of ligand substitution. Gold(III) complexes 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. Surprisingly, despite this strict similarity, little literature data exist on the use of gold(III) complexes as anticancer drugs. The paucity of data on gold(III) complexes probably derives from their high redox potential and relatively poor stability, which make their use rather problematic under physiological conditions. In recent years, gold(III) complexes with polyamines, polypyridines [6], porphyrins [7,8] and dithiocarbamate [9–11], have been synthesized and characterized, and showed sufficient stability under physiologically relevant conditions by introducing chelating ligands. Intriguingly, some of these gold(III) compounds displayed *in vitro* cytotoxicity comparable to or even greater than cisplatin toward a series of established human tumor cell lines, and only a minimal cross-resistance with the reference drug was observed. The ligands generally coordinated to metal ions by nitrogen or sulfur atom in these stable complexes. Guo et al. reported the cytotoxicity of three gold complexes [Au(Quinpy)Cl]Cl, [Au(Quingly)Cl]Cl and [Au(Quinala)Cl]Cl with three Au–N bonds. The cytotoxicity of [Au(Quinpy)Cl]Cl against A549 cells is about 3 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} [12]. Ronconi et al. synthesized a series of gold(III) complexes with Au–S bonds, most of them are much more cytotoxic *in vitro* than cisplatin [9]. In our present work, a series of gold(III) complexes with one Au–S and two Au–N bonds were synthesized, all of them have cytotoxicity against HeLa cell line, especially the complexes **1**, **4**, **5**, **7** and **8** show higher cytotoxicity than cisplatin.

Abbreviations: HQuinpy, *N*-(8-quinolyl)pyridine-2-carboxamide; HQuingly, *N*-(8-quinolyl)glycine-carboxamide; HQuinala, *N*-(8-quinolyl)-l-alanine-carboxamide; py, pyridine dhpy dihydropyrazole; DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OD, optical density; SD, standard deviation.

* Corresponding authors. Tel./fax: +86 0312 5079361.
E-mail addresses: wsx@hbu.edu.cn (S. Wang), jczhang6970@yahoo.com.cn (J. Zhang).

0223-5234/$ – see front matter Crown Copyright © 2011 Published by Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.02.031

2. Results and discussion

The complexes **1**–**8** were prepared by the reaction of $K[AuCl_4]\cdot H_2O$ with 5-aryl-3-(pyridin-2-yl)-4,5-dihydropyrazole-1-carbothioamide ($\mathbf{L_1}$–$\mathbf{L_8}$) in CH_3CN (as shown in Scheme 1).

The elemental analysis data of the complexes **1**–**8** are in good agreement with the calculated values. The molar conductances (80.3–101.3 Ω^{-1} cm^2 mol^{-1}) for the complexes **1**–**8** correspond to 1:1 electrolytes [13].

The IR spectra of the complexes **1**–**8** are similar. The band of υ_{NH} in the complexes **1**–**8** shifted to lower frequencies than those of free amide group, the band of $\upsilon_{C=S}$ disappeared and a new band appeared at 1593–1614 cm^{-1} (C=N–H), indicating that the thione have evolved to thiol. After complexation, the strong and sharp band of υ_{C-H} (py) in 3139–3167 cm^{-1} became to a weak broad band in 3035–3092 cm^{-1}, the bands of $\upsilon_{C=N}$ (py) in 1585–1606 cm^{-1} and $\upsilon_{C=N}$ (dhpy) in 1526–1566 cm^{-1} shifted to 1612–1620 and 1531–1593 cm^{-1}, a new band corresponding to υ_{Au-N} appeared in 410–424 cm^{-1} [14], suggesting that py and dhpy coordinated with gold through nitrogen atom. The bands of υ_{Au-S} and υ_{Au-Cl} are not observed for being in far-infrared region [15].

The UV–Vis spectra of ligands and complexes in methanol were measured. At a concentration of 1×10^{-4} M, three main absorption peaks at about 209, 230 and 329 nm for ligands are assigned to internal π-π^* type transition of substituted benzene, C=S and the conjugated system of py with C=N (dhpy), respectively. After the formation of complexes, the peak at about 209 nm has little change, confirming that the coordination has negligible effect on the UV characteristic of substituted benzene. The disappearance of the peak at about 230 nm may be caused by ligands stabilisation in the thiol form. The peak at 329 nm blue shifts by 7 nm, indicates that py and dhpy coordinate with gold through nitrogen atom. A shoulder peak appears at about 334 nm may be caused by charge transfer transition (metal ~ ligand) from gold *d*-orbital to a π^* orbital of the conjugated system of py with C=N (dhpy). Then the concentration is raised to 8×10^{-4} M, a new peak is found at about 407 nm which could be assigned to *d*–*d* transition of gold(III).

In ^{1}H NMR spectra, the δ of protons of the complexes **1**–**8** shifts to down field compared to free ligands. The two broad singlets in 8.13–8.24 and 6.94–8.09 ppm regions of $-NH_2$ (2H) are shifted to 8.09–9.04 and 8.76–8.87 ppm regions attributed to >C=NH (1H), which indicated that the thione of ligand tautomerized to thiol after complexation (See Supporting Information Fig. S1 and S2). The ^{13}C NMR spectra further provide support for the structure of the complexes. The δ of the C atom (**C**=S) shifted to down filed after metallation (See Supporting Information Fig S3 and S4), which is due to the thione/thiol evolution (in which a C=S double bond changes to a more shielding C=N double bond [16]).

