Article

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Synthesis, Structural Characterization and Biological Activity of Cu(II) Compounds Incorporating Pyrazole-derived Ligand: Effect on Cell Growth in Human Colorectal Carcinoma

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A series of Cu(II) compounds containing neutral multi-dentate ligand [2,6-diisopropylphenyl]-bis[(1-H-pyrazol-1-yl)methyl]amine (**L_1**) and pyrazole dimethoxethyl ligand [(1-H-pyrazol-1-yl)methyl]-bis(2-methoxyethyl)amine (**L_2**) were synthesized. Reactions of **L_1** and **L_2** with copper(II) chloride generate L_1CuCl_2 (**1**) and L_2CuCl_2 (**2**), respectively. Compounds **1** and **2** have been characterized by elemental analysis and X-ray single crystal diffractometry. The effects of compounds **1** and **2** on the cell viability of various human cancer cells (including A549, COLO 205, HT-29, Hep3B, HepG2, Huh7, and PCL5 cells) were investigated. The results indicate that compound **2** has a strong inhibitory effect on cell growth in human colorectal carcinoma cells (COLO 205 cells and HT-29 cells).

Keywords: Copper; Pyrazole; Carcinoma.

INTRODUCTION

The increasing interest in chelating polydentate nitrogen-donor ligands, especially heterocyclic compounds, is based on the occurrence of such systems in nature.[1] Most attention is given to model systems containing imidazoles, benzimidazoles, and pyridines.[2] In this connection, attention is also paid to pyrazole derivative,[3] because of the similarity of pyrazoles and imidazoles due to their significant involvement in numerous stoichiometric and catalytic reactions in the development of organometallic chemistry. Pyrazoles have been extensively used as N-donor ligands to metals.[4] The structure and properties of copper pyrazolates are of significant interest.[5] These compounds have been found to exhibit a variety of structures with exo-bidentate coordination of the pyrazolate ligand to the copper centers.[6] Thus, the coordination chemistry of copper incorporating pyrazole-based ligands have drawn attention because of their interesting unusual structural features, remarkable physical and chemical properties and biological activity.[7]

The control of tumor cell proliferation by inhibition of the cell cycle and induction of apoptosis could provide a therapeutic strategy for the treatment of cancer.[8] Programmed cell death plays an important role in the regulation of cellular homeostasis.[9] In cells responsive to apoptotic stimuli, there are two major apoptotic pathways that involve intrinsic (mitochondrion-dependent pathway) and extrinsic (death receptor-dependent pathway) signaling pathways.[10] Marín-Hernández et al.[11] indicated that some mixed chelate copper-based drugs have potent antitumor activity than cisplatin in *in vivo* and *in vitro* studies of a variety of tumor cells. However, human cancer cell lines are a useful model to study cell growth inhibition of tumor cells by natural compounds or newly-synthesized compounds.

We now develop our research using new pyrazolyl ligands, [2,6-diisopropylphenyl]-bis[(1-H-pyrazol-1-yl)-methyl]amine (**L_1**) and pyrazole dimethoxethyl ligand [(1-H-pyrazol-1-yl)methyl]-bis(2-methoxyethyl)amine (**L_2**) and their corresponding Cu(II) derivatives, $\mathbf{L_1}CuCl_2$ (**1**) and L_2CuCl_2 (**2**). The aim of the present study has been to investigate the effect of compounds **1** and **2** on the cell

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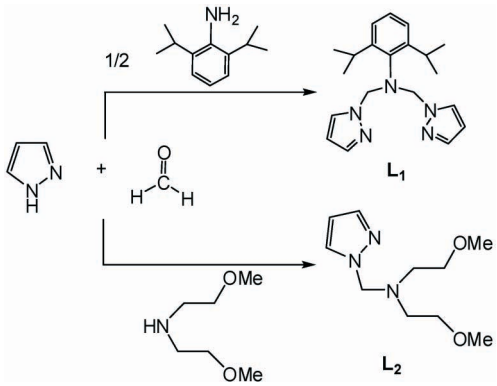
viability of various human cancer cells.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Compounds [2,6-diisopropylphenyl]-bis[(1-H-pyrazol-1-yl)methyl]-amine (L_1) and [(1-H-pyrazol-1-yl)methyl]-bis(2-methoxyethyl)amine (L_2)

The ligands, [2,6-diisopropylphenyl]-bis[(1-H-pyrazol-1-yl)methyl]amine ($\mathbf{L_1}$) and [(1-H-pyrazol-1-yl)-methyl]-bis(2-methoxyethyl)amine ($\mathbf{L_2}$), were prepared by condensation between 2,6-diisopropylaniline or bis(2-methoxyethyl)amine with pyrazole in presence of formaldehyde in ethanol (Scheme I). Both of the ligands $\mathbf{L_1}$ and $\mathbf{L_2}$ were isolated with satisfactory yield and used for complexation without further purification.

