Full Paper

Applied Organometallic Chemistry

Received: 11 December 2012 Revised: 26 February 2013 Accepted: 3 March 2013 Published online in Wiley Online Library: 21 May 2013

(wileyonlinelibrary.com) DOI 10.1002/aoc.2988

Synthesis, characterization and cytotoxicity of Pt(II), Pd(II), Cu(II) and Zn(II) complexes with 4′-substituted terpyridine

Shuxiang Wang[a,b]*, Wenhao Chu[a], Yuechai Wang[a], Siyuan Liu[a], Jinchao Zhang[a,b], Shenghui Li[a], Haiying Wei[a], Guoqiang Zhou[a] and Xinying Qin[a]

Eight novel Pt(II), Pd(II), Cu(II) and Zn(II) complexes with 4′-substituted terpyridine were synthesized and characterized by elemental analysis, UV, IR, NMR, electron paramagnetic resonance, high-resolution mass spectrometry and molar conductivity measurements. The cytotoxicity of these complexes against HL-60, BGC-823, KB and Bel-7402 cell lines was evaluated by MTT assay. All the complexes displayed cytotoxicity with low IC_{50} values (<20 μM) and showed selectivity. Complexes 3, 5, 7 and 8 exerted 9-, 5-, 12- and 7-fold higher cytotoxicity than cisplatin against Bel-7402 cell line. The cytotoxicity of complexes 3, 5, 6, 7 and 8 was higher than that of cisplatin against BGC-823 cell line. Complexes 3, 7 and 8 showed similar cytotoxicity to cisplatin against KB cell line. Complex 7 exhibited higher cytotoxicity than cisplatin against HL-60 cell line. Among these complexes, complex 7 demonstrated the highest *in vitro* cytotoxicity, with IC_{50} values of 1.62, 3.59, 2.28 and 0.63 μM against HL-60, BGC-823, Bel-7402 and KB cells lines, respectively. The results suggest that the cytotoxicity of these complexes is related to the nature of the terminal group of the ligand, the metal center and the leaving groups.

Keywords: Pt(II), Pd(II), Cu(II) and Zn(II) complexes; 4′-substituted terpyridine; cytotoxicity

Introduction

Cisplatin is one of the most effective drugs in the treatment of testicular, ovarian, small cell lung, bladder, cervical, head and neck carcinomas.[1–5] Regardless of the achievements of current platinum drugs, there are some major drawbacks, including effectiveness against only a limited range of cancers, drug resistance and severe side effects.[6,7] These problems provide an incentive to discover new metal-based anticancer drugs.

Transition metal complexes as antitumor agents have been extensively used following the success of cisplatin.[8] Among the non-platinum compounds for cancer treatment, palladium (II) derivatives have been reported to possess antitumor activity at least comparable to cisplatin, while they exhibit less kidney toxicity.[9–11] Khan *et al.* [7] reported a series of mixed-ligand dithiocarbamate Pd(II) complexes. The antitumor screening of these compounds showed them to be highly active against cisplatin-resistant DU145 human prostate cancer cells. Ulukaya *et al.*[8] reported a Pd(II) complex based on terpyridine (tpy) and saccharin, which exhibited a more powerful antigrowth effect than its Pt(II) analog and cisplatin against human non-small cell lung cancer cells. Copper(II) is known to be involved in many biological processes and its role in increasing antitumor efficacy of organic molecules has been established.[12,13] Roy *et al.*[14] synthesized copper(II) complexes with terpyridyl and phenanthroline bases in a distorted square-pyramidal coordination geometry; these copper(II) complexes showed significant cytotoxicity with low IC_{50} value. Patel *et al.*[15] reported some copper(II) complexes based on tpy and norfloxacin, and all the complexes exhibited good cytotoxic activity. Zinc(II) is a vital component, an essential cofactor, critical for numerous cellular processes, and may be a major regulatory ion in the metabolism of cells.[16] The synthesis of new Zn(II) complexes, study of their pharmacological properties and their screening as antitumor agents constitute a matter of current interest.[17] Stanojkovic *et al.*[18] reported several zinc(II) complexes based on 2-acetylpyridine thiosemicarbazone derivatives, with IC_{50} values ranging from 26 to 90 nM, against HeLa (cervical adenocarcinoma), K562 (chronic myelogenous leukemia), and MDA-MB-361 and MDA-MB-453 (breast cancer) cell lines.

Coordination chemistry of multidentate α-tpy-based ligands is one of the most interesting research subjects because of their application in molecular probes for biochemical research.[4] Metal complexes of tpy can efficiently intercalate into nucleic acid to be effectively performed as potential antitumor agents.[5] Modification of tpy generated a variety of derivatives and structural variation of tpy may have a significant impact on the antitumor activity of their complexes.[13–19] In order to further explore the structure–activity relationships and discover new metal-based

* *Correspondence to: Shuxiang Wang, Key Laboratory of Chemical Biology of Hebei Province, College of Chemistry and Environmental Science, Hebei University, Baoding 071002, Hebei, People's Republic of China. Email: wsx@hbu.edu.cn*

a *Key Laboratory of Chemical Biology of Hebei Province, College of Chemistry and Environmental ScienceHebei University, Baoding 071002, People's Republic of China*

b *Key Laboratory of Medicinal Chemistry and Molecular Diagnosis of Ministry of Education, Hebei University, Baoding 071002, People's Republic of China*

anticancer drugs, the synthesis, characterization and cytotoxicity of eight new metal (Pt(II), Pd(II), Cu(II) and Zn(II)) complexes with 4′-substitued tpy are described in the present work for the first time. The results of the cytotoxicity experiments indicated that the metal (Pt(II), Pd(II), Cu(II) and Zn(II)) complexes with 4′-substitued tpy exerted cytotoxic effects against HL-60 (immature granulocyte leukemia), BGC-823 (gastrocarcinoma), Bel-7402 (liver carcinoma) and KB (nasopharyngeal carcinoma) cell lines.

Experimental

Materials and Measurements

All chemicals and reagents were of analytical grade. DMSO-d_6 was purchased from Separation (Beijing) Technology Co. Ltd. RPMI-1640 medium, trypsin and fetal bovine serum were purchased from Gibco. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), benzyl penicillin and streptomycin were from Sigma. Four different human carcinoma cell lines – HL-60, BGC-823, Bel-7402 and KB – were obtained from Peking University Health Science Center.

Elemental analyses were determined on an Elementar Vario EL III elemental analyzer. The UV spectra were measured using a TU-1901 double-beam UV-visible spectrophotometer (Beijing Purkinje General Instrument Co. Ltd). The IR spectra were recorded using KBr pellets and a PerkinElmer Model-683 spectrophotometer. The far-IR spectra were recorded on NICOLET 750. The ^{1}H NMR spectra were recorded on a Bruker AVIII 600 NMR spectrometer. Electron paramagnetic resonance (EPR) was recorded on Bruker E500. Electrospray ionization (ESI) high-resolution mass spectrometry (HRMS) was performed by apex ultra 7.0T US+. Molar conductance at room temperature was measured in 1×10^{-3} M methanol or DMF solution using a DDS-12DW type conductivity meter. Optical density (OD) was measured on a microplate spectrophotometer (Bio-RadModel 680, USA).