As listed in Table 1, the thermal behaviour of the complexes **1**–**8** are similar. The mass loss (5.88–7.76 and 5.05–7.37%) in the first two stages (79–220 and 155–245 °C) corresponds to the loss of two HCl molecules. The 51.09–54.96% mass loss in the last stage at 225–940 °C coincides with the organic ligands of the complexes. The 64.57–68.66% total mass loss suggests the residue may be gold.

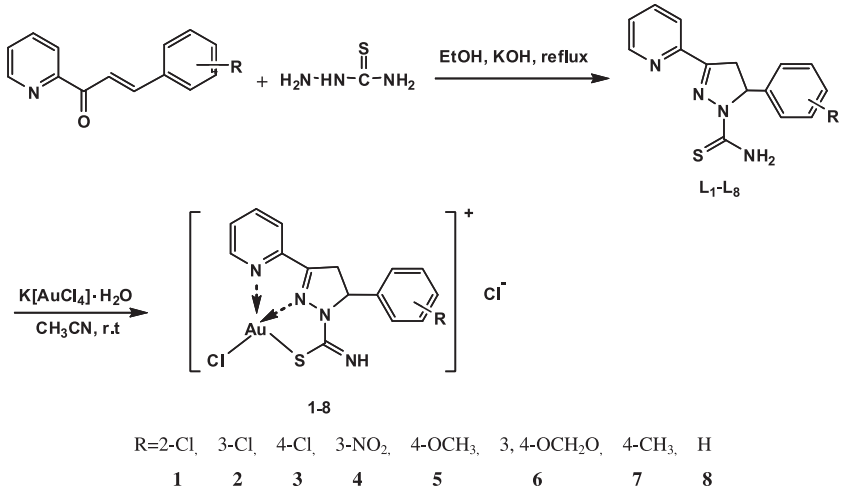
Based on all the above physical and spectral studies and relative literatures [13–17], we propose a tentative coordination mode for the complexes (Fig. 1).

As listed in Table 2, it can be seen that the complexes **1**–**8** exert cytotoxic effects against tested carcinoma cell lines with a lower IC_{50} value. The cytotoxicities of the complexes **1**–**8** are less active than that of cisplatin against A549 cell line. The complexes **2**, **3** and **6** have weaker cytotoxicity than cisplatin, but the complexes **1**, **4**, **5**, **7** and **8** have higher cytotoxicity than cisplatin against HeLa cell line.

In addition, the position and electronic effects of substituent group (*R*) on benzene of complexes shows important influence on cytotoxicity. For A549 cell line, when *R* is electron-withdrawing groups on *meta*-position (**2** and **4**) or electron-donating groups on *para*-position (**5**), the complexes show higher cytotoxicity. For HeLa cell line, when *R* are electron-withdrawing groups on *ortho*-position or *meta*-position (**1** and **4**) or *R* are electron-donating groups on *para*-position (**5** and **7**) have higher cytotoxicity, the complex whose benzene is unsubstituted (**8**) also exhibits high cytotoxicity toward the HeLa cell line. The effect of substituent groups on py and dhpy remained to be further studied.

3. Conclusion

In conclusion, the gold(III) complexes of 5-aryl-3-(pyridin-2-yl)-4,5-dihydropyrazole-1-carbothioamide derivatives have showed cytotoxicity against HeLa and A549 cell lines. It is to be pointed that, some of the complexes (**1**, **4**, **5**, **7**, and **8**) show higher cytotoxicity against HeLa cells than cisplatin. In addition, the position and electronic effects of substituent group (*R*) on benzene of complexes shows important influence on cytotoxicity. The results indicated that gold(III) complexes with 4,5-dihydropyrazole derivative might be a promising source of metal-based antitumor agents. Current studies are ongoing in our laboratory in order to gain a better insight in the mechanism of action of these gold(III) complexes,



Scheme 1. The synthetic routes of the complexes (**1**–**8**).

Table 1
Thermal analytical data of the complexes (**1**–**8**) with increasing rate of temperature of 10 °C min^{-1}.

Complex	Temp. range (°C)	Mass loss/% Found (calc.)	temp. range (°C)	Mass loss/% Found (calc.)	Temp. range (°C)	Mass loss/% Found (calc.)	Total mass loss/% Found (calc.)	Residue
1	79–210	5.88 (6.08)	210–240	6.68 (6.08)	240–940	53.81 (54.07)	66.37 (66.23)	Au
2	95–155	5.91 (6.08)	155–225	7.11 (6.08)	225–625	54.96 (54.07)	67.98 (66.23)	Au
3	90–205	6.69 (6.08)	205–225	5.53 (6.08)	225–930	52.64 (54.07)	64.86 (66.23)	Au
4	84–220	6.23 (5.98)	220–235	5.05 (5.98)	235–870	57.38 (54.88)	68.66 (66.84)	Au
5	100–155	7.76 (6.13)	155–245	5.16 (6.13)	245–920	51.63 (53.71)	64.57 (65.97)	Au
6	83–215	6.40 (5.99)	215–235	7.43 (5.99)	235–800	53.97 (54.81)	67.80 (66.79)	Au
7	85–190	6.54 (6.31)	190–235	7.37 (6.31)	235–635	51.09 (52.40)	65.00 (65.02)	Au
8	90–190	6.06 (6.44)	190–235	6.94 (6.44)	235–620	53.98 (51.18)	66.98 (64.06)	Au

which may be helpful for the design of new metal-based antitumor agents.