Scheme I Formation of ligands $\mathbf{L_1}$ and $\mathbf{L_2}$

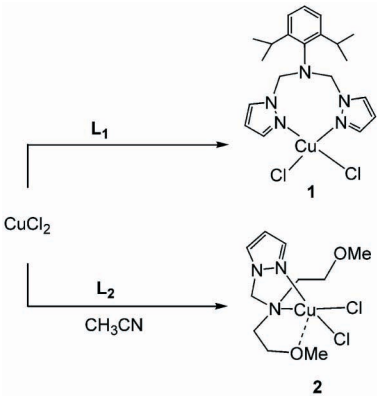


The ^{1}H and ^{13}C NMR spectra of $\mathbf{L_1}$ show the methylene fragments at δ 5.49 and 69.6, respectively. The ^{1}H NMR spectrum of $\mathbf{L_2}$ is also consistent with the structure in solution having one singlet appeared at δ 3.28 for the two methoxy groups. Two triplets appeared at δ 2.76 and 3.45, are assigned as the two methylene groups of the pendant $N(CH_2CH_2OMe)_2$ fragment and a singlet at δ 5.05 is assigned as the methylene protons between pyrazole and amine.

Syntheis of L_1CuCl_2 (1) and L_2CuCl_2 (2)

Reactions of $\mathbf{L_1}$ and $\mathbf{L_2}$ with one equivalent of anhydrous $CuCl_2$ in acetonitrile or methylene chloride generate L_1CuCl_2 (**1**) and L_2CuCl_2 (**2**), respectively in satisfactory yield. Both of compounds **1** and **2** are paramagnetic compounds and therefore no NMR spectra could be obtained. Both of the compounds were characterized on the basis of elemental analysis and X-ray structure determination.

Scheme II Cu-complexation with $\mathbf{L_1}$ and $\mathbf{L_2}$



Crystal Structures of L_1, 1, and 2

Crystals of $\mathbf{L_1}$ suitable for X-ray diffraction analysis were obtained from a toluene solution at -20 °C. The Summary of data collection and selected bond lengths and angles are shown in Table 1 and 2, respectively. The molecular geometry of $\mathbf{L_1}$ is shown in Fig. 1. Apparently, the N(1) nitrogen atom uses sp^2 hybrid orbitals to overlap with the orbitals of the phenyl carbon and two pyrazol-1-ly methyl carbons and the lone pair electrons locating on the unhybrid p orbital of the N(1) nitrogen atom may delocalize to the phenyl ring. This results the total bond angle around the aniline nitrogen N(1) closes to 360° (118.13, 118.00, and 116.73).

Crystals of **1** suitable for X-ray diffraction analysis were obtained from a CH_3CN solution at -20 °C. The molecular geometry of **1** is shown in Fig. 2. The geometry of **1** can be described as a highly distorted tetrahedral. The bond angle of Cl(1)–Cu(1)–Cl(2) (131.23(5)°) is larger than 109.5°, presumably due to the large lone pair interaction of the two chlorine atoms. The $\mathbf{L_1}$ ligand binds to copper atom through N(1) and N(5) forming a eight-member ring with the bond angle of N(1)–Cu(1)–N(5) at 129.99(13)°. The

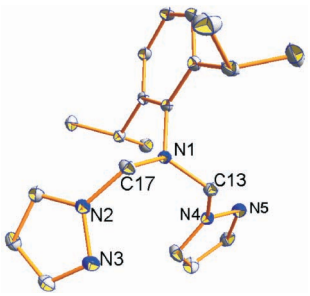


Fig. 1. The molecular structure of $\mathbf{L_1}$. The atoms are drawn with 30% probability ellipsoids and the hydrogen atoms are omitted for clarity.