Synthesis of Ligands

*4′-(4-Hydroxyphenyl)-2,2′,6′,2″-terpyridine (**L_a**)*

4-Hydroxybenzaldehyde (2.44 g, 20 mmol) was dissolved in 100 ml of 95% ethanol solution, following addition of 2-acetylpyridine (4.84 g, 40 mmol) and KOH (4.2 g). After stirring for a few minutes, aqueous NH_3 (50 ml, 25%) was added to the solution and the mixture was stirred at 50°C overnight. A large amount of green solid was obtained when the pH of the solution was changed to 3–4 followed by evaporation of ethanol in the solution. The precipitate was filtrated and washed with ethanol (3×10 ml) and recrystallized with methanol. A green solid was obtained after drying. Yield 2.23 g, 34.3%; m.p. > 250°C; UV (methanol, nm) 203, 229, 251, 285; IR (KBr, cm^{-1}) 3435 (OH), 3071–3039 (C—H), 1521 (C=N); ^{1}H NMR (CD_3OD, 600 MHz) δ/ppm: 9.08 (m, 4H, 6,6″-H, 3,3″-H, tpy), 8.91 (s, 2H, 3′,5′-H, tpy), 8.72 (td, 2H, J = 7.8, 1.2 Hz, 4,4″-H, tpy), 8.14 (td, 2H, J = 7.8, 1.2 Hz, 5,5″-H, tpy), 8.02 (d, 2H, J = 9.0 Hz, 3,5-H, ph), 7.05 (d, 2H, J = 9.0 Hz, 2,6-H, ph); ^{13}C NMR (CD_3OD, 150 MHz) δ/ppm: 160.5 (1-C, ph), 156.3 (2C, 2′,6′-C, tpy), 155.9 (2C, 2,2″-C, tpy), 149.8 (4′-C, tpy), 149.1 (2C, 6,6″-C, tpy), 136.8 (2C, 4,4″-C, tpy), 131.7 (4-C, ph), 129.4 (2C, 3′,5′-C, tpy), 123.7 (2C, 3,3″-C, tpy), 121.2 (2C, 5,5″-C, tpy), 118.4 (2C, 3′,5′-C, tpy), 116.5 (2C, 2,6-C, ph); Anal. Calcd for $C_{21}H_{15}N_3O$: C, 77.52; H, 4.65; N, 12.91. Found: C, 77.54; H, 4.63; N, 12.88; HRMS (+)-ESI m/z $[M+H]^+$ calcd for $[C_{21}H_{16}N_3O]^+$: 326.1288; found: 326.1287.

*4′-(4-Carboxymethylphenyl)-2,2′,6′,2″-terpyridine (**L_b**)*

The synthesis of **L_b** was carried out in a similar manner to **L_a** starting from chloroacetic acid (3.2 g, 32 mmol),4-hydroxybenzaldehyde (4.88 g, 40 mmol), 2-acetylpyridine (2.03 g, 16.8 mmol) and aqueous NH_3 (40 ml, 25%). White solid; yield 1.04 g, 32.4%; m.p. > 250°C; UV (methanol, nm) 206, 227, 251, 285; IR (KBr, cm^{-1}) 3059–3029 (C—H), 1521 (C=N); ^{1}H NMR (CD_3OD, 600 MHz) δ/ppm: 8.76 (d, 2H, J = 4.8 Hz, 6,6″-H, tpy), 8.67 (s, 2H, 3′,5′-H, tpy), 8.65 (dd, 2H, J = 7.8, 1.2 Hz, 3,3″-H), 8.02 (td, 2H, J = 7.8, 1.2 Hz, 4,4″-H, tpy), 7.87 (d, 2H, J = 9.0 Hz, 3,5-H, ph), 7.52 (ddd, 2H, J = 7.8, 4.8, 1.2 Hz, 5-5″-H, tpy), 7.13 (d, 2H, J = 9.0 Hz, 2,6-H, ph), 4.79 (s, 2H, CH_2);^{13}C NMR (CD_3OD, 150 MHz) δ/ppm: 174.8 (COOH), 160.1 (1-C, ph), 156.1 (2C, 2′,6′-C, tpy), 155.7 (2C, 2,2″-C, tpy), 150.0 (4′-C, tpy), 148.7 (2C, 6,6″-C, tpy), 137.4 (2C, 4,4″-C, tpy), 130.1 (4-C, ph), 127.8 (2C, 3,5-C, ph), 124.0 (2C, 3,3″-C, tpy), 121.6 (2C, 5,5″-C, tpy), 117.8 (2C, 3′,5′-C, tpy), 115.1 (2C, 2,6-C, ph), 67.1 (CH_2); Anal. Calcd for $C_{23}H_{17}N_3O_3$: C, 72.05; H, 4.47; N, 10.96. Found: C, 72.09; H, 4.47; N, 10.95; HRMS (+)-ESI m/z $[M+H]^+$ calcd for $[C_{23}H_{18}N_3O_3]^+$: 384.1343; found: 384.1343.

*4′-(4-(2-Morpholinoethoxy)phenyl)-2,2′,6′,2″-terpyridine (**L_c**)*

The synthesis of **L_c** was carried out in a similar manner to **L_a** starting from 4-hydroxybenzaldehyde (2.44 g, 20 mmol),*N*-(2-Chloroethyl) morpholine hydrochloride (3.68 g, 20 mmol), 2-acetylpyridine (4.2 g, 40 mmol) and aqueous NH_3 (40 ml, 25%). White solid; yield 2.94 g, 33.6%; m.p. 165–166.1°C; UV (methanol, nm) 203, 227, 251, 285; IR (KBr, cm^{-1}) 3059–3039 (C—H), 1521 (C=N); ^{1}H NMR ($CDCl_3$, 600 MHz) δ/ppm: 8.71 (d, 2H, J = 4.8 Hz, 6,6″-H, tpy), 8.69 (s, 2H, 3′,5′-H), 8.64 (d, 2H, J = 7.8 Hz, 3,3″-H, tpy), 7.84 (m, 4H, 4,4″-H, tpy, 3,5-H, ph), 7.31 (dd, 2H, J = 7.8, 4.8 Hz, 5,5″-H, tpy), 7.01 (d, 2H, J = 8.4 Hz, 2,6-H, ph), 4.15 (t, 2H, J = 3.0 Hz, O-CH_2), 3.75 (t, 4H, J = 3.0 Hz, O-CH_2, morpholine), 2.81 (t, 2H, J = 3.0 Hz, N-CH_2), 2.60 (t, 4H, J = 3.0 Hz, N-CH_2, morpholine); ^{13}C NMR ($CDCl_3$, 150 MHz) δ/ppm: 159.7 (1-C, ph), 156.4 (2C, 2′,6′-C, tpy), 155.9 (2C, 2,2″-C, tpy), 149.7 (4′-C, tpy), 149.1 (2C, 6,6″-C, tpy), 136.8 (2C, 4,4″-C, tpy), 131.0 (4-C, ph), 128.6 (2C, 3,5-C, ph), 123.8 (2C, 3,3″-C, tpy), 121.4 (2C, 5,5″-C, tpy), 118.3 (2C, 3′,5′ -C, tpy), 115.0 (2C, 2,6-C, ph), 67.0 (2C, O—CH_2, morpholine), 66.0 (O—CH_2), 57.7 (N—CH_2), 54.2 (2C, N—CH_2, morpholine); Anal. Calcd for $C_{27}H_{26}N_4O_2$: C, 73.95; H, 5.98; N, 12.78. Found: C, 73.93; H, 5.95; N, 12.76; HRMS (+)-ESI m/z $[M+H]^+$ calcd for $[C_{27}H_{27}N_4O_2]^+$: 439.2129; found: 439.2128.

