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Synthesis, crystal structure, and in vitro antitumor activities of copper(II) complexes containing tetradentate pyridine-based ligands

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Abstract Several new Cu(II) complexes of Schiff bases obtained by condensation of 2-[N-(α-picolyl)-amino]-benzophenone with different chiral amino acids were synthesized and characterized by physico-chemical and spectroscopic methods. The crystal structure of one of the complexes was determined using single crystal X-ray diffraction. The ligands were coordinated to the metal atom in a tetradentate manner with ONNN donor sets using the carboxyl oxygen, azomethine nitrogen, CON^{-}, and pyridine nitrogen. The cytotoxicities of the complexes were evaluated against human cancer cells. The substituents on the aromatic rings strongly influenced the cytotoxicities of the complexes. The complex with bromine substituents on the pyridine rings showed the highest cytotoxicity. The antitumor activities against tumor cell lines were assayed in vitro, and the complexes were found to be highly effective, with six of the nine complexes having inhibition ratios better than that of 5-Fluorouracil. This behavior is indicative of a high ability to circumvent the cellular drug resistance mechanisms.

Introduction

Schiff bases are usually formed by the condensation of a primary amine with an active carbonyl compound [1, 2]. A large number of Schiff base complexes have been used as ligands in metal coordination chemistry. Some Schiff base metal complexes have been found to possess biological activity [3, 4]. Our interest in the study of Schiff bases and their complexes containing sulfur and nitrogen donor atoms arises from their significant anticancer activities [5–8]. Among them, amino acid (such as glycine, α-valine, β-alanine) Schiff bases and their first-row transition metal complexes were reported to exhibit antitumor activities [9–16]. The clinical success of cisplatin in the treatment of several human malignant tumors was motivated major research efforts toward the discovery of alternative metal complexes with potential as anticancer drugs [17, 18]. So far, some of the most promising results have been reported for copper, which exhibits antitumor activity even in the form of simple copper(II) salts [19]. However, the use of copper(II) salts as anticancer drugs presents some drawbacks, which are mainly related to their toxicity and relatively poor bioavailability. To overcome these limitations, copper(II) complexes of multidentate chelate ligands have been evaluated in recent years as alternatives to simple copper salts. In this paper, we report the synthesis of several new copper(II) complexes of various amino acid-derived Schiff base ligands. It is well known that 5-Fluorouracil (5-FU) remains the most commonly used anticancer drug for the treatment of solid tumors, although objective response rates are as low as 20%. Biological activity data showed that the complexes obtained in this study were more cytotoxic than 5-FU against tumor cell lines.

Experimental

All the reagents and solvents employed were of the best grade available and used without further purification. The

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elemental analyses were carried out with a YANACO CHN CORDER MT-3 analyzer. Melting points were determined using an electrothermal apparatus and are uncorrected. Mass spectra were obtained using a ThermoFinnigan TRACE-DSQ electrospray ionization (ESI) mass spectrometer. IR spectra were recorded as KBr disks on a Nicolet 560 ESP FTIR spectrometer, while ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AC-P300 instrument in $CDCl_3$. The following abbreviations were used to designate chemical shift mutiplicities: s = singlet, d = doublet, t =triplet, m = multiplet, br = broad. Tumor cells were cultured at 37 °C under a humidified atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal serum and dispersed in replicate 96-well plates with 1×10^4 cells per well. Complexes were then added. After 48 h exposure to the toxins, cell viability was determined by the MTT colorimetric assay by measuring the absorbance at 570 nm with an ELISA reader. Each test was performed in triplicate.

Synthesis of 2-[*N*-(α-picolyl)amino]-benzophenone (**1**)

Thionyl chloride (3.5 mL, 50 mmol) was added dropwise to a stirred solution of picolinic acid (6.2 g, 50 mmol) and Et_3N (10.4 mL, 75 mmol) in CH_2Cl_2 (35 mL) at 0 °C. The mixture was stirred for 20 min, and then a solution of o-aminobenzophenone (9.9 g, 50 mmol) in CH_2Cl_2 (20 mL) was added at 0 °C. Stirring was continued for another 2 h at room temperature, and the reaction mixture was left standing overnight. A saturated aqueous solution of Na_2CO_3 (20 mL) was then added slowly over a period of 30 min while stirring. The organic layer was separated, washed with water, and dried under vacuum. The crude product was recrystallized from an acetone–benzene mixture to afford **1**. Yield: 94%, m.p 156–157 °C. $C_{19}H_{14}N_2O_2$: Found: C, 75.6; H, 4.6; N, 9.3. Calcd: C, 75.5; H, 4.7; N, 9.3. ^{1}H-NMR ($CDCl_3$): $\delta = 12.72$ (br, s, 1H), 8.90 (d, $J = 8.3$ Hz, 1H), 8.76 (ddd, $J = 4.7$, 1.7, 0.8 Hz, 1H), 8.29 (br, d, $J = 7.8$ Hz, 1H), 7.90 (td, $J = 7.7$, 1.6 Hz, 1H), 7.76–7.78 (m, 2H), 7.56–7.66 (m, 3H), 7.45–7.49 (m, 3H), 7.15 (m, 1H) ppm.