Table 1. Crystal data and structure refinements for $\mathbf{L_1}$, **1** and **2** (**2a** and **2b**)

	$\mathbf{L_1}$	**1**	**2a**	**2b**
Empirical formula	$C_{20}H_{27}N_5$	$C_{20}H_{27}N_5CuCl_2$	$C_{42}H_{76}N_{13}O_8Cu_4Cl_8$	$C_{10}H_{19}N_3O_2CuCl_2$
Formula weight	337.47	471.91	1428.92	347.72
Temperature (K)	150(2)	150(2)	150(2)	150(2)
Wavelength (Å)	0.71073	0.71073	0.71073	0.71073
Crystal system	Orthorhombic	Trigonal	Monoclinic	Monoclinic
Space group	Pbca	R-3	C2/c	$P2_1/n$
Unut cell dimensions	a = 13.6830(5) Å	*a* = 30.548(2) Å	*a* = 31.379(2) Å	*a* = 6.91060(10) Å
	b = 14.9767(6) Å	*b* = 30.548(2) Å	*b* = 12.6377(7) Å	*b* = 18.2887(3) Å
	c = 18.8372(6) Å	*c* = 13.8507(10) Å	*c* = 31.1777(18) Å	*c* = 11.4727(2) Å
	α = 90°	α = 90°	α = 90°	α = 90°
	β = 90°	β = 90°	β = 102.449(3)°	β = 96.7610 (10)°
	γ = 90°	γ = 120°	γ = 90°	γ = 90°
Volume ($Å^3$), Z	3860.2(2), 8	11193.8(15), 18	12072.9(13), 8	1439.90(4), 4
Density (calculated) (Mg/m^3)	1.161	1.260	1.572	1.604
Absorption coefficient (mm^{-1})	0.071	1.106	1.802	1.885
F(000)	1456	4410	5880	716
Crystal size (mm^3)	0.50 × 0.46 × 0.26	0.30 × 0.15 × 0.11	0.40 × 0.21 × 0.15	0.18 × 0.16 × 0.15
θ range for data collection	2.16 to 29.07°	1.33 to 29.00°	1.33 to 29.08°	2.11 to 29.03°
Reflections collected	42668	80890	129877	16453
Independent reflections	5156; R_{int} = 0.0340	6596; R_{int} = 0.0679	16164; R_{int} = 0.0584	3840; R_{int} = 0.0259
Max. and min. transmission	0.9817 and 0.9651	0.8880 and 0.7325	0.7738 and 0.5327	0.7652 and 0.7278
Data/restraints/parameters	5156 / 0 / 235	6596 / 0 / 257	16164 / 0 / 679	3840 / 0 / 165
Goodness-of-fit on F^2	1.026	1.085	1.045	1.037
Final Rindices	R_1 = 0.0414	R_1 = 0.0548	R_1 = 0.0351	R_1 = 0.0237
[I > 2σ(I)]	wR_2 = 0.0995	wR_2 = 0.1908	wR_2 = 0.00738	wR_2 = 0.0635
R indices (all data)	R_1 = 0.0627	R_1 = 0.0904	R_1 = 0.0672	R_1 = 0.0288
	wR_2 = 0.1118	wR_2 = 0.2181	wR_2 = 0.0898	wR_2 = 0.0661
Largest diff. peak and hole ($e.Å^{-3}$)	0.290 and -0.199	3.135 and -0.458	0.615 and -0.573	0.439 and -0.497

[a] $R_1 = \Sigma \left| F_0 \right| - \left| F_c \right| / \Sigma \left| F_0 \right|$; [b] $wR_2 = [\Sigma[\omega(F_0^2 - F_c^2)^2] / \Sigma[\omega(F_0^2)^2]]^{1/2}$.

bond length of Cu(1)-N(1) and Cu(1)-N(5) are 1.981(3) and 1.997(3) Å, respectively.

Two types of green crystals of **2** were obtained from CH_3CN solution depending on the concentration and crystallization time. Crystals of **2a** were obtained directly from

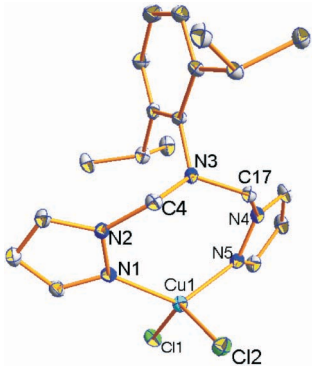


Fig. 2. The molecular structure of compound **1**. The atoms are drawn with 30% probability ellipsoids and the hydrogen atoms are omitted for clarity.