*4′-(4-(2-(Piperidin-1-yl)ethoxy)phenyl)-2,2′,6′,2″-terpyridine) (**L_d**)*

The synthesis of **L_d** was carried out in a similar manner to **L_a** starting from 4-hydroxybenzaldehyde (2.44 g, 20 mmol), *N*-(2-chloroethyl) piperidine hydrochloride (3.68 g, 20 mmol), 2-acetylpyridine (4.2 g, 40 mmol) and aqueous NH_3 (40 ml, 25%). White solid; yield 3.13 g, 35.9%; m.p. 134.7–135.3°C; UV (methanol, nm) 203, 227, 251, 285; IR (KBr, cm^{-1}) 3059–3039 (C—H), 1590 (C=N); ^{1}H NMR ($CDCl_3$, 600 MHz) δ/ppm: 8.76 (d, 2H, J = 4.8 Hz, 6,6″-H, tpy), 8.67 (s, 2H, 3′,5′-H, tpy), 8.65 (d, 2H, J = 7.8 Hz, 3,3″-H, tpy), 8.03 (t, 2H, J = 7.8 Hz, 4,4″-H, tpy), 7.87 (d, 2H, J = 8.4 Hz, 3,5-H, ph), 7.52 (dd, 2H, J = 7.8, 4.8 Hz, 5,5″-H, tpy), 7.13 (d, 2H, J = 8.4 Hz, 2,6-H, ph), 4.13 (t, 2H, J = 6.0 Hz, O—CH_2), 2.68 (t, 2H, J = 6.0 Hz, N-CH_2), 2.44 (s, 4H, N—CH_2, piperidine), 1.50 (m, 4H, —CH_2, piperidine), 1.38 (s, 2H, —CH_2, piperidine); ^{13}C NMR ($CDCl_3$, 150 MHz) δ/ppm: 159.8 (1-C, ph), 156.4 (2C, 2′,6′-C, tpy), 155.9 (2C, 2,2″-C, tpy), 149.8 (4′-C, tpy), 149.1 (2C, 6,6″-C, tpy), 136.8 (2C, 4,4″-C, tpy), 130.8 (4-C, ph), 128.5 (2C, 3,5-C, ph), 123.7 (2C, 3,3″-C, tpy), 121.4 (2C, 5,5″-C, tpy), 118.3 (2C, 3′,5′-C, tpy), 115.0 (2C, 2,6-C, ph),

66.2 (O−CH_2), 57.9 (N−CH_2), 55.1 (2C, N−CH_2, piperidine), 26.0 (2C, 2,4-C, piperidine), 24.2 (3-C, piperidine); Anal. Calcd for $C_{28}H_{28}N_4O$: C, 77.04; H, 6.46; N, 12.83. Found: C, 77.09; H, 6.49; N, 12.79; HRMS (+)-ESI *m/z* $[M+H]^+$ calcd for $[C_{28}H_{29}N_4O]^+$: 437.2336; found: 437.2335.

Synthesis of Metal Complexes

*[Pt(II)L_aCl]Cl (**1**)*

To 42 mg (0.1 mmol) K_2PtCl_4 dissolved in 10 ml water, 33 mg (0.1 mmol) **L_a** was added, the mixture was stirred at 30°C for 3 h before filtering, and the residue was washed with water. An orange solid was obtained after drying; yield 32 mg, 54.2%; UV (water, nm) 268, 324, 338 (IL), 386 (LMCT); IR (KBr, cm^{-1}) 3139–3049 (C−H), 1602 (C=N), 3447 (OH); far-IR (Nujol mull, cm^{-1}) 423.1 (Pt−N); ^{1}H NMR (DMSO-d_6, 600 MHz) δ/ppm:9.12 (d, 2H, J=4.8 Hz, 6,6″-H, tpy), 8.99 (d, 2H, J=4.8 Hz, 3,3″-H, tpy), 8.90 (s, 2H, 3′,5′-H, tpy), 8.57 (t, 2H, J=4.8 Hz, 4,4″-H, tpy), 8.02 (d, 2H, J=8.4 Hz, 3,5-H, ph), 7.98 (t, 2H, J=4.8 Hz, 5,5″-H, tpy), 7.01 (d, 2H, J=8.4 Hz, 2,6-H, ph); ^{13}C NMR (DMSO-d_6, 150 MHz) δ/ppm: 160.4 (1-C, ph), 159.5 (2C, 2′,6′-C, tpy), 158.4 (2C, 2,2″-C, tpy), 151.3 (4′-C, tpy), 149.8 (2C, 6,6″-C, tpy), 136.8 (2C, 4,4″-C, tpy), 131.6 (4-C, ph), 129.5 (2C, 3′,5′-C, tpy), 124.5 (2C, 3,3″-C, tpy), 121.4 (2C, 5,5″-C, tpy), 119.3 (2C, 3′,5′-C, tpy), 116.4 (2C, 2,6-C, ph); Anal. Calcd for [Pt(II)L_aCl]Cl · 3.5H_2O: C, 38.54; H, 3.39; N, 6.42. Found: C, 38.02; H, 3.49; N, 6.33; HRMS (+)-ESI *m/z* $[M-Cl^-]^+$ calcd for $[C_{21}H_{15}ClN_3OPt]^+$: 555.0547; found: 555.0552; Λ_m (DMF, S m^2 mol^{-1}) 69.2.