Synthesis of 2-[N-(α-picolyl)amino]-acetophenone (**2**)

Using a procedure similar to that described elsewhere, reaction of picolinic acid with o-amino-acetophenone in dichloromethane afforded ligand **2**. Yield: 89%, m.p 110–112 °C. $C_{14}H_{12}N_2O_2$: Found: C, 69.6; H, 5.0; N, 11.6. Calcd: C, 69.9; H, 5.1; N, 11.7. ^{1}H-NMR ($CDCl_3$): $\delta = 13.55$ (s, 1H), 9.03 (d, $J = 8.2$ Hz, 1H), 8.81 (br, s, 1H), 8.29 (d, $J = 7.4$ Hz, 1H), 7.97 (d, $J = 7.4$ Hz, 1H), 7.90 (t, $J = 7.3$ Hz, 1H), 7.63 (t, $J = 7.3$ Hz, 1H), 7.49 (br, s, 1H), 7.19 (t, $J = 7.2$ Hz, 1H), 2.73 (s, 3H) ppm.

Synthesis of 2-[N-(α-picolyl)amino]-2′,5-dichlorobenzophenone (**3**)

Using a procedure similar to that described elsewhere, reaction of picolinic acid with 2-amino-2′,5-dichlorobenzophenone in CH_2Cl_2 afforded ligand **3**. Yield: 84%, m.p 223–225 °C. $C_{19}H_{12}Cl_2N_2O_2$: Found: C, 61.6; H, 3.3; N, 7.6. Calcd: C, 61.5; H, 3.4; N, 7.7. ^{1}H-NMR ($CDCl_3$): $\delta = 13.34$ (s, 1H), 9.06 (d, $J = 9.1$ Hz, 1H), 8.79 (d, $J = 4.6$ Hz, 1H), 8.31 (d, $J = 7.9$ Hz, 1H), 7.92 (td, $J = 7.7$, 1.5 Hz, 1H), 7.61 (dd, $J = 9.1$, 2.5 Hz, 1H), 7.50 (m, 3H), 7.41 (dd, $J = 3.9$, 1.9 Hz, 2H), 7.37 (d, $J = 2.5$ Hz, 1H) ppm. ^{13}C-NMR ($CDCl_3$): $\delta = 197.5$, 163.9, 149.9, 148.8, 139.5, 138.3, 137.5, 135.2, 133.4, 131.7, 131.2, 130.3, 129.1, 127.7, 126.9, 126.7, 124.2, 122.8, 122.6 ppm.

5-bromo-pyridine-2-carboxylic acid (2-benzoyl-phenyl)-amide (**4**)

Using a procedure similar to that described elsewhere, reaction of 5-bromo-2-pyridine carboxylic acid with o-amino-benzophenone in dichloromethane afforded ligand **4**. Yield: 80%, m.p 148–150 °C. $C_{19}H_{13}BrN_2O_2$: Found: C, 59.6; H, 3.5; N, 7.3. Calcd: C, 59.9; H, 3.4; N, 7.4. ^{1}H-NMR ($CDCl_3$): $\delta = 12.65$ (s, 1H), 8.86 (d, $J = 8.4$ Hz, 1H), 8.82 (d, $J = 1.8$ Hz, 1H), 8.18 (d, $J = 8.3$ Hz, 1H), 8.02 (dd, $J = 8.4$, 2.2 Hz, 1H), 7.77 (m, 2H), 7.63 (m, 3H), 7.49 (t, $J = 7.6$ Hz, 2H), 7.16 (m, 1H) ppm. ^{13}C-NMR ($CDCl_3$): $\delta = 198.9$, 162.8, 149.9, 148.7, 140.1, 139.6, 138.7, 133.9, 133.5, 132.5, 130.1, 128.3, 124.8, 124.3, 124.0, 122.7, 121.7 ppm.