a saturated CH_3CN solution at -20 °C after reacting $\mathbf{L_2}$ with $CuCl_2$. Crystals of **2b** were obtained in a similar procedure excepting the volatiles were removed first after reaction complete and then re-crystallized again from a saturated CH_3CN solution at -20 °C. The molecular geometries of **2a** and **2b** are shown in Figs. 3 and 4, respectively. Considering the unit cell of **2a**, there are eight asymmetry units in a unit cell and there are four different molecules in every asymmetry unit. Fig. 3 shows the geometries of these four different molecules. The hydrogen's of the acetonitrile cannot be added because they exist on special positions but they do not affect the quality of the structure data. Even though these four molecules are different in **2a**, there bond lengths and angles are rather similar. All the four geometries of **2a** can be described as square pyramidal. The bond lengths of Cu to oxygen atoms of methoxy fragments are spanned in a large range from short-end 2.411 Å to long-end 2.852 Å indicating the bonding to nonbonding interaction of Cu to oxygen atoms. The average bond lengths of

Table 2. The selected bond lengths (Å) and angles (°) for compounds **L_1**, **1** and **2** (**2a** and **2b**)

L_1			
C(1)–N(1)	1.4425(13)	C(13)–N(1)	1.4405(13)
C(17)–N(1)	1.4495(13)	C(1)–N(1)–C(13)	118.13(8)
C(13)–N(1)–C(17)	118.00(9)	C(1)–N(1)–C(17)	116.73(8)
N(1)–C(13)–N(4)	113.96(9)	N(1)–C(17)–N(2)	113.81(9)
1			
Cu(1)–Cl(1)	2.212(11)	Cu(1)–Cl(2)	2.221(11)
Cu(1)–N(1)	1.981(3)	Cu(1)–N(5)	1.997(3)
N(1)–Cu(1) –N(5)	125.99(13)	N(1)–Cu(1)–Cl(1)	100.66(10)
N(5)–Cu(1) –Cl(1)	98.36(10)	N(1)–Cu(1)–Cl(2)	100.61(10)
N(5)–Cu(1) –Cl(2)	103.56(10)	Cl(1)–Cu(1)–Cl(2)	131.23(5)
2a			
Cu(1)–Cl(1)	2.273(7)	Cu(1)–Cl(2)	2.244(6)
Cu(1)–N(1)	1.991(2)	Cu(1)–N(3)	2.123(2)
Cu(2)–Cl(3)	2.261(7)	Cu(2)–Cl(4)	2.246(7)
Cu(2)–N(4)	1.978(2)	Cu(2)–N(6)	2.141(2)
Cu(2)–O(3)	2.372(19)		
N(1)–Cu(1)–N(3)	81.01(8)	N(1)–Cu(1)–Cl(2)	172.70(6)
N(3)–Cu(1)–Cl(2)	93.57(5)	N(1)–Cu(1)–Cl(1)	91.15(6)
N(3)–Cu(1)–Cl(1)	171.54(6)	Cl(2)–Cu(1)–Cl(1)	93.96(3)
N(4)–Cu(2)–N(6)	80.06(8)	N(4)–Cu(2)–Cl(4)	164.45(7)
N(6)–Cu(2)–Cl(4)	93.51(6)	N(4)–Cu(2)–Cl(3)	91.79(6)
N(6)–Cu(2)–Cl(3)	170.10(6)	Cl(4)–Cu(2)–Cl(3)	95.75(3)
N(4)–Cu(2)–O(3)	100.31(8)	N(6)–Cu(2)–O(3)	76.82(8)
Cl(4)–Cu(2)–O(3)	91.87(5)	Cl(3)–Cu(2)–O(3)	99.34(6)
2b			
Cu(1)–Cl(1)	2.237(4)	Cu(1)–Cl(2)	2.261(4)
Cu(1)–N(3)	1.988(12)	Cu(1)–N(1)	2.133(12)
Cu(1)–O(2)	2.340(10)		
N(1)–Cu(1)–N(3)	81.62(5)	N(3)–Cu(1)–Cl(1)	170.10(4)
N(1)–Cu(1)–Cl(1)	92.55(3)	N(3)–Cu(1)–Cl(2)	91.15(4)
N(1)–Cu(1)–Cl(2)	172.59(3)	Cl(1)–Cu(1)–Cl(2)	94.84(14)
N(3)–Cu(1)–O(2)	89.07(4)	N(1)–Cu(1)–O(2)	79.97(4)
Cl(1)–Cu(1)–O(2)	97.83(3)	Cl(1)–Cu(1)–O(2)	98.35(3)

Cu to pyrazole-N atom (1.986 Å), is shorter than the corresponding value involving the amine-N atom (2.132 Å). The Cu-N(pyrazole) bond lengths are comparable with other previously reported Cu-pyrazole complexes, [1.966(5) and 1.985(2) Å],[12] [1.944(4), 1.954(4) and 1.942(4) Å],[13] [1.933(2) and 2.013(2) Å][14] and [1.951(4) and 1.955(5) Å].[15] The bond lengths of Cu to chlorine atoms are ranged from 2.244 to 2.273 Å.