*[Pd(II)L_bCl]Cl (**2**)*

To 33 mg K_2PdCl_4 (0.1 mmol) dissolved in 10 ml water, 38 mg **L_b** (0.1 mmol) in 5 ml methanol was added, the mixture was stirred at room temperature for 3 h before filtering, and the residue was washed with water and methanol. A yellow solid was obtained after drying; yield 35 mg, 57.1%; UV (water, nm) 244, 278, 349 (IL), 365 (LMCT); IR (KBr, cm^{-1}) 3071–3024 (C−H), 1602 (C=N); far-IR (Nujol mull, cm^{-1}) 438.5 (Pd−N); ^{1}H NMR (DMSO-d_6, 600 MHz) δ/ppm:8.79 (d, 2H, J=4.8 Hz, 6,6″-H, tpy), 8.68 (d, 2H, J=4.8 Hz, 3,3″-H, tpy), 8.64 (s, 2H, 3′,5′-H, tpy), 8.01 (t, 2H, J=4.8 Hz, 4,4″-H, tpy), 7.78 (d, 2H, J=7.8 Hz, 3,5-H, ph), 7.46 (t, 2H, J=4.8 Hz, 5,5″-H, tpy), 7.10 (d, 2H, J=7.8 Hz, 2,6-H, ph), 4.62 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz) δ/ppm: 174.6 (COOH), 160.1 (1-C, ph), 158.9 (2C, 2′,6′-C, tpy), 157.9 (2C, 2,2″-C, tpy), 149.7 (4′-C, tpy), 149.1 (2C, 6,6″-C, tpy), 138.5 (2C, 4,4″-C, tpy), 130.2 (4-C, ph), 127.4 (2C, 3,5-C, ph), 125.8 (2C, 3,3″-C, tpy), 121.7 (2C, 5,5″-C, tpy), 117.8 (2C, 3′,5′-C, tpy), 115.2 (2C, 2,6-C, ph), 67.3 (CH_2); Anal. Calcd for [Pd(II)L_bCl]Cl · H_2O: C, 47.73; H, 3.31; N, 7.26. Found: C, 48.09; H, 3.10; N, 7.18; HRMS (+)-ESI *m/z* $[M-Cl^-]^+$ calcd for $[C_{23}H_{17}ClN_3O_3Pd]^+$: 525.9992; found: 525.9997; Λ_m (DMF, S m^2 mol^{-1}) 68.1.

*Cu(II)L_aCl_2 (**3**)*

To 17 mg (0.1 mmol) $CuCl_2.2H_2O$ dissolved in 10 ml methanol, 33 mg **L_a** was added, the mixture was stirred at room temperature for 3 h before filtering, and the residue was washed with methanol. Green solid; yield 31 mg, 67.4%; UV (water, nm) 223, 265, 286 (IL), 333 (LMCT); IR (KBr, cm^{-1}) 3105–3024 (C−H), 1595 (C=N), 3401 (OH); far-IR (Nujol mull, cm^{-1}) 419.6 (Cu−N); Anal. Calcd for Cu(II)L_aCl_2: C, 54.85; H, 3.29; N, 9.14. Found: C, 54.86; H, 3.37; N, 8.95; HRMS (+)-ESI *m/z* $[M-Cl^-]^+$ calcd for $[C_{21}H_{15}ClCuN_3O]^+$: 423.0200; found: 423.0194; Λ_m (DMF, S m^2 mol^{-1}) 32.4.

*Zn(II)L_b(CH_3COO)$_2$ (**4**)*

The synthesis of **4** was carried out in a similar manner to **3** starting from **L_b** (38 mg, 0.1 mmol) and $Zn(CH_3COO)_2.2H_2O$ (22 mg, 0.1 mmol) in methanol. White solid; yield 34 mg, 61.2%; UV (water, nm) 233, 265, 284 (IL), 326 (LMCT); IR (KBr, cm^{-1}) 3071–3049 (C−H), 1594 (C=N); far-IR (Nujol mull, cm^{-1}) 413.2 (Zn−N); ^{1}H NMR (DMSO-d_6, 600 MHz) δ/ppm: 8.77 (d, 2H, J=4.8 Hz, 6,6″-H, tpy), 8.67 (m, 4H, 3,3″-H, tpy, 3′,5′-H, tpy), 8.04 (t, 2H, J=4.8 Hz, 4,4″-H, tpy), 7.83 (d, 2H, J=9.0 Hz, 3,5-H, ph), 7.53 (t, 2H, J=4.8 Hz, 5,5″-H, tpy), 7.01 (d, 2H, J=9.0 Hz, 2,6-H, ph), 4.20 (s, 2H, CH_2), 1.67 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6, 150 MHz) δ/ppm: 179.0 (2C, C=O), 174.6 (−COOH), 160.2 (1-C, ph), 157.2 (2C, 2′,6′-C, tpy), 156.6 (2C, 2,2″-C, tpy), 149.8 (4′-C, tpy), 148.9 (2C, 6,6″-C, tpy), 137.8 (2C, 4,4″-C, tpy), 130.1 (4-C, ph), 127.7 (2C, 3,5-C, ph), 124.5 (2C, 3,3″-C, tpy), 121.7 (2C, 5,5″-C, tpy), 117.5 (2C, 3′, 5′-C, tpy), 115.1 (2C, 2,6-C, ph), 67.2 (CH_2), 21.7 (2C, CH_3); Anal. Calcd for Zn(II)L_b(CH_3COO)$_2$: C, 57.21; H, 4.09; N, 7.41. Found: C, 57.48; H, 3.85; N, 7.91; HRMS (+)-ESI *m/z* $[M-Ac^-]^+$ calcd for $[C_{25}H_{20}N_3O_5Zn]^+$: 506.0694; found: 506.0698; Λ_m (DMF, S m^2 mol^{-1}) 26.2.

*Zn(II)L_c(CH_3COO)$_2$ (**5**)*

The synthesis of **5** was carried out in a similar manner to **3** starting from **L_c** (44 mg, 0.1 mmol) and $Zn(CH_3COO)_2.2H_2O$ (22 mg, 0.1 mmol) in methanol. White solid; yield 34 mg, 54.7%; yield 34 mg, 61.2%; UV (water, nm) 232, 264, 284 (IL), 326 (LMCT); IR (KBr, cm^{-1}) 3083–3049 (C−H), 1594 (C=N); far-IR (Nujol mull, cm^{-1}) 413.2 (Zn−N); ^{1}H NMR (DMSO-d_6, 600 MHz) δ/ppm: 8.88 (s, 2H, 6,6″-H, tpy), 8.79 (m, 4H, 3,3″-H, 3′,5′-H, tpy), 8.19 (m, 4H, 4,4″-H, tpy, 3,5-H, ph), 7.75 (t, 2H, J=5.4 Hz, 5,5″-H, tpy), 7.11 (d, 2H, J=7.8 Hz, 2,6-H, ph), 4.21 (t, 2H, J=5.4 Hz, −O−CH_2−), 3.63 (t, 4H, J=5.4 Hz, −O−CH_2−, morpholine), 2.77 (t, 2H, J=6.0 Hz, N−CH_2−), 2.53 (t, 4H, J=5.4 Hz, N−CH_2−, morpholine), 1.67 (s, 6H, CH_3−COO^-); ^{13}C NMR ($CDCl_3$, 150 MHz) δ/ppm: 179.0 (2C, C=O), 161.0 (1-C, ph), 157.6 (2C, 2′,6′-C, tpy), 156.8 (2C, 2,2″-C, tpy), 149.6 (4′-C, tpy), 149.2 (2C, 6,6″-C, tpy), 140.0 (2C, 4,4″-C, tpy), 131.4 (4-C, ph), 128.6 (2C, 3,5-C, ph), 126.4 (2C, 3,3″-C, tpy), 121.8 (2C, 5,5″-C, tpy), 117.9 (2C, 3′,5′-C, tpy), 114.8 (2C, 2,6-C, ph), 66.3 (2C, O−CH_2, morpholine), 65.4 (O−CH_2), 57.2 (N−CH_2), 53.9 (2C, N−CH_2, morpholine), 21.7 (2C, CH_3); Anal. Calcd for Zn(II)L_c(CH_3COO)$_2$ · 1.5H_2O: C, 57.37; H, 5.44; N, 8.63. Found: C, 57.06; H, 5.18; N, 8.69; HRMS (+)-ESI *m/z* $[M-Ac^-]^+$ calcd for $[C_{29}H_{29}N_4O_4Zn]^+$: 561.1474; found: 561.1483; Λ_m (methanol, S m^2 mol^{-1}) 52.3.