Preparation of Cu(II) complex (**5**) of glycine Schiff base with ligand **1**

A solution of NaOH (1.6 g, 40 mmol) in methanol (40 mL) was added to a stirred solution of ligand **1** (6.0 g, 20 mmol), glycine (7.5 g, 100 mmol), and $CuCl_2 \cdot 2H_2O$ (6.8 g, 40 mmol) in methanol (100 mL). The mixture was refluxed for 4 h, cooled to room temperature, and a solution of ice water (100 mL) and glacial acetic acid (10 mL) was added, then the reaction mixture was stirred for an additional 10 min. The solid was filtered off, washed with hexanes, and dried under vacuum to afford the desired product **5**: Yield: 85%, m.p > 270 °C. $C_{21}H_{15}CuN_3O_3$: Found: C, 59.9; H, 3.6; N, 9.9. Calcd: C, 59.8; H, 3.5; N, 9.8. ESI/MS (m/z): 421.3 [M + 1].

Preparation of Cu(II) complex (**6**) of alanine Schiff base with ligand **1**

Using a procedure similar to that described elsewhere, complex **6** was obtained. Yield: 86%, m.p > 270 °C. $C_{22}H_{17}CuN_3O_3$: Found: C, 60.8; H, 3.9; N, 9.7. Calcd: C, 60.9; H, 3.8; N, 9.8. ESI/MS (m/z): 435.4 [M + 1].

Preparation of Cu(II) complex (**7**) of valine Schiff base with ligand **1**

Using a procedure similar to that described elsewhere, complex **7** was obtained. Yield: 76%, m.p > 270 °C. $C_{24}H_{21}CuN_3O_3$: Found: C, 62.3; H, 4.6; N, 9.1. Calcd: C, 62.4; H, 4.7; N, 9.2. ESI/MS (m/z): 463.4 [M + 1].

Preparation of Cu(II) complex (**8**) of leucine Schiff base with ligand **1**

Using a procedure similar to that described elsewhere, complex **8** was obtained. Yield: 78%, m.p > 270 °C. $C_{25}H_{23}CuN_3O_3$: Found: C, 62.9; H, 4.9; N, 8.8. Calcd: C, 62.7; H, 5.0; N, 8.7. ESI/MS (m/z): 477.4 [M + 1].

Preparation of Cu(II) complex (**9**) of phenylalanine Schiff base with ligand **1**

Using a procedure similar to that described elsewhere, complex **9** was obtained. Yield: 75%, m.p > 270 °C. $C_{28}H_{21}CuN_3O_3$: Found: C, 65.8; H, 4.2; N, 8.2. Calcd: C, 65.7; H, 4.3; N, 8.3. ESI/MS (m/z): 511.6 [M + 1].

Preparation of Cu(II) complex (**10**) of glutamic acid Schiff base with ligand **1**

Using a procedure similar to that described elsewhere, complex **10** was obtained. Yield: 73%, m.p > 270 °C. $C_{24}H_{19}CuN_3O_5$: Found: C, 58.5; H, 3.9; N, 8.5. Calcd: C, 58.6; H, 4.0; N, 8.4. ESI/MS (m/z): 493.2 [M + 1].

Preparation of Cu(II) complex (**11**) of alanine Schiff base with ligand **2**

Using a procedure similar to that described elsewhere, complex **11** was obtained. Yield: 87%, m.p > 270 °C. $C_{17}H_{15}CuN_3O_3$: Found: C, 54.8; H, 4.1; N, 11.3. Calcd: C, 54.9; H, 4.0; N, 11.4. ESI/MS (m/z): 373.4 [M + 1].

Preparation of Cu(II) complex (**12**) of valine Schiff base with ligand **3**

Using a procedure similar to that described elsewhere, complex **12** was obtained. Yield: 67%, m.p > 270 °C. $C_{24}H_{19}Cl_2CuN_3O_3$: Found: C, 54.2; H, 3.6; N, 7.9. Calcd: C, 54.3; H, 3.7; N, 8.0. ESI/MS (m/z): 531.3 [M + 1].

Preparation of Cu(II) complex (**13**) of valine Schiff base with ligand **4**

Using a procedure similar to that described elsewhere, complex **13** was obtained. Yield: 65%, m.p > 270 °C. $C_{24}H_{20}BrCuN_3O_3$: Found: C, 53.2; H, 3.7; N, 7.8. Calcd: C, 53.3; H, 3.8; N, 7.9. ESI/MS (m/z): 541.1 [M + 1].