4. Experimental section

4.1. Materials

All chemicals and reagents were of analytical grade. RPMI-1640 medium, trypsin and fetal bovine serum were purchased from Gibco. MTT, penicillin and streptomycin were from sigma. Two different human carcinoma cell lines: HeLa (cervix carcinoma) and A549 (lung cancer) were obtained from American Type Culture Collection.

4.2. Instrumentation and measurement

Melting points were determined on an XT-4 microscopic melting-point spectrometer and are uncorrected. Elemental analyses were determined on an Elementar Vario EL III elemental analyzer. Molar conductances at room temperature were measured in 10^{-3} M methanol solutions using a DDS-12DW type conductivity meter. The IR spectra were recorded using KBr pellets and a Perkin–Elmer Model-683 spectrophotometer. The UV–Vis spectra at room temperature were measured in methanol solutions using a TU-1901 double beam UV–Vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVIII 600 NMR spectrometer. The mass spectra were measured by LC-MS apparatus Agilent 1200–6310. The thermal analysis was conducted using Netzsch (Germany) TG209c (N_2, 10 °C min^{-1}, Al_2O_3). The OD was measured on a microplate spectrophotometer (Bio-Rad Model 680, USA).

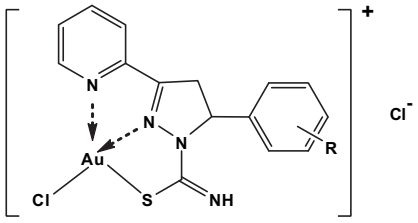
4.3. Synthesis of ligands

The azachalcones have been prepared as described in the literature [18].

The ligands $\mathbf{L_1}$–$\mathbf{L_8}$ was prepared as described in the literature [19]. A mixture of 0.243 g (0.001 mol) of (*E*)-3-(2-chlorophenyl)-1-(pyridin-2-yl) prop-2-en-1-one and 0.182 g (0.002 mol) of thiosemicarbazide is refluxed in 5 mL ethanol under vigorous stirring. After complete dissolution of the reactants, a solution of 0.112 g (0.002 mol) KOH in 2 mL ethanol is added dropwise. The solution is refluxed for a further 1 h. A precipitate is formed when the solution is cool, which is filtered off and crystallized from ethanol to give $\mathbf{L_1}$ as a pure product. $\mathbf{L_1}$: yield: 88.5%; m.p:221–223 °C; yellow solid; IR (KBr, cm^{-1}): 3403–3260 (N–H), 3148 (C–H, py), 1593 (C=N, py), 1563 (C=N, dhpy), 1349 (C=S); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.58–8.59 (m, 1H, py-H), 8.28 (d, 2H, py-H), 8.13 (br s, 1H, NH), 7.89–7.92 (m, 1H, py-H), 7.44–7.49 (m, 2H, ph-H), 7.26–7.29 (m, 2H, ph-H), 6.94 (br s, 1H, NH), 6.15 (dd,1H, J_{Ax} = 3.8 Hz, J_{Bx} = 11.6 Hz, H_x), 4.03 (dd,1H, J_{Bx} = 11.6 Hz, J_{AB} = 18.4 Hz, H_B), 3.04 (dd,1H, J_{Ax} = 3.8 Hz, J_{AB} = 18.4 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 41.6, 61.5, 122.0, 125.5, 127.9, 129.2, 130.2, 130.7, 137.2, 140.0, 150.0, 150.3, 156.3, 177.0; ESI-MS: 339.0 $[M + Na]^+$.

$\mathbf{L_2}$ was carried out in an identical manner to $\mathbf{L_1}$. $\mathbf{L_2}$: yield: 85.9%; m.p:168–169 °C; light yellow solid; IR (KBr, cm^{-1}): 3425–3260 (N–H), 3152 (C–H, py), 1593 (C=N, py), 1563 (C=N, dhpy), 1349 (C=S); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.59–8.61 (m, 1H, py-H), 8.29 (d, 1H, py-H), 8.24 (br s, 1H, NH), 8.09 (br s, 1H, NH),7.90–7.93 (m, 1H, py-H), 7.45–7.47 (m, 1H, py-H), 7.36 (t, 1H, ph-H), 7.29–7.31 (m, 1H, ph-H), 7.17 (t, 1H, ph-H), 7.10–7.11 (m, 1H, ph-H), 5.94 (dd,1H, J_{Ax} = 3.7 Hz, J_{Bx} = 11.6 Hz, H_x), 4.03 (dd,1H, J_{Bx} = 11.6 Hz, J_{AB} = 18.7 Hz, H_B), 3.04 (dd,1H, J_{Ax} = 3.7 Hz, J_{AB} = 18.7 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 42.3, 62.6, 121.6, 123.9, 124.9, 125.2, 126.9, 130.6, 133.0, 136.7, 145.3, 149.5, 149.9, 155.7, 176.5; ESI-MS: 339.1 $[M + Na]^+$.