*Cu(II)L_c(CH_3COO)$_2$ (**6**)*

The synthesis of **6** was carried out in a similar manner to **3** starting from **L_c** (44 mg, 0.1 mg) and $Cu(CH_2COO)_2.H_2O$ (20 mg, 0.1 mmol) in methanol. Blue solid; yield 41 mg, 66.1%; UV (water, nm) 223, 266, 286 (IL), 334 (LMCT); IR (KBr, cm^{-1}) 3071–3049 (C−H), 1595 (C=N); far-IR (Nujol mull, cm^{-1}) 419.6 (Cu − N); Anal. Calc. for Cu(II)L_c(CH_3COO)$_2$.3H_2O: C, 55.23; H, 5.68; N, 8.31. Found: C, 54.90; H, 5.57; N, 8.24; HRMS (+)-ESI *m/z* $[M-Ac^-]^+$ calcd for $[C_{29}H_{29}CuN_4O_4]^+$: 560.1485; found: 560.1478; Λ_m (methanol, S m^2 mol^{-1}) 56.2.

*Cu(II)L_cCl_2 (**7**)*

The synthesis of **7** was carried out in a similar manner to **3** starting from **L_c** (44 mg, 0.1 mg) and $CuCl_2.2H_2O$ (17 mg, 0.1 mmol) in methanol. Green solid; yield 33 mg, 57.6%; UV (water, nm) 223,

265, 286 (IL), 334 (LMCT); IR (KBr, cm^{-1}) 3059–3039 (C−H), 1595 (C=N); far-IR (Nujol mull, cm^{-1}) 419.6 (Cu−N); Anal. Calcd for Cu (II)$L_cCl_2.2H_2O$: C, 53.25; H, 4.97; N, 9.20; Found: C, 53.42; H, 4.61; N, 8.96; HRMS (+)-ESI *m/z* $[M-Cl^-]^+$ calcd for $[C_{27}H_{26}ClCuN_4O_2]^+$: 536.1040; found: 536.1035; Λ_m (methanol, S m^2 mol^{-1}) 58.6.

*Cu(II)L_dCl_2 (**8**)*

The synthesis of **8** was carried out in a similar manner to **3** starting from $\mathbf{L_d}$ (44 mg, 0.1 mmol) and $CuCl_2.2H_2O$ (17 mg, 0.1 mmol) in methanol. Green solid; yield: 39 mg, 63.2%; yield 63.2%; UV (water, nm) 223, 265, 287 (IL), 332 (LMCT); IR (KBr, cm^{-1}) 3059–3039 (C−H), 1595 (C=N); far-IR (Nujol mull, cm^{-1}) 419.6 (Cu−N); Anal. Calc. for Cu(II)$L_dCl_2.H_2O$: C, 57.10; H, 5.13; N, 9.51. Found: C, 56.59; H, 4.66; N, 9.23; HRMS (+)-ESI *m/z* $[M-Cl^-]^+$ calcd for $[C_{28}H_{28}N_4OClCu]^+$: 534.1248; found: 534.1243; Λ_m (methanol, S m^2 mol^{-1}) 58.3.

Cytotoxic Studies

Cell culture

Four different human carcinoma cell lines – HL-60, BGC-823, KB, 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 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.

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^{-1}) 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 percent viability against dose of compounds added.

Results and Discussion

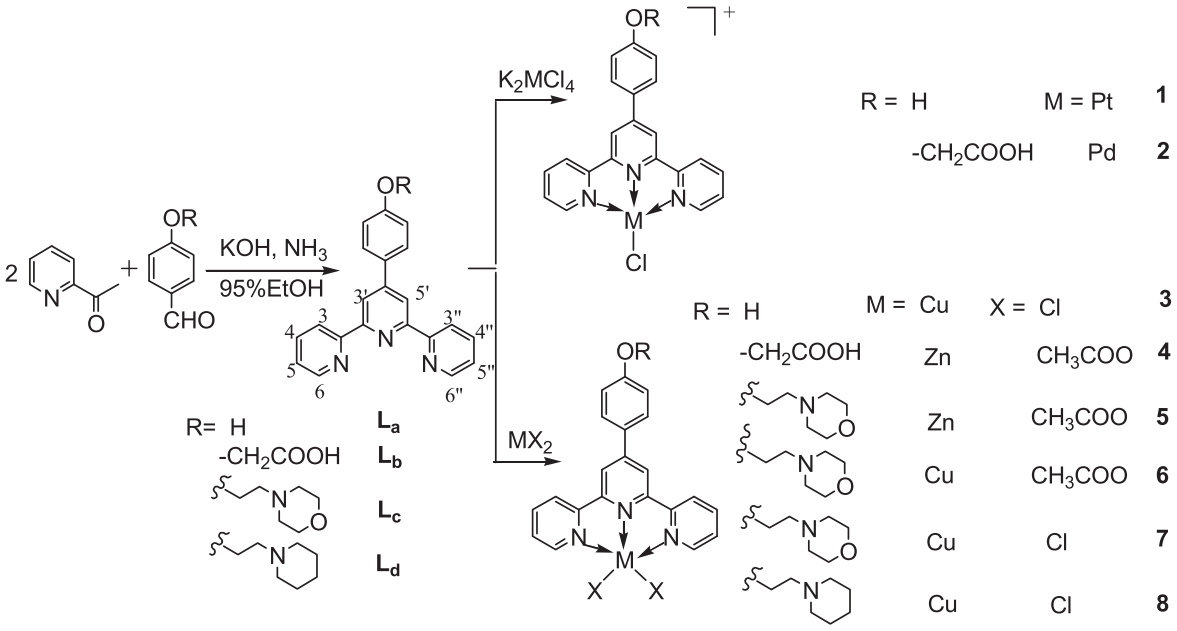
Synthesis and Characterization

The ligands $\mathbf{L_a}$–$\mathbf{L_d}$ were prepared following reported procedures,[19,21] and their structures and purity were identified by melting points, IR, NMR data, HRMS and element analysis.