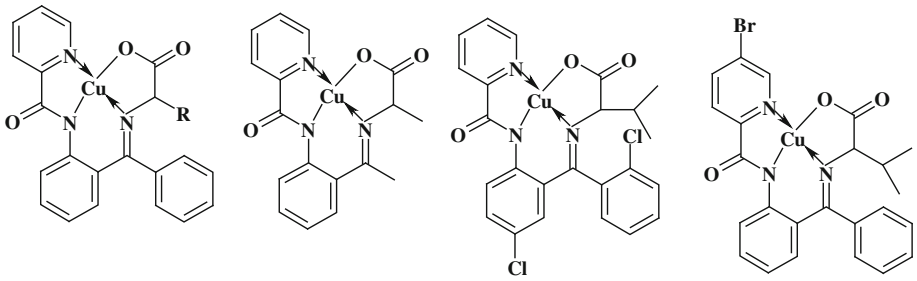
X-ray crystal structure determination

Single crystals suitable for X-ray diffraction were obtained from the slow evaporation of acetonitrile solutions at room temperature. For complex **7**, a single crystal of suitable size was mounted on a Bruker SMART CCD area-detector diffractometer with graphite monochromated Mo–Ka radiation ($\lambda = 0.71073$ Å) at 113(2) K. Corrections were made for Lorentz and Polarization factors as well as for absorption (numerical). Structures were solved with direct methods using SHELXL-97 and refined by full matrix least-squares methods on F^2 with SHELXL-97. Non-hydrogen atoms were refined with anisotropic thermal parameters. All hydrogen atoms were geometrically fixed and allowed to refine using a riding model. Atomic coordinates, bond angles, bond lengths, and thermal parameters

Table 1 Crystal data and structure refinement for complex **7**

Complex	**7**
Empirical formula	$C_{24}H_{21}N_3O_3Cu$
Formula weight	462.98
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	$a = 11.130$ (2) Å, $\alpha = 90°$
	$b = 11.875$ (2) Å, $\beta = 90°$
	$c = 32.284$ (7) Å, $\gamma = 90°$
Volume	4,266.6 (15) Å^3
Z	8
Density (calculated)	1.442 mg/m^3
Absorption coefficient	1.055 mm^{-1}
$F(000)$	1,912
Crystal size	0.20 × 0.18 × 0.12 mm^3
T	113(2) K
θ Range for data collection	2.51–25.02̊
Index ranges	$-13 \leq h \leq 10$
	$-14 \leq k \leq 13$
	$-32 \leq l \leq 38$
Reflections collected	28,611
Independent reflections	7,512 [R(int) = 0.0427]
Completeness to θ	99.8%
Max. and min. transmission	0.8839 and 0.8168
Data/restraints/parameters	7512/0/563
Goodness-of-fit on F^2	0.989
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0310$, $\omega R2 = 0.0699$
R indices (all data)	$R1 = 0.0338$, $\omega R2 = 0.0714$
Largest diffraction peak and hole	0.231 and −0.291 eÅ^{-3}

Scheme 1 Synthesis of complexes



11 **12** **13**

associated with complex **7** have been deposited at the Cambridge Crystallographic Data Center (CCDC code is 754970). The crystal data and structure refinement parameters for complex **7** are given in Table 1.

Results and discussion

When the Schiff base ligands [R = H, methyl, isopropyl, $-CH_2CH(CH_3)_2$, benzyl, or $-CH_2CH_2COOH$] were reacted with $CuCl_2{\cdot}2H_2O$ in refluxing methanol for 4 h, the corresponding complexes were obtained. When alanine Schiff base with ligand **2**, valine Schiff base with ligand **3** and ligand **4** were reacted with $CuCl_2{\cdot}2H_2O$ in refluxing methanol for 4 h, the corresponding complexes were obtained, respectively (Scheme 1).

The IR spectra of copper complexes are all very similar, and all show two strong carbonyl absorptions at 1,600–1,700 cm^{-1} and a strong imine band at 1,605–1,607 cm^{-1}.