$\mathbf{L_3}$ was carried out in an identical manner to $\mathbf{L_1}$. $\mathbf{L_3}$: yield: 91.0%; m.p:185–187 °C; white solid; IR (KBr, cm^{-1}): 3414–3270 (N–H),3156 (C–H, py), 1602 (C=N, py), 1565 (C=N, dhpy), 1343 (C=S); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.60–8.61 (m, 1H, py-H), 8.29 (d, 1H, py-H), 8.24 (br s, 1H, NH), 8.08 (br s, 1H, NH),7.90–7.93 (m, 1H, py-H), 7.45–7.47 (m, 1H, py-H), 7.36–7.39 (m, 2H, ph-H), 7.15–7.18 (m, 2H, ph-H), 5.93 (dd,1H, J_{Ax} = 3.7 Hz, J_{Bx} = 11.6 Hz, H_x), 3.95 (dd,1H, J_{Bx} = 11.6 Hz, J_{AB} = 18.6 Hz, H_B), 3.13 (dd,1H, J_{Ax} = 3.7 Hz, J_{AB} = 18.6 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 42.2, 62.5, 121.6, 124.9, 127.3, 128.5, 131.4, 136.7, 141.8, 149.5, 149.9, 155.7, 176.5; ESI-MS: 339.0 $[M + Na]^+$.



R=2-Cl, 3-Cl, 4-Cl, 3-NO_2, 4-OCH_3, 3, 4-OCH_2O, 4-CH_3, H

Fig. 1. Tentative coordination mode of the complexes.

Table 2
The cytotoxicity of the complexes (**1**–**8**) against A549 and HeLa cell lines.

Complex	IC_{50} (μM) ($\bar{x} \pm SD$)	
	A549	HeLa
cisplatin	4.66 ± 0.07	6.86 ± 0.18
1	36.23 ± 1.04	3.86 ± 0.13
2	7.18 ± 0.22	8.60 ± 0.15
3	34.21 ± 0.38	8.70 ± 0.49
4	10.04 ± 0.39	3.91 ± 0.10
5	12.69 ± 0.30	2.55 ± 0.08
6	12.73 ± 0.13	13.82 ± 0.42
7	29.22 ± 0.76	5.14 ± 0.18
8	51.18 ± 3.21	4.41 ± 0.37

L_4 was carried out in an identical manner to **L_1**. **L_4**: yield: 69.7%; m.p:226–227 °C; yellow solid; IR (KBr, cm^{-1}): 3416–3295 (N–H), 3167 (C–H, py), 1596 (C=N, py), 1526 (C=N, dhpy), 1344 (C=S); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.60–8.61 (m, 1H, py-H), 8.31 (d, 1H, py-H), 8.16 (br s, 1H, NH), 8.10–8.13 (m, 1H, py-H), 7.99 (t, 1H, py-H), 7.92–7.94 (t, 1H, ph-H), 7.69 (br s, 1H, NH), 7.63–7.66 (m, 2H, ph-H), 7.46–7.48 (m, 1H, ph-H), 6.10 (dd,1H, J_{Ax} = 4.2 Hz, J_{Bx} = 11.8 Hz, H_x), 3.95 (dd, 1H, J_{Bx} = 11.8 Hz, J_{AB} = 18.8 Hz, H_B), 3.13 (dd,1H, J_{Ax} = 4.2 Hz, J_{AB} = 18.8 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 42.1, 62.5, 120.2, 121.6, 122.0, 125.0, 130.2, 132.3, 136.7, 145.0, 147.8, 149.5, 149.8, 155.8, 176.6; ESI-MS: 350.0 $[M + Na]^+$.

L_5 was carried out in an identical manner to **L_1**. **L_5**: yield: 66.9%; m.p:197–199 °C; light yellow solid; IR (KBr, cm^{-1}): 3397–3262 (N–H), 3162 (C–H, py), 1606 (C=N, py), 1559 (C=N, dhpy), 1345 (C=S); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.60–8.61 (m, 1H, py-H), 8.29 (d, 1H, py-H), 8.12 (br s, 1H, NH), 7.99 (br s, 1H, NH), 7.90–7.93 (m, 1H, py-H), 7.45–7.47 (m, 1H, py-H), 7.05–7.08 (m, 2H, ph-H), 6.86–6.88 (m, 2H, ph-H), 5.89 (dd, 1H, J_{Ax} = 3.7 Hz, J_{Bx} = 11.5 Hz, H_x), 3.90 (dd, 1H, J_{Bx} = 11.5 Hz, J_{AB} = 18.6 Hz, H_B), 3.72 (s, 3H, OCH_3), 3.13 (dd,1H, J_{Ax} = 3.7 Hz, J_{AB} = 18.6 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 42.9, 55.5, 63.1, 114.3, 122.0, 125.3, 127.1, 135.4, 137.2, 150.0, 150.6, 156.3, 158.7, 177.0; ESI-MS: 335.1 $[M + Na]^+$.