All complexes were synthesized as described in the experimental section (as shown in Scheme 1). Treatment of the free 4′-sustituted tpy ligands ($\mathbf{L_a}$–$\mathbf{L_d}$) with corresponding salts in a methanol or methanol/water mixture at different temperatures afforded complexes (**1**–**8**) in ~60% yields. These complexes are air-stable powders at room temperature. They can be dissolved in methanol, water, DMF and DMSO. Attempts to obtain a single crystal suitable for X-ray determination were unsuccessful. The structures of the synthesized complexes were established with the help of elemental analyses data, IR, UV, NMR, EPR and HRMS.

The elemental analysis data of the complexes are in agreement with the calculated values. The molar conductance values in methanol or DMF for the complexes **1** and **2** correspond to 1:1 electrolyte type, while other complexes correspond to non-electrolyte.[22]

The UV-visible spectra of ligands in methanol and complexes in water were measured at a concentration of 1×10^{-4} mol L^{-1}. The essential absorption of prepared complexes appeared red shifted compared with free ligands. The peaks of Pt(II) complex **1** at 324 and 338 nm were attributed to a π–π^* intraligand (IL) transition of tpy, whereas the peak at 386 nm was assigned to



Scheme 1. Synthesis of the ligands and their complexes.

metal-to-ligand charge transfer (MLCT) transition. Peaks of Pd(II) complex **2** at 244 and 278 nm were assigned to the π–π^* IL transition and the 349 and 365 nm absorptions were assigned to MLCT transition.[23,24] The bands of Cu(II) and Zn(II) complexes (**3–8**) between 223 and 286 nm were assigned to IL transition and 326–334 nm absorption bands were assigned to MLCT transition.[15,18,25] The red shift indicated that the metal coordinated with the ligand through nitrogen atoms.

Comparison of the IR spectra of the ligands and complexes provided information about the mode of bonding of the ligands in complexes. The band of $u_{C=N}$ at 1590 cm^{-1} of free ligands shifted to higher frequency by 4–12 cm^{-1} after coordination. far-IR spectra of the complexes were recorded, and bands assigned to u_{M-N} appeared at 413.2–438.5 cm^{-1}, which suggested the coordination of the metal ion with the nitrogen atom of pyridyl.[17]

The ^{1}H NMR and ^{13}C NMR spectra of ligands (**L_a–L_d**) and complexes **1**, **2**, **4** and **5** were recorded in DMSO-d_6. The ^{1}H NMR spectra of the complexes showed down-field shift for the 6,6″-tpy***H***s (δ 0.04, 0.03, 0.01 and 0.17 ppm, respectively) compared to the ligands. The chemical shift for the 2′,6′-tpy***C***s moved down-field by 3.2, 2.8, 1.1 and 1.2 ppm, respectively. Similar situations were found for 2,2″-tpy***C***s (2.5, 2.2, 0.9 and 0.9 ppm, respectively) and 6,6″-tpy***C***s (0.7, 0.4, 0.1 and 0.1 ppm, respectively). The results indicate the coordination of the ligands with the metal ions.

The ^{1}H NMR spectra of complexes **4** and **5** provided information about the bonding mode of acetate in Zn(II) complexes. The acetate anion can act as a monodentate ligand or a bidentate ligand. These Zn(II) complexes may choose a six-coordinate geometry around metal ion through one tridentate tpy, one bidentate acetate and one monodentate acetate ligand, or a five-coordinate geometry around metal ion through one tridentate tpy and two monodentate acetate ligands. Two peaks of methyl due to bidentate and monodentate acetate ligands should be obvious at about 2.1 and 1.8 ppm for six-coordinate geometry.[26–29] However, only a single peak (s, 6H, CH_3) was found at 1.67 ppm for complexes **4** or **5**, which suggested the five-coordinate geometry around Zn(II) ion through one tridentate tpy and two monodentate acetate ligands.

EPR spectroscopy was used to further confirm the identity of the Cu(II) complex. The X-band EPR spectrum of a powdered complex **7** recorded at room temperature (298 K) is shown in Fig. 1. It is accordance with the existence around the Cu(II) ion of the square-pyramidal geometry.[30,31] For complex **7**, $g_{\|}$ (2.253) $> g_{\perp}$(2.082) > 2, and G value ($G = (g_{\|} - 2)/(g_{\perp} - 2)$) falling in the range 2–4 is consistent with a dx^2–y^2 ground state, which suggests the existence of considerable exchange interaction in the solid complex.

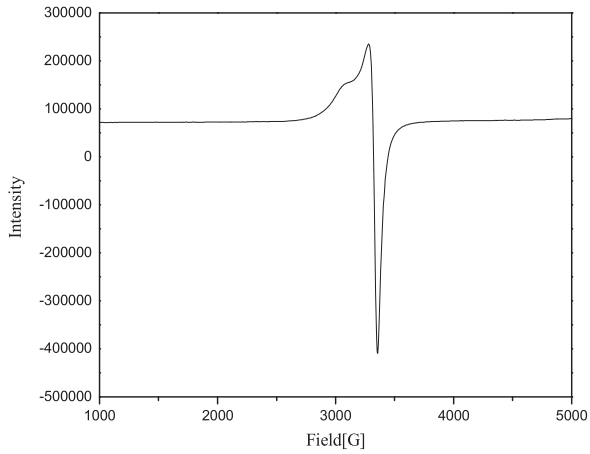


Figure 1. EPR (X-band) spectrum of powdered sample of complex **7** at room temperature (298 K) ($u = 9.689838$ GHz).

The formation of complexes was further supported by positive mode ESI-HRMS spectrometry, which documented a base peak corresponding to the $[M - Cl^-]^+$ or $[M - Ac^-]^+$. The HRMS spectra of Pt(II) complex **1**, Pd(II) complex **2**, Zn(II) complex **4** and **5** and Cu(II) complexes **3** and **6–8** were recorded in MeOH. The molecular ion peaks appeared (m/z) at 555.05525 $[M - Cl^-]^+$, 525.9997 $[M - Cl^-]^+$, 423.0194 $[M - Cl^-]^+$, 506.0698 $[M - Ac^-]^+$, 561.14838 $[M - Ac^-]^+$, 560.1478 $[M - Ac^-]^+$, 536.1035 $[M - Cl^-]^+$ and 534.1243 $[M - Cl^-]^+$ for complexes **1–8**, respectively, which indicated the formation of these complexes. As shown in Fig. 2, the obtained experimental data of complex **3** perfectly match theoretical expectations, therefore confirming our hypotheses on the chemical structure.

Attempts to obtain a single crystal suitable for X-ray determination were unsuccessful. The crystal structures of the analogues of title complexes, ZnL_1Cl_2, $ZnL_1(CH_3COO)_2$ (L_1 = 4′-phenyl-terpyridine)[17] and $[Pt(II)L_2Cl]^+$ (L_2 = 4′-(2-morpholinoethoxy)-2,2′,6′,2″-terpyridine, 4′-(2-(piperidin-1-yl)ethoxy)-2,2′,6′,2″-terpyridine)[32] have revealed that the metals are coordinated by three nitrogen atoms from ligands.