The compound **2b** (Fig. 4) consists of a five-coordinated copper center that achieves a square pyramidal geometry on coordination from a tetradentate ligand, binding through its NNO donor set like tridentate fashion and two adjacent *cis* chlorine atoms. The geometry of the Cu(1) center is best described as a distorted square based pyramid. The four atoms constituting the basal plane are the two nitrogen [N(1) and N(3)] atoms of the amine and pyrazole and the two chlorine atoms [Cl(1) and Cl(2)] where the bond angle of N(1)–Cu(1)–Cl(2) and N(3)–Cu(1)–Cl(1) are 172.59(3)º and 170.10(4)º, respectively. The Cu(1) atom is deviated from the square basal plane for *ca*. 0.15 Å. The axial site is occupied by the O(2) atom with bond length of Cu(1)–O(2) = 2.340(10) Å. In the basal plane the average bond distances are Cu(1)–N(1), 2.133(12); Cu(1)–N(3), 1.988(12); Cu(1)–Cl(1), 2.237(4) and Cu(1)–Cl(2), 2.261(4) Å.

UV-vis and ERP spectra measurements for compounds 1 and 2

The UV-vis spectral data for **1** and **2**, were recorded in methylene chloride and acetonitrile, respectively within a range of 200–1000 nm. The observed UV absorption bands exhibit charge-transfer transitions (CT)[16] in the range 217

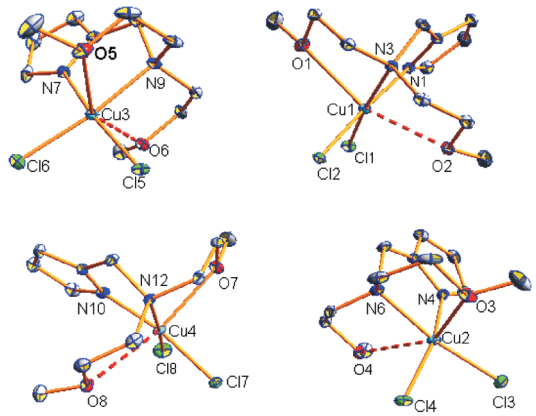


Fig. 3. The molecular structure of compound **2a**. The atoms are drawn with 30% probability ellipsoids and the acetonitrile and hydrogen atoms are omitted for clarity.

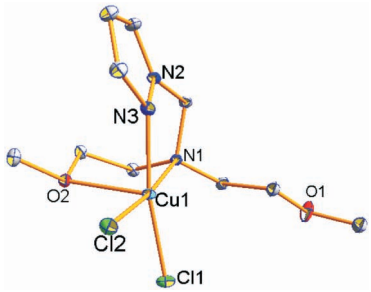


Fig. 4. The molecular structure of compound **2b**. The atoms are drawn with 30% probability ellipsoids and the hydrogen atoms are omitted for clarity.

and 281 nm for **1**, and 233 and 298 nm for **2**. The UV absorption band observed at 395 and 399 nm for **1** and **2**, respectively, is attributed to the ligand-to–Cu(II) charge-transfer transition (LMCT).[16] The EPR spectra of powdered compounds **1** and **2** at 77 K are shown in Fig. 5. Compounds **1** and **2** show a g value at 2.75 and 2.06, respectively, which are typical for isolated Cu(II) compounds with one unpaired electron.

Magnetic Measurements of 2

The magnetic moment of compound **2** was obtained by SQUID magnetometry with 10,000 Gausses of external magnetic field over the temperature range 5-350 K, as shown in Fig. 6. The effective magnetic moment (μ_{eff} ~ 1.78 B.M) is quite stable in the temperature range 5-350 K, which is close to the spin only value, μ_{eff} = 1.73 B.M., expected for the S = 1/2 spin state of d^9 Cu(II) species.