L_6 was carried out in an identical manner to **L_1**. **L_6**: yield: 90.8%; m.p:178–179 °C; white solid; IR (KBr, cm^{-1}): 3431–3253 (N–H), 3139 (C–H, py), 1585 (C=N, py), 1567 (C=N, dhpy), 1350 (C=S); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.60–8.61 (m, 1H, py-H), 8.28 (d, 1H, py-H), 8.18 (br s, 1H, NH), 8.03 (br s, 1H, NH), 7.90–7.93 (m, 1H, py-H), 7.45–7.47 (m, 1H, py-H), 6.84 (d, 1H, ph-H), 6.67 (d, 1H, ph-H), 6.61 (dd, 1H, ph-H), 5.98 (d, 2H, OCH_2O), 5.85 (dd, 1H, J_{Ax} = 3.8 Hz, J_{Bx} = 11.5 Hz, H_x), 3.83 (dd, 1H, J_{Bx} = 11.5 Hz, J_{AB} = 18.6 Hz, H_B), 3.12 (dd,1H, J_{Ax} = 3.8 Hz, J_{AB} = 18.6 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 42.4, 62.9, 100.9, 105.9, 108.2, 118.3, 121.5, 124.8, 136.7, 136.8, 146.1, 147.3, 149.5, 150.0, 155.8, 176.5; ESI-MS: 349.0 $[M + Na]^+$.

L_7 was carried out in an identical manner to **L_1**. **L_7**: yield: 81.1%; m.p:153–154 °C; white solid; IR (KBr, cm^{-1}): 3407–3276 (N–H), 3155 (C–H, py), 1597 (C=N, py), 1566 (C=N, dhpy), 1342 (C=S); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.59–8.60 (m, 1H, py-H), 8.29 (d, 1H, py-H), 8.16 (br s, 1H, NH), 8.03 (br s, 1H, NH), 7.90–7.93 (m, 1H, py-H), 7.44–7.46 (m, 1H, py-H), 7.11 (d, 2H, ph-H), 7.02 (d, 2H, ph-H), 5.90 (dd, 1H, J_{Ax} = 3.7 Hz, J_{Bx} = 11.5 Hz, H_x), 3.91 (dd, 1H, J_{Bx} = 11.5 Hz, J_{AB} = 18.6 Hz, H_B), 3.11 (dd,1H, J_{Ax} = 3.7 Hz, J_{AB} = 18.6 Hz, H_A), 2.26 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ 20.6, 42.4, 62.8, 121.5, 124.8, 125.1, 129.0, 136.0, 136.7, 139.9, 149.0, 150.0, 155.7, 176.5; ESI-MS: 319.1 $[M + Na]^+$.

L_8 was carried out in an identical manner to **L_1**. **L_8**: yield: 94.3%; m.p:210–211 °C; light yellow solid; IR (KBr, cm^{-1}): 3378–3269 (N–H), 3156 (C–H, py), 1604 (C=N, py), 1565 (C=N, dhpy), 1345 (C=S); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.59–8.60 (m, 1H, py-H), 8.29 (d, 1H, py-H), 8.17 (br s, 1H, NH), 8.04 (br s, 1H, NH), 7.90–7.93 (m, 1H, py-H), 7.44–7.46 (m, 1H, py-H),, 7.31 (t, 2H, ph-H), 7.23 (t, 1H, ph-H), 7.14 (d, 2H, ph-H), 5.95 (dd, 1H, J_{Ax} = 3.6 Hz, J_{Bx} = 11.4 Hz, H_x), 3.94 (dd, 1H, J_{Bx} = 11.4 Hz, J_{AB} = 18.6 Hz, H_B), 3.13 (dd,1H, J_{Ax} = 3.6 Hz, J_{AB} = 18.6 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 42.9, 63.6, 122.0, 125.4, 125.7, 127.4, 129.0, 137.2, 143.3, 150.0, 156.2, 177.0; ESI-MS: 305.1 $[M + Na]^+$.

4.4. Synthesis of gold(III) complexes

To a rapidly stirred solution of $K[AuCl_4]\cdot H_2O$ (40 mg, 0.1 mmol) in 3 mL CH_3CN, the solution of **L_1** (31 mg, 0.1 mmol) in 3 mL CH_3CN was added in room temperature. After further 6 h, the resulting yellow precipitate was filtered. The collected solid was washed with cold CH_3CN (3 × 3 mL) and dried to give **1**. **1**: Yield: 51.4%; IR (KBr, cm^{-1}): 3089 (C–H, py), 1617 (C=N, py), 1590 (C=N, dhpy), 1612 (C=N, >C=N–H), 412 (Au–N); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.04 (s, 0.5H, NH), 8.87 (s, 0.5H, NH), 8.66 (d, 1H, py-H), 8.37 (d, 1H, py-H), 8.11–8.17 (m, 1H, py-H), 8.01–8.04 (m, 1H, py-H), 7.56–7.58 (m, 1H, ph-H), 7.53–7.55(m, 1H, ph-H), 7.34–7.39 (m, 2H, ph-H),6.14 (dd,1H, J_{Ax} = 3.6 Hz, J_{Bx} = 11.4 Hz, H_x), 4.19 (dd,1H, J_{Bx} = 11.4 Hz, J_{AB} = 19.2 Hz, H_B), 3.22 (dd,1H, J_{Ax} = 3.6 Hz, J_{AB} = 19.2 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 42.3, 61.8, 122.6, 125.9, 127.9, 129.5, 130.2, 130.5, 137.7, 148.5, 149.2, 149.3, 151.0, 197.5; Anal. Calc. for $C_{15}H_{12}N_4AuCl_3S$: C, 30.84; H, 2.06; N, 9.59. Found: C, 30.81; H, 1.77; N, 9.41.