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

Cytotoxicity Study

As shown in Table 1, the complexes (**1–8**) display cytotoxic effects with low IC_{50} values (<20 μM) and show selective

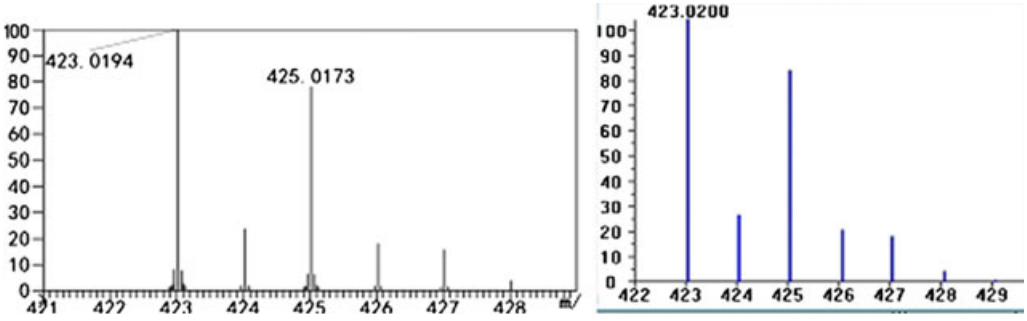


Figure 2. Experimental and theoretical high-resolution mass spectra of complex **3**: (A) experimental spectrum; (B) theoretical spectrum.

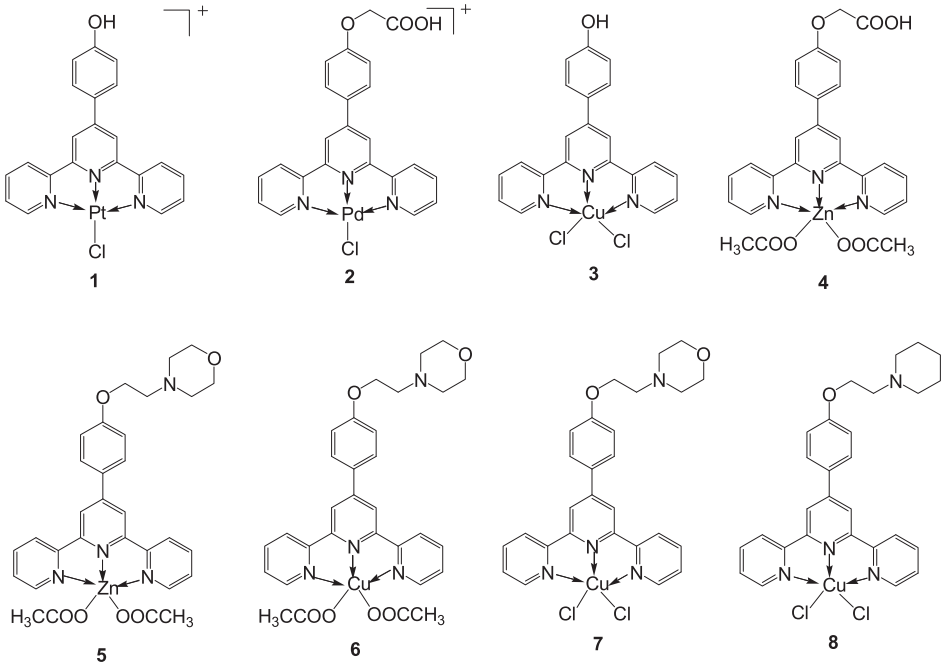


Figure 3. Tentative structures of complexes **1–8**.

Table 1. IC_{50} values of the complexes against HL-60, BGC-823, KB and Bel-7402 cell lines

Complex	HL-60	BGC-823	KB	Bel-7402
1	13.09 ± 1.27	9.93 ± 0.52	6.71 ± 0.38	3.66 ± 0.14
2	10.32 ± 0.73	15.24 ± 1.26	7.57 ± 0.27	5.58 ± 0.24
3	4.77 ± 0.15	4.54 ± 0.24	2.74 ± 0.16	0.89 ± 0.12
4	39.14 ± 1.14	21.26 ± 1.26	11.87 ± 0.82	16.23 ± 1.36
5	17.71 ± 1.32	2.12 ± 0.23	5.99 ± 0.36	1.42 ± 0.28
6	5.02 ± 0.12	4.34 ± 0.21	7.70 ± 0.52	4.43 ± 0.25
7	1.62 ± 0.19	3.59 ± 0.16	2.28 ± 0.27	0.63 ± 0.08
8	3.40 ± 0.23	3.73 ± 0.26	2.37 ± 0.18	1.09 ± 0.12
Cisplatin[33]	2.89 ± 0.34	6.48 ± 0.81	2.65 ± 0.33	8.12 ± 0.97

cytotoxicity against HL-60, BGC-823, Bel-7402 and KB cell lines. The cytotoxicity of these complexes against Bel-7420 cell line is better than that against the other three cell lines. Complexes **1–3** and **5–8** exhibit better cytotoxicity than cisplatin against Bel-7402 cell line. Complexes **3**, **5**, **7** and **8** demonstrate 9-, 5-, 12- and 7-fold higher cytotoxicity than cisplatin, respectively. The cytotoxicity of complexes **3**, **5**, **6**, **7** and **8** is also higher than that of cisplatin against BGC-823 cell line. Complex **7** exerts higher cytotoxicity than cisplatin against HL-60 cell line. Complexes **3**, **7** and **8** show similar cytotoxicity to cisplatin against KB cell line. Among these complexes, complex **7** displays the highest *in vitro* cytotoxicity, with IC_{50} values of 1.62, 3.59, 2.28 and 0.63 μM against HL-60, BGC-823, Bel-7402 and KB cells lines, respectively.

The structure–activity relationships are summarized as follows. (i) The terminal group of the complex plays an important role in the cytotoxicity. For the tested cell lines, complex **7** demonstrates higher cytotoxicity than complexes **3** and **8**; complex **5** exhibits a higher cytotoxicity than complex **4**. It was shown that complex **5** or **7** with morpholinyl as the terminal group has higher cytotoxicity than complexes (**3**, **8** or **4**) with the other three terminal groups. (ii) The metal center of the complexes has an important effect on the cytotoxicity. For example, with the same ligand $\mathbf{L_a}$, the cytotoxicity of complexes **1** and **3** against the four cell lines decreases in the sequence Cu > Pt. In addition, with the same ligand $\mathbf{L_c}$, the cytotoxicity of complexes **5** and **6** against HL-60 cell lines decreases in the sequence Cu > Zn; the cytotoxicity against BGC-823, Bel-7402 and KB cell lines decreases in the sequence Zn > Cu. (iii) The leaving group of the complexes also plays an important role in cytotoxicity. For example, with the same metal center and the same carrying group, the cytotoxicity of complexes **6** and **7** against the four cell lines decreases in the sequence: $Cl^- > Ac^-$.

