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Anticancer activity, structure, and theoretical calculation of N-(1-phenyl-3-methyl-4-propyl-pyrazolone-5)-salicylidene hydrazone and its copper(II) complex

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ABSTRACT

The pyrazolone derivative N-(1-phenyl-3-methyl-4-propyl-pyrazolone-5)-salicylidene hydrazone (H_2L) and its copper(II) complex $[Cu_2L_2CH_3OH]\cdot 2CH_3OH$ have been both synthesized and characterized by elemental analyses, IR spectroscopy, X-ray crystallography, theoretical calculation and pharmacological testing. It's found that the Cu(II) complex possesses more powerful anticancer effectivity than that of the ligand. In order to make its anticancer principium clearly, we investigate their structures. In ligand there are several coordination spots, such as N, O atoms, which are close to biological environment. The crystallographic structural analysis of the complex reveals that the two Cu centers display two different coordination patterns. O1, O2, N3, and N4 from the ligand take part in the coordination with Cu atoms, resulting in the formation of the double-nuclear complex. The pharmacological testing results show that the coordination effect improves the antitumor activity of the ligand. The calculated Fukui function for H_2L and its deprotonated form L^{2-} predicts that the most probable reactive sites for electrophilic attack are oxygen atoms. The result is agreement well with the experimental data of the crystal structure analyses.

1. Introduction

Recently, one important branch of pharmaceutical chemistry – synthesis and research of the Schiff base and their metal complexes has attracted many attention [1–4]. Pyrazolone, as a prominent structural motif, is found in numerous active compounds. Due to the easy preparation and its rich biological activity of broad-spectrum antibacterial action, antitumor, antisepsis [1,5–7], pyrazolone and its complexes have both received considerable attention in coordination chemistry and medicinal chemistry.

4-Acyl pyrazolone derivatives containing salicylidene hydrazone not only hold many coordinated atoms but also exhibit diversiform coordination modes and their phenoxy derivatives are capable of chelate and bridge properties [8]. What's more, the ligands and their metal complexes also possess strong biological activity. Recently, our group has been actively engaged in studying the synthesis, structural characterization and properties of 4-acyl pyrazolone derivatives and their complexes [9–15]. Among these works, 4-acyl pyrazolone derivatives exhibit various coordination patterns and properties [9,11–15]. However, to our knowledge, very little has appeared on the biological activity derived from 4-acylpyrazole and salicylidene hydrazone. Herein, we report the synthesis, structural characterization and biological activity of a binuclear copper(II) complex. The pharmacological testing has proved that the coordination effect improves the antitumor activity of the copper(II) complex, which is much greater than that of the ligand. Theoretical calculation performed on the ligand predicts its coordination ability. The results consist with the crystal structural analyses of the complex.

2. Experimental

2.1. Physical measurements and materials

IR spectra were recorded on BRUKER EQUINOX-55 spectrophotometer within 400–4000 cm^{-1} using the samples prepared as pellets with KBr. The crystal structure was performed using Rigaku *R*-axis Spider IP diffractometer. The calculations were performed using the Gaussian 03 program. The anticancer activity was tested by State Key Laboratory of Oncology, Sun Yat-Sen University.

All reagents purchased from commercial sources were directly used without purification. 1-Phenyl-3-methyl-4-propyl-

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pyrazolone-5 (PMPP) and salicylidene hydrazone (SAH) were prepared according to the literatures [16,17].

2.2. Preparation of H_2L and its copper(II) complex

The ligand H_2L was prepared following the literature method [18]. Yield: 81%. *Anal. Calc.* for $C_{20}H_{19}N_4O_2$: C, 69.15; H, 5.51; N, 16.13. Found: C, 68.95; H, 5.77; N, 16.11%.

The complex $[Cu_2L_2CH_3OH]\cdot 2CH_3OH$ was prepared as follows. To 50 mL methanol solution of the ligand (0.3 mmol) was added a methanol solution (5 mL) of $Cu(OAc)_2\cdot H_2O$ (0.3 mmol), and the mixture was stirred and refluxed at 70 °C for 4 h. The resulting solution was cooled to room temperature and then filtered off. The dark green powders were obtained. Single crystals suitable for X-ray diffraction analysis were obtained by slow evaporation of the filtrate. This crystal must be protected by mother liquor as a result of airslaking. Yield: 52%. *Anal. Calc.* for $C_{43}H_{48}Cu_2N_8O_7$: C, 57.81; H, 4.73; N, 13.15. Found: C, 57.15; H, 5.15; N, 14.19%.

2.3. X-ray crystallography

The crystallographic data were collected on an imaging plate system (Rigaku *R*-axis spider) with a graphite monochromatized Mo Kα radiation ($\lambda = 0.71073$ Å). The crystal was mounted with grease on the top of a glass fiber and quench to 153 K in a liquid nitrogen stream. Cell constants and orientation matrix for data collection were obtained by least-squares refinement of the diffraction data in the range of 3.24–27.48°. Crystal structures were solved by direct method and refined on F^2 by full-matrix least-squares method with the SHELXTL-97 program [19]. All non-H atoms were refined anisotropically. The H atoms on oxygen atoms were located from the Fourier maps, and all of the other H atoms were placed in geometrically idealized positions. The crystal data and structure refinement details are given in Table 1. Selected bond lengths and bond angles are listed in Table 2.

Table 1
Crystallographic data for complex.