The synthesis of **2** was carried out in an identical manner to **1**. **2**: Yield: 61.6%; IR (KBr, cm^{-1}): 3088 (C–H, py), 1619 (C=N, py), 1591 (C=N, dhpy), 1614 (C=N, >C=N–H), 414 (Au–N); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.94 (s, 0.5H, NH), 8.75 (s, 0.5H, NH), 8.68 (d, 1H, py-H), 8.37 (d, 1H, py-H), 8.06 (t, 1H, py-H), 7.60 (t, 1H, py-H), 7.41 (t, 1H, ph-H), 7.36 (d, 1H, ph-H), 7.27 (s, 1H, ph-H), 7.15 (d, 1H, ph-H), 5.95 (dd,1H, J_{Ax} = 3.6 Hz, J_{Bx} = 11.4 Hz, H_x), 4.10 (dd, 1H, J_{Bx} = 11.4 Hz, J_{AB} = 18.6 Hz, H_B), 3.22 (dd,1H, J_{Ax} = 3.6 Hz, J_{AB} = 18.6 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 43.3, 63.5, 122.8, 124.0, 125.4, 125.9, 127.7, 130.9, 133.4, 138.06, 138.1, 148.2, 148.8, 206.6; Anal. Calc. for $C_{15}H_{12}N_4AuCl_3S$: C, 30.84; H, 2.06; N, 9.59. Found: C, 30.38; H, 2.24; N, 9.50.

The synthesis of **3** was carried out in an identical manner to **1**. **3**: Yield: 58.2%; IR (KBr, cm^{-1}): 3082 (C–H, py), 1619 (C=N, py), 1593 (C=N, dhpy), 1613 (C=N, >C=N–H), 410 (Au–N); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.01 (s, 0.5H, NH), 8.84 (s, 0.5H, NH), 8.69 (d, 1H, py-H), 8.38 (d, 1H, py-H), 8.06 (t, 1H, py-H), 7.59–7.61 (m, 1H, py-H), 7.44 (d, 2H, ph-H), 7.23 (d, 2H, ph-H), 5.94 (dd,1H, J_{Ax} = 3.6 Hz, J_{Bx} = 11.4 Hz, H_x), 4.11 (dd, 1H, J_{Bx} = 11.4 Hz, J_{AB} = 19.2 Hz, H_B), 3.29 (dd,1H, J_{Ax} = 3.6 Hz, J_{AB} = 19.2 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 43.4, 63.5, 122.8, 125.9, 127.4, 128.9, 132.3, 137.9, 138.0, 148.3, 148.9, 149.0, 203.2; Anal. Calc. for $C_{15}H_{12}N_4AuCl_3S$: C, 30.84; H, 2.06; N, 9.59. Found: C, 30.39; H, 2.31; N, 9.33.

The synthesis of **4** was carried out in an identical manner to **1**. **4**: Yield: 62.3%; IR (KBr, cm^{-1}): 3090 (C–H, py), 1620 (C=N, py), 1531 (C=N, dhpy), 1594 (C=N, >C=N–H), 412 (Au–N); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.04 (s, 0.5H, NH), 8.87 (s, 0.5H, NH), 8.69 (d, 1H, py-H), 8.19 (d, 1H, py-H), 8.16–8.18 (m, 1H, py-H), 8.06–8.07 (m, 2H, a py-H and a ph-H), 7.67–7.70 (m, 2H, ph-H), 7.59–7.62 (m, 1H, ph-H), 6.12 (dd,1H, J_{Ax} = 3.6 Hz, J_{Bx} = 11.4 Hz, H_x), 4.14 (dd, 1H, J_{Bx} = 11.4 Hz, J_{AB} = 18.6 Hz, H_B), 3.36 (dd,1H, J_{Ax} = 3.6 Hz, J_{AB} = 18.6 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 43.0, 63.2, 115.1, 120.5, 122.6, 122.7, 122.9, 125.9, 130.6, 130.8, 132.3, 148.0, 148.2, 148.3, 203.4; Anal. Calc. for $C_{15}H_{12}O_2N_5AuCl_2S$: C, 30.33; H, 2.02; N, 11.78. Found: C, 30.43; H, 2.06; N, 11.55; ESI-MS: 635.4 $[M + H_2O + Na]^+$.