Crystal and molecular structure of complex 7

X-ray quality crystals of the copper(II) complex were grown from acetonitrile at room temperature for several days. The solid-state structure of $C_{24}H_{21}CuN_3O_3$ is shown in the Fig. 1. X-ray crystal data are summarized in Table 1; selected bond lengths and angles are listed in Table 2. Complex **7** crystallizes in orthorhombic chiral space group $P2_12_12_1$. The central copper(II) atom adopts a slightly distorted square-planar geometry in a N_3–O donor environment. Bond lengths around the Cu atom are 1.982(2) Å to N(1), 1.921(2) Å to N(2), 1.926(2) Å to N(3). The deviation of average trans angles (177.56° and 161.02°) from 180° indicates slight tetrahedral distortion from a basically square-planar geometry. The Cu(1)···Cu(2) distance is 3.399 Å in the one asymmetric unit, which makes weak Cu(1)···O(4) [2.611 Å] and Cu(2)···N(1) [3.170 Å] interactions possible. Besides, the interdimer interactions involve C–H···O and C–H···N intermolecular contacts. The adjacent chains are connected through C–H···O hydrogen bonds from C–H of CH_3 in valine, together with carboxylic O(3) atom, and carboxylic O(2) atom with C–H of the benzene ring, leading to a 3D infinite hydrogen-bond network.

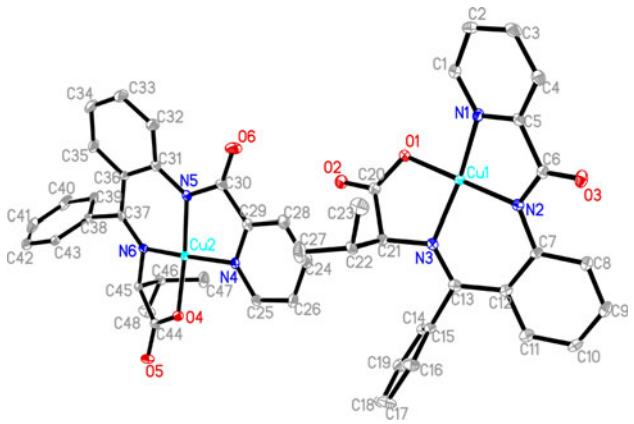


Fig. 1 ORTEP diagram of complex **7** with atom labeling. All H atoms are omitted for clarity

Table 2 Selected bond lengths (Å) and angles (°) for complex **7**

Bond length (Å)		Bond angle (°)	
Cu(1)–N(2)	1.921(2)	N(2)–Cu(1)–N(3)	94.29(9)
Cu(1)–N(3)	1.926(2)	N(2)–Cu(1)–O(2)	177.56(9)
Cu(1)–O(2)	1.9267	N(3)–Cu(1)–O(2)	85.81(8)
Cu(1)–N(1)	1.982(2)	N(2)–Cu(1)–N(1)	84.61(9)
Cu(2)–N(6)	1.911(2)	N(3)–Cu(1)–N(1)	161.02(9)
Cu(2)–O(5)	1.9163	O(2)–Cu(1)–N(1)	96.08(8)
Cu(2)–N(5)	1.925(2)	N(6)–Cu(2)–O(5)	86.18(8)
Cu(2)–N(4)	1.960(2)	N(6)–Cu(2)–N(5)	95.38(9)
N(1)–C(1)	1.342(3)	O(5)–Cu(2)–N(5)	171.88(9)
N(4)–C(25)	1.339(4)	N(6)–Cu(2)–N(4)	170.54(10)
O(1)–C(6)	1.233(3)	O(5)–Cu(2)–N(4)	94.15(8)
O(4)–C(30)	1.217(3)	N(5)–Cu(2)–N(4)	85.63(9)
N(2)–C(6)	1.369(3)	C(20)–O(2)–Cu(1)	114.00(17)
N(5)–C(30)	1.367(3)	C(44)–O(5)–Cu(2)	114.23(17)

Table 3 Percentage inhibition of tumor cell lines (A-549, HCT-8, and Bel-7402)

Complexes	A-549	HCT-8	Bel-7402
5	79.9	80.4	74.5
6	77.8	74.0	81.3
7	75.0	76.1	65.3
8	12.8	17.7	9.3
9	75.8	55.3	73.9
10	7.6	9.0	0.2
11	86.8	68.1	72.9
12	89.8	90.8	87.4
13	89.9	92.0	92.2
5-FU	61.1	78.5	63.9

Antitumor activities

Cytotoxicity assays were carried out on three kinds of cell lines (A-549, HCT-8, Bel-7402). From the data in Table 3, it can be inferred that the copper complexes all have antitumor activities. The substituents of the aromatic rings strongly influence the cytotoxicities of the complexes. Complex **13**, which contains bromide substituents on the pyridine rings, shows the highest cytotoxicity. Complexes are more cytotoxic than 5-FU against A-549, HCT-8, and Bel-7402 tumor cell lines.

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Appendix: Supplementary data

CCDC 754970 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, e-mail: deposit@ccdc.cam.ac.uk.

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