Conclusion

Eight new complexes with 4′-substitued tpy have been synthesized and characterized, and their cytotoxicity against four human cell lines has been investigated. All the complexes display high cytotoxicity

and selectivity against HL-60, BGC-823, Bel-7402 and KB cell lines; some complexes display higher cytotoxicity than cisplatin. In particular, complex **7** demonstrates the highest *in vitro* cytotoxicity against HL-60, BGC-823, Bel-7402 and KB cell lines. In addition, the cytotoxicity of these complexes is related to the terminal group of the ligands, the metal center and the leaving group. The results indicate that a complex with 4′-substitued tpy may be a promising source of metal-based antitumor agent.

Acknowledgments

This work was supported by Hebei Province Nature Science Fund for Distinguished Young Scholars (Grant No. B2011201164), the Nature Science Fund of Hebei Province (Grant No. B2011201135), the Key Basic Research Special Foundation of Science Technology Ministry of Hebei Province (Grant No. 11966412D, 12966418D), the Key Research Project Foundation of the Department of Education of Hebei Province (Grant No. ZH2012041), the Pharmaceutical Joint Research Foundation of the Natural Science Foundation of Hebei Province and China Shiyao Pharmaceutical Group Co. Ltd (B2011201174) and the Technology Research and Development Foundation of Hebei Province (No. 11276431).

References

[1] V. Angerer, *Keppler* **1993**, 75–83.
[2] R. P. Perez, T. C. Hamilton, R. F. Ozols, R. C. Young, *Cancer* **1993**, *71*, 1571.
[3] Y. P. Ho, S. C. Au-Yeung, K. K. To, *Med. Res. Rev.* **2003**, *23*, 633.
[4] L. X. Zhao, J. Sherchan, J. K. Park, Y. Jahng, B. S. Jeong, T. C. Jeong, C. S. Lee, E. S. Lee, *Arch. Pharm. Res.* **2006**, *29*, 1091.
[5] Z. Naseri, A. Nemati Kharat, A. Banavand, A. Bakhoda, S. Foroutannejad, *Polyhedron* **2012**, *33*, 396.
[6] J. Vázquez, S. Bernès, P. Sharma, J. Pérez, G. Hernández, A. Tovar, U. Peña, R. Gutiérrez, *Polyhedron* **2011**, *30*, 2514.
[7] H. Khan, A. Badshah, G. Murtaz, M. Said, Z. Rehman, C. Neuhausen, M. Todorova, B. J. Jean-Claude, I. S. Butler, *Eur. J. Med. Chem.* **2011**, *46*, 4071.
[8] E. Ulukaya, F. Ari, K. Dimas, M. Sarimahmut, E. Guney, N. Sakellaridis, *J. Cancer Res. Clin. Oncol.* **2011**, *137*, 1425.
[9] F. V. Rocha, C. V. Barra, A. V. G. Netto, A. E. Mauro, I. Z. Carlos, R. C. G. Frema, S. R. Ananias, M. B. Quilles, A. Stevanato, M. C. Rocha, *Eur. J. Med. Chem.* **2010**, *45*, 1698.
[10] N. T. A. Ghani, A. M. Mansour, *Spectrochim. Acta, Part A* **2011**, *81*, 529.
[11] E. Ulukaya, F. Ari, K. Dimas, E. I. Ikitimur, E. Guney, V. T. Yilmaz, *Eur. J. Med. Chem.* **2011**, *46*, 4957.
[12] H. A. El-Boraey, S. M. Emam, D. A. Tolan, A. M. El-Nahas, *Acta A* **2011**, *78*, 360.
[13] A. Kumar, J. P. Chinta, A. K. Ajay, M. K. Bhat, *Dalton Trans.* **2011**, *40*, 10865.
[14] S. Roy, S. Saha, R. Majumdar, R. R. Dighe, A. R. Chakravarty, *Polyhedron* **2010**, *29*, 2787.
[15] M. N. Patel, H. N. Joshi, C. R. Patel, *Polyhedron* **2012**, *40*, 159.
[16] Q. Jiang, J. H. Zhu, Y. M. Zhang, N. Xiao, Z. J. Guo, *Biometals* **2009**, *22*, 297.
[17] Z. Ma, Y. Q. Cao, Q. S. Li, M. F. C. G. Silva, J. J. R. F. Silva, A. J. L. Pombeiro, *J. Inorg. Biochem.* **2010**, *104*, 704.
[18] T. P. Stanojkovic, D. Kovala-Demertzi, A. Primikyri, I. Garcia-Santos, A. Castineiras, Z. Juranic, M. A. Demertzis, *J. Inorg. Biochem.* **2010**, *104*, 467.
[19] P. F. Shi, Q. Jiang, Y. M. Zhao, Y. M. Zhang, J. Lin, L. P. Lin, J. Ding, Z. J. Guo, *J. Biol. Inorg. Chem.* **2006**, *11*, 745.
[20] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.
[21] M. N. Patel, D. S. Gandhi, P. A. Parmar, *Spectrochim. Acta, Part A* **2011**, *84*, 243.
[22] W. J. Geary, *Coord. Chem. Rev.* **1971**, *7*, 81.
[23] I. Eryazici, C. N. Moorefield, G. R. Newkome, *Chem. Rev.* **2008**, *108*, 1834.
[24] E. Guney, V. T. Yilmaz, A. Sengul, *Inorg. Chim. Acta* **2010**, *363*, 438.
[25] S. Roy, S. Saha, R. Majumdar, R. R. Dighe, A. R. Chakravarty, *Polyhedron* **2010**, *29*, 3251.
[26] A. Arbaoui, C. Redshaw, N. M. Sanchez-Ballester, M. R. J. Elsegood, D. L. Hughes, *Inorg. Chim. Acta* **2011**, *365*, 96.
[27] Q. F. Mokuolu, C. A. Kilner, P. C. McGowan, M. A. Halcrow, *Inorg. Chim. Acta* **2007**, *360*, 4025.
[28] J. S. Klitzke, T. Roisnel, J. F. Carpentier, O. L. Casagrande Jr., *Inorg. Chim. Acta* **2009**, *362*, 4585.
[29] S. I. Orysyk, V. V. Bon, O. O. Obolentseva, Y. L. Zborovskii, V. V. Orysyk, V. I. Pekhnyo, V. I. Staninets, V. M. Vovk, *Inorg. Chim. Acta* **2012**, *382*, 127.
[30] H. Elliott, B. J. Hathaway, R. C. Slater, *J. Chem. Soc. A* **1966**, 1443.
[31] B. J. Hathaway, D. E. Billing, *Coord. Chem. Rev.* **1970**, *5*, 143.
[32] K. Suntharalingam, A. J. P. White, R. Vilar, *Inorg. Chem.* **2009**, *48*, 9427.
[33] J. C. Zhang, L. W. Li, L. W. Wang, F. F. Zhang, X. L. Li, *Eur. J. Med. Chem.* **2010**, *45*, 5337.