The synthesis of **5** was carried out in an identical manner to **1**. **5**: Yield: 55.3%; IR (KBr, cm^{-1}): 3092 (C–H, py), 1612 (C=N, py), 1577 (C=N, dhpy), 1595 (C=N, >C=N–H), 421 (Au–N); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.92 (s, 0.5H, NH), 8.76 (s, 0.5H, NH), 8.68 (d, 1H, py-H), 8.38 (d, 1H, py-H), 8.03 (t, 1H, py-H), 7.57–7.59 (m, 1H, py-H), 7.12 (d, 2H, ph-H), 6.92 (d, 2H, ph-H), 5.86 (dd,1H, J_{Ax} = 3.6 Hz, J_{Bx} = 10.8 Hz, H_x), 4.08 (dd, 1H, J_{Bx} = 10.8 Hz, J_{AB} = 18.6 Hz, H_B), 3.76 (s, 3H, OCH_3),3.27 (dd,1H, J_{Ax} = 3.6 Hz, J_{AB} = 18.6 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 43.5, 55.1, 63.6, 114.2, 122.6, 125.8, 126.8, 137.7, 148.6, 149.1, 149.2, 154.7, 158.7, 187.1; Anal. Calc. for $C_{16}H_{15}ON_4AuCl_2S$: C, 33.16; H, 2.59; N, 9.67. Found: C, 32.86; H, 2.78; N, 9.47.

The synthesis of **6** was carried out in an identical manner to **1**. **6**: Yield: 56.7%; IR (KBr, cm^{-1}): 3036 (C–H, py), 1620 (C=N, py), 1569 (C=N, dhpy), 1593 (C=N, >C=N–H), 413 (Au–N); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.02 (s, 0.5H, NH), 8.83 (s, 0.5H, NH), 8.67 (d, 1H, py-H), 8.38 (d, 1H, py-H), 8.00–8.03 (m, 1H, py-H), 7.56–7.58 (m, 1H, py-H), 6.89 (d, 1H, ph-H), 6.75 (d, 1H, ph-H), 6.67(dd, 1H, ph-H), 6.02 (d, 2H, OCH_2O), 5.82 (dd,1H, J_{Ax} = 3.6 Hz, J_{Bx} = 11.4 Hz, H_x), 4.08 (dd, 1H, J_{Bx} = 11.4 Hz, J_{AB} = 18.6 Hz, H_B), 3.28 (dd, 1H, J_{Ax} = 3.6 Hz, J_{AB} = 18.6 Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 43.7, 63.1, 101.2,

105.9, 106.8, 108.2, 118.8, 122.6, 125.8, 137.4, 137.5, 146.8, 147.8, 148.7, 149.3, 149.4, 178.8; Anal. Calc. for $C_{16}H_{14}N_4O_2AuCl_2S$: C, 32.38; H, 2.19; N, 9.44. Found: C, 31.92; H, 2.47; N, 9.14; ESI-MS: 634.3 $[M + H_2O + Na]^+$.

The synthesis of **7** was carried out in an identical manner to **1**. **7**: Yield: 64.1%; IR (KBr, cm^{-1}): 3080 (C—H, py), 1620 (C═N, py), 1593 (C═N, dhpy), 1614 (C═N, >C═N—H), 410 (Au—N); ^1H NMR (600 MHz, DMSO-d_6) δ 8.99 (s, 0.5H, NH), 8.83 (s, 0.5H, NH), 8.69 (d, 1H, py-H), 8.39 (d, 1H, py-H), 8.07 (t, 1H, py-H), 7.60–7.62 (m, 1H, py-H), 7.17 (d, 2H, ph-H), 7.08 (d, 2H, ph-H), 5.89 (dd,1H, $J_{Ax} = 3.6$ Hz, $J_{Bx} = 11.4$ Hz, H_x), 4.09 (dd, 1H, $J_{Bx} = 11.4$ Hz, $J_{AB} = 18.6$ Hz, H_B), 3.27 (dd, 1H, $J_{Ax} = 3.6$ Hz, $J_{AB} = 18.6$ Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 19.6, 42.3, 62.8, 121.8, 124.3, 124.8, 125.2, 128.3, 136.0, 137.1, 137.2, 147.0, 147.6, 203.0; Anal. Calc. for $C_{16}H_{15}N_4AuCl_2S$: C, 34.10; H, 2.66; N, 9.95. Found: C, 34.44; H, 3.11; N, 10.25.