Effects of Compounds 1 and 2 on Cell Viability of Human Cancer Cells

The inhibitory effects of compounds **1** and **2** on the cell population growth of human lung carcinoma cells (A549 cells), human colorectal carcinoma cells (COLO 205 cells and HT-29 cells), human hepatoblastoma cell (Hep3B cells and HepG2 cells), and human hepatocellular carcinoma cells (Huh7 cells and PLC5 cells) were determined by MTT assays. Various human cancer cells were treated with 50 μM compounds **1** and **2** for 48 h. Table 3 shows that compound **2** had a strong inhibitory effect on cell growth in human colorectal carcinoma cells (COLO 205 cells and HT-29 cells). The cell viability values of compound **2** on COLO 205 cells and HT-29 cells were 19.0% and 38.9%, respectively. Marín-Hernández et al.[11] indicated that some mixed chelate copper-based compounds have been tested for anticancer activity. We would further investigate the anticancer effects of compound **2** on intrinsically and extrinsically mediated pathways in human colorectal carcinoma cells.

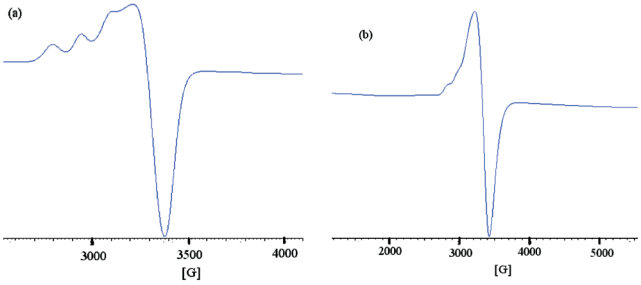


Fig. 5. The X-band EPR spectra of compounds **1** (a) and **2** (b) observed at 77 K using THF as solvent.

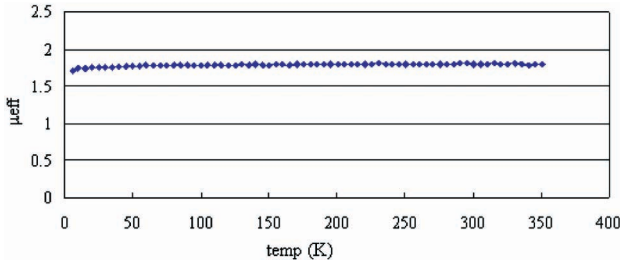


Fig. 6. Temperature dependence of the effective magnetic moments for a microcrystalline sample of **2** in the range of 5-350 K.

Table 3. Effects of compounds **1** and **2** on the cell viability of various human cancer cells

Cell line	Untreated control	Treated with compound	
		1	**2**
A549 cell	100.0 ± 4.8	85.8 ± 2.2	53.7 ± 4.7
COLO 205 cell	100.0 ± 10.5	99.5 ± 14.2	19.0 ± 1.5
HT-29 cell	100.0 ± 4.3	110.4 ± 15.2	38.9 ± 4.1
Hep3B cell	100.0 ± 4.8	67.4 ± 5.1	77.5 ± 2.7
HepG2 cell	100.0 ± 6.9	113.2 ± 5.6	101.8 ± 4.9
Huh7 cell	100.0 ± 4.9	119.4 ± 12.1	62.0 ± 8.5
PLC5 cell	100.0 ± 3.5	101.6 ± 3.2	54.8 ± 2.8

Cells were treated with 50 μM compounds **1** and **2** for 48 h. The reported values are the means ± SD (n = 3).

CONCLUSION

In this article, we have demonstrated the coordination behavior of potentially neutral multi-dentate pyrazole ligands [2,6-diisopropylphenyl]-bis[(1-H-pyrazol-1-yl)-methyl]amine ($\mathbf{L_1}$) and pyrazole dimethoxethyl ligand [(1-H-pyrazol-1-yl)methyl]-bis(2-methoxyethyl)amine ($\mathbf{L_2}$) with copper chloride. Single crystal X-ray diffraction analysis shows that **1** and **2** have tetra- or penta-coordination of the central copper atom. The results from an MTT assay demonstrated that compound **2** had a strong inhibitory effect on cell growth in human colorectal carcinoma cells (COLO 205 cells and HT-29 cells). Future work will explore the coordination mode and modification of the current system using different substituents concerning the pyrazole derivative to develop new generations of transition metal ions and characterize their activities as well as the stereochemical control during the course of the reaction.

EXPERIMENTAL SECTION

Materials and Physical Measurements

All the reactions were performed using standard

Schlenk techniques under an atmosphere of high purity nitrogen or in glove box. $CuCl_2$ (Aldrich), 2,6-diisopropylaniline, bis(2-methoxyethyl)amine, formaldehyde and pyrazole (Strem Chemical) were obtained commercially and used as received. All solvents were distilled and stored in solvent reservoirs which contained 4 Å molecular sieves and were purged with nitrogen. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer. Chemical shifts for ^{1}H and ^{13}C spectra were recorded in ppm relative to the residual protons of $CDCl_3$ (7.24, 77.0). Elemental analyses were performed on a Heraeus CHN-OS Rapid Elemental Analyzer at the Instrument Center of the NCHU. EPR spectra were recorded on a Bruker EMS spectrometer using X-band frequency and UV-vis spectra were measured on a Agilent 8453 spectrometer.