The synthesis of **8** was carried out in an identical manner to **1**. **8**: Yield: 54.7%; IR (KBr, cm^{-1}): 3035 (C—H, py), 1620 (C═N, py), 1591 (C═N, dhpy), 1614 (C═N, >C═N—H), 412 (Au—N); ^1H NMR (600 MHz, DMSO-d_6) δ 9.00 (s, 0.5H, NH), 8.83 (s, 0.5H, NH), 8.69 (d, 1H, py-H), 8.39 (d, 1H, py-H), 8.07 (t, 1H, py-H), 7.60–7.62 (m, 1H, py-H), 7.38 (t, 2H, ph-H), 7.31(t, 1H, ph-H), 7.20 (d, 2H, ph-H), 5.94 (dd,1H, $J_{Ax} = 3.6$ Hz, $J_{Bx} = 11.4$ Hz, H_x), 4.12 (dd, 1H, $J_{Bx} = 11.4$ Hz, $J_{AB} = 18.6$ Hz, H_B), 3.29 (dd, 1H, $J_{Ax} = 3.6$ Hz, $J_{AB} = 18.6$ Hz, H_A); ^{13}C NMR (150 MHz, DMSO-d_6) δ 43.5, 64.1, 122.8, 125.3, 125.9, 126.3, 127.8, 128.9, 138.1, 140.2, 148.2, 148.9, 202.8; Anal. Calc. for $C_{15}H_{13}N_4AuCl_2S$: C, 32.79; H, 2.37; N, 10.20. Found: C, 32.43; H, 2.54; N, 9.86.

4.5. Cell culture

Two human carcinoma cell lines were used for cytotoxicity determination: HeLa and A549. They were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/mL of penicillin and 100 μg/mL of streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air.

4.6. Solutions

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

4.7. 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 described by Mosmann [20]. Upon completion of the incubation for 44 h, stock MTT dye solution (20 mL, 5 mg/mL) was added to each well. After 4 h incubation, 2-propanol (100 mL) was added to solubilize the MTT formazan. The OD of each well was measured on a microplate spectrophotometer at a wavelength of 570 nm. The IC_{50} value was determined from plot of % viability against dose of compounds added.

Acknowledgements

This work was supported by the Special Foundation for State Major New Drug Research Program of China (Grant No. 2009ZX09103-139), the National Basic Research 973 Program (Grant No. 2010CB534913), Natural Science Foundation of Hebei Province (Grant No. B2011201135), Nature Science Foundation for Distinguished Young Scholars of Hebei Province (Grant No. B2011201164), the Key Research Project Foundation of Department of Education of Hebei Province (Grant No. ZD2010142).

Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.02.031.

References

[1] S. Komeda, M. Lutz, A. Spek, M. Chikuma, J. Reedijk, Inorg. Chem. 39 (2000) 4230–4236.
[2] J. Reedijk, Chem. Commun. (1996) 801–806.
[3] J. Reedijk, Chem. Rev. 99 (1999) 2499–2510.
[4] S. Van Zutphen, J. Reedijk, Coord. Chem. Rev. 249 (2005) 2845–2853.
[5] J. Van der Veer, G. Van der Marel, H. Van den Elst, J. Reedijk, Inorg. Chem. 26 (1987) 2272–2275.
[6] L. Messori, F. Abbate, G. Marcon, P. Orioli, M. Fontani, E. Mini, T. Mazzei, S. Carotti, T. O'Connell, P. Zanello, J. Med. Chem. 43 (2000) 3541–3548.
[7] Y.F. To, R.W.Y. Sun, Y.X. Chen, V.S.F. Chan, W.Y. Yu, P.K.H. Tam, C.M. Che, C.L.S. Lin, Int. J. Cancer 124 (2009) 1971–1979.
[8] R.W.Y. Sun, C.K.L. Li, D.L. Ma, J.J. Yan, C.N. Lok, C.H. Leung, N.Y. Zhu, C.M. Che, Chem. Eur. J. 16 (2010) 3097–3113.
[9] L. Ronconi, L. Giovagnini, C. Marzano, F. Bettio, R. Graziani, G. Pilloni, D. Fregona, Inorg. Chem. 44 (2005) 1867–1881.
[10] L. Ronconi, C. Marzano, P. Zanello, M. Corsini, G. Miolo, C. Macca, A. Trevisan, D. Fregona, J. Med. Chem. 49 (2006) 1648–1657.
[11] L. Giovagnini, L. Ronconi, D. Aldinucci, D. Lorenzon, S. Sitran, D. Fregona, J. Med. Chem. 48 (2005) 1588–1595.
[12] T. Yang, C. Tu, J.Y. Zhang, L.P. Lin, X.M. Zhang, Q. Liu, J. Ding, Q. Xu, Z.J. Guo, Dalton Trans. (2003) 3419–3424.
[13] W.J. Geary, Coord. Chem. Rev. 7 (1971) 81–122.
[14] D. Kovala-Demertzi, Bull. Chem. Soc. Jpn. 64 (1991) 744–746.
[15] J.S. Casas, M.V. Castaño, M.C. Cifuentes, J.C. Garcia-Monteagudo, A. Sánchez, J. Sordo, U. Abram, J. Inorg. Biochem. 98 (2004) 1009–1016.
[16] J.D. Curry, R.J. Jandacek, J. Chem. Soc. Dalton Trans. (1972) 1120–1123.
[17] L. Pizzuti, L.A. Piovesan, A.F.C. Flores, F.H. Quina, C.M.P. Pereira, Ultrason. Sonochem. 16 (2009) 728–731.
[18] Z. Zhang, Y.W. Dong, G.W. Wang, Chem. Lett. 32 (2003) 966–967.
[19] F. Chimenti, E. Maccioni, D. Secci, J. Med. Chem. 48 (2005) 7113–7122.
[20] T. Mosmann, J. Immunol. Meth. 65 (1983) 55–63.