Synthesis of Ligands and Metal Complexes

Synthesis of Ligand, [2,6-diisopropylphenyl]-bis[(1-H-pyrazol-1-yl)methyl]amine (L_1)

A 250 mL Schlenk flask charged with 2,6-diisopropylaniline (24.44 g, 0.127 mol) was cooled to 0 °C and was added 37% formaldehyde (20.57 g, 0.254 mol). The resulting solution was added dropwise a ethanol solution (70 mL) of pyrazole (17.62 g, 0.254 mol) and stirred for 12 h. The volatiles were removed under vacuum at 70 °C and the resulting solid was recrystallized from a toluene solution to yield white crystals of product. Yield, 30.23 g (70.6%). ^{1}H NMR ($CDCl_3$): 1.01 (d, 12H, C*H*$_3$), 2.69 (m, 2H, PhC*H*Me$_2$), 5.49 (s, 4H, NC*H*$_2$N), 6.19 (m, 2H, pyrazole C*H*), 7.05-7.18 (m, 3H, phenyl C*H*), 7.27 (m, 2H, pyrazole C*H*), 7.53 (m, 2H, pyrazole C*H*). ^{13}C{^{1}H} NMR ($CDCl_3$): 24.5 (q, J_{CH} = 126 Hz, *C*H$_3$), 27.9 (d, J_{CH} = 130 Hz, Ph*C*HMe$_2$), 69.6 (t, J_{CH} = 150 Hz, N*C*H$_2$N), 105.6 (d, J_{CH} = 176 Hz, pyrazole *C*H), 124.3 (d, J_{CH} = 156 Hz, phenyl *C*H), 127.6 (d, J_{CH} = 159 Hz, phenyl *C*H), 129.0 (d, J_{CH} = 186 Hz, pyrazole *C*H), 139.7 (d, J_{CH} = 185 Hz, pyrazole *C*H), 140.6 (s, phenyl C_{ipso}), 148.0 (s, phenyl C_{ipso}). Anal. Calc. for $C_{20}H_{27}N_5$: C, 71.18; H, 8.06; N, 20.75. Found; C, 70.89; H, 7.82; N, 20.28%.

Synthesis of Ligand [(1-H-pyrazol-1-yl)methyl]-bis(2-methoxyethyl)amine (L_2)

10 mL ethanolic solution of pyrazole (5.0 g, 72.0 mmol) was added to a mixture of bis(2-methoxyethyl)-amine (9.68 g, 72.0 mmol) and formaldehyde (5.84 g, 72.0 mmol) in ethanol (50 mL) at 0 °C. The mixture was stirred for 24 h at room temperature. The solvent was then evaporated under vacuum to obtain the pure pale yellow liquid **L_2**. Yield, 14.75 g (96%). ^{1}H NMR ($CDCl_3$, ppm): δ 2.62 (t, 4H, NC*H*$_2$CH$_2$O), 3.13 (s, 6H, O*Me*), 3.29 (t, 4H, NCH$_2$C*H*$_2$O), 4.91 (s, 2H, NC*H*$_2$N), 6.03 (m, 1H, pyrazole C*H*), 7.29 (m, 1H, pyrazole C*H*), 7.35 (m, 1H, pyrazole C*H*). ^{13}C NMR ($CDCl_3$, ppm): δ 51.3 (t, J_{CH} = 133 Hz, NC*H*$_2$CH$_2$O), 58.2 (q, J_{CH} = 141 Hz, O*Me*), 69.4 (t, J_{CH} = 151 Hz, NC*H*$_2$N), 70.9 (t, J_{CH} = 141 Hz, NCH$_2$C*H*$_2$O), 104.6 (d, J_{CH} = 175 Hz, pyrazole C*H*), 129.5 (d, J_{CH} = 190 Hz, pyrazole C*H*), 138.5 (d, J_{CH} = 184 Hz, pyrazole C*H*). Anal. Calc. for $C_{10}H_{19}N_3O_2$: C, 56.32; H, 8.98; N, 19.70. Found, C, 56.11; H, 9.31; N, 19.80%.

Synthesis of L_1CuCl_2 (1)

A 50 mL Schlenk flask charged wtih **L_1** (0.251 g, 0.744 mmol) and 10 mL of methylene chloride. The solution was added to a 10 mL methylene chloride solution of anhydrous $CuCl_2$ (0.1 g, 0.744 mmol) at 0 °C. The resulting solution was stirred for 3 h and filtered through celite. The resulting yellowish solid was recrystallized from a CH_3CN solution to generate 0.223 g of yellow crystals. Yield, 63.5%. Anal. Calc. for $C_{20}H_{27}N_5CuCl_2$: C, 50.90; H, 5.77; N, 14.84. Found, C, 51.09; H, 5.88; N, 15.04%.

Synthesis of L_2CuCl_2 (2)

A 50 mL Schlenk flask was charged with **L_2** (1.586 g, 7.44 mmol) in 15 mL CH_3CN. The solution was added dropwise to a 15 mL CH_3CN solution of anhydrous $CuCl_2$ (1.0 g, 7.44 mmol) at 0 °C. The solution was stirred for 3 h at room temperature and filtered through Celite to remove unreacted $CuCl_2$. The green filtrate was concentrated to small amount and stored at -20 °C to afford 2.42 g of green crystals of 2. Yield, 94%. Anal. Calc. for $C_{10}H_{19}CuN_3O_2Cl_2$: C, 34.54; H, 5.51; N, 12.08. Found, C, 34.25; H, 5.46; N, 11.99%.

X-ray Crystallography

Good diffraction quality air stable single crystals of **L_1**, **1** and **2** were mounted on a Bruker SMART CCD diffractometer equipped with graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å). Crystal data were collected at 150 K with an Oxford Cryosystems Cryostream. No significant crystal decay was found. Data were corrected for absorption empirically by means of ψ scans. All non-hydrogen atoms were refined with anisotropic displacement parameters. For both the structures, the hydrogen atom positions were calculated and they were constrained to idealized geometries and treated as riding where the H atom displacement parameter was calculated from the equivalent isotropic displacement parameter of the bound atom. Data were processed using the CRYSALIS-CCD and –RED programs.[17] The structures of both com-

plexes were determined by direct methods procedures in SHELXS,[18] and refined by full-matrix least-squares methods, on F^2's, in SHELXL.[19]

Cell Culture

Human lung carcinoma cells (A549 cells) and human hepatoblastoma cell (Hep3B cells and HepG2 cells) were obtained from the Bioresource Collection and Research Center (BCRC, Food Industry Research and Development Institute, Hsinchu, Taiwan). Human hepatocellular carcinoma cells (Huh7 cells and PLC5 cells) were obtained from the American Type Culture Collection (ATCC, Bethesda, MD, USA). Human colorectal carcinoma cells (COLO 205 cells and HT-29 cells) were provided by Dr Min-Hsiung Pan (National Kaohsiung Marine University, Kaohsiung, Taiwan). A549 cells were grown in a medium consisting of 90% RPMI 1640 with 10% fetal bovine serum supplemented with 0.1 mM nonessential amino acid, 2 mM L-glutamine, 1 mM sodium pyruvate, and 100 units/mL penicillin/streptomycin. COLO 205 cells and HT-29 cells were grown in 90% RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. Hep3B cells, HepG2 cells, Huh7 cells, and PLC5 cells were grown in 90% Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, 100 units/mL penicillin, and 100 μg/mL streptomycin. Human cancer cells were cultured at 37 °C in a humidified 5% CO_2 incubator. Human cancer cells were treated with 50 μM compounds **1** and **2** for 48 h.

Cell Viability by MTT Assay

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma Chemical Co., St. Louis, MO, USA) assay was performed according to the method of Mosmann.[20] Cancer cells were plated into 96-well microtiter plates at a density of 1×10^4 cells/well. After 24 h, culture medium was replaced by 200 μL (50 μM) of compounds **1** and **2**, and the cells were incubated for 48 h. The final concentration of solvent was less than 0.1% in cell culture medium. Culture medium was removed and replaced by 90 μL fresh culture medium. Ten microliters of sterile filtered MTT solution (5 mg/mL) in phosphate buffered saline (PBS, pH = 7.4) was added to each well, thereby reaching a final concentration of 0.5 mg MTT/mL. After 5 h, unreacted dye was removed, and the insoluble formazan crystals were dissolved in 200 μL/well DMSO and measured spectrophotometrically in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 570 nm. The relative cell viability (%) related to control wells containing cell culture medium without samples was calculated by $A_{570\ nm}$ [sample]/$A_{570\ nm}$ [control] × 100.

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