Empirical formula	$C_{43}H_{48}N_8O_7Cu_2$
Formula weight	915.97
Crystal system	triclinic
Space group	$P\bar{1}$
Unit cell dimensions	
a (Å)	11.6905(4)
b (Å)	13.0063(4)
c (Å)	14.4352(5)
α (°)	103.8900(10)
β (°)	93.9400(10)
γ (°)	99.2750(10)
Volume, *Z* (Å^3)	2089.58(12), 2
Crystal size (mm)	0.33 × 0.15 × 0.12
Density (calculated) (g/cm^3)	1.456
Absorption coefficient (mm^{-1})	1.079
F(0 0 0)	952
Limiting indices	$-15 \le h \le 15$, $-16 \le k \le 16$, $-18 \le l \le 18$
Absorption correction	empirical
Refinement method	full-matrix least-squares on F^2
Data/restraints/parameters	9490/3/560
Goodness-of-fit (GOF) on F^2	1.082
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0281$, $\omega R_2 = 0.0817$
R indices (all data)	$R_1 = 0.1529$, $\omega R_2 = 0.1561$
Largest differences in peak and hole	0.388 and −0.393 e. Å^{-3}

Table 2
Selected bond lengths (Å) and bond angles (°) for the complex.

Cu(1)–O(1A)	1.927(1)	Cu(1)–O(1)	1.934(1)
Cu(1)–N(3)	1.977(1)	Cu(1)–N(3A)	1.998(2)
Cu(1)–O(3)	2.330(2)	Cu(2)–O(2A)	1.892(1)
Cu(2)–O(2)	1.902(1)	Cu(2)–N(4)	1.939(2)
Cu(2)–N(4A)	1.942(1)	O(1A)–C(7A)	1.277(2)
O(1)–C(7)	1.278(2)		
O(1A)–Cu(1)–O(1)	83.82(5)	O(1A)–Cu(1)–N(3)	171.86(6)
O(1)–Cu(1)–N(3)	89.95(6)	O(1A)–Cu(1)–N(3A)	89.90(6)
O(1)–Cu(1)–N(3A)	166.21(6)	N(3)–Cu(1)–N(3A)	95.04(6)
O(1A)–Cu(1)–O(3)	92.55(6)	O(1)–Cu(1)–O(3)	98.50(6)
N(3)–Cu(1)–O(3)	93.55(6)	N(3A)–Cu(1)–O(3)	94.03(6)
O(2A)–Cu(2)–O(2)	90.42(6)	O(2A)–Cu(2)–N(4)	152.91(7)
O(2)–Cu(2)–N(4)	94.11(6)	O(2A)–Cu(2)–N(4A)	92.84(6)
O(2)–Cu(2)–N(4A)	155.49(6)	N(4)–Cu(2)–N(4A)	93.97(6)

3. Results and discussion

3.1. Structural description

The title complex crystallized in triclinic with space group of $P\bar{1}$. The crystal structure of $[Cu_2L_2CH_3OH]\cdot 2CH_3OH$ with non-carbon atomic numbering scheme is illustrated in Fig. 1. The crystallographic structural analysis of the copper complex reveals that the whole structure exhibits axisymmetric way and the two central metal atoms adopt different coordination patterns. The distance between Cu1 and Cu2 is 3.867 Å.

Cu1 locates in a square–pyramidal geometry. Four atoms (O1, O1A, N3, N3A) define a basal plane of the square pyramid with the least-square mean plane deviation of 0.0573 Å. Cu1 ion strays from the bottom plane with the deviation of 0.1579 Å. The apical position is occupied by the O3 atom of the methanol molecule. Furthermore, the bond angles of O1–Cu–N3A and O1A–Cu–N3 are 166.21(6)° and 171.86(6)°, which are slightly deviated from the theoretical value of 180°. All these observations indicate that the coordination geometry of the Cu1(II) ion is a little distorted square pyramid.

However, the Cu2 ion exists in a square–planar geometry with two oxygen atoms O2, O2A and two nitrogen atoms N4, N4A from two ligands. The four coordinated atoms O2, O2A, N4 and N4A deviate badly from their least-square mean plane with 0.4370, −0.4437, −0.4091 and 0.4158 Å, respectively. The bond angles of O(2)–Cu(2)–N(4A), O(2A)–Cu(2)–N(4), N(4)–Cu(2)–N(4A), O(2A)–Cu(2)–O(2), O(2)–Cu(2)–N(4), O(2A)–Cu(2)–N(4A) are 155.49(6)°, 152.91(7)°, 93.97(6)°, 90.42(6)°, 94.11(6)°, 92.84(6)°, respectively. So the geometry of the Cu2(II) ion is a seriously distorted square plane.

In addition, there are two methanol molecules in the structure of the complex. From the Fig. 2, it can be seen that an intermolecular hydrogen bond (O4A···H–O5B) exists in two methanol molecules and there are two intermolecular hydrogen bonds (O3B–H···O5B, O4A–H···O2A) between methanol and complex molecules. A dimer is formed owing to the interaction of the intermolecular hydrogen bonds.

3.2. IR spectra studies

The bonding mode of the ligand coordinated to copper(II) ion was further elucidated by comparison of the IR spectra of H_2L and Cu-complex.

The molecular structure of the ligand [20] is shown in Fig. 3. From the crystal structure, we can see the ligand exists as the keto form [21]. The broad peak at 2975 cm^{-1} in the free ligand corresponds to the ν(N–H) [22]. And the strong bands at 1591 and 1623 cm^{-1} are assigned to ν(C=N) and ν(C=O) of the pyrazolone-ring [22,23], which bears out that ligand exists as the keto form in the solid state, consisting with the crystal structure. The band at 3054 and 1267 cm^{-1} can be attributed to the OH [22,24] and C–O stretching vibration of the salicyl on the lateral chain [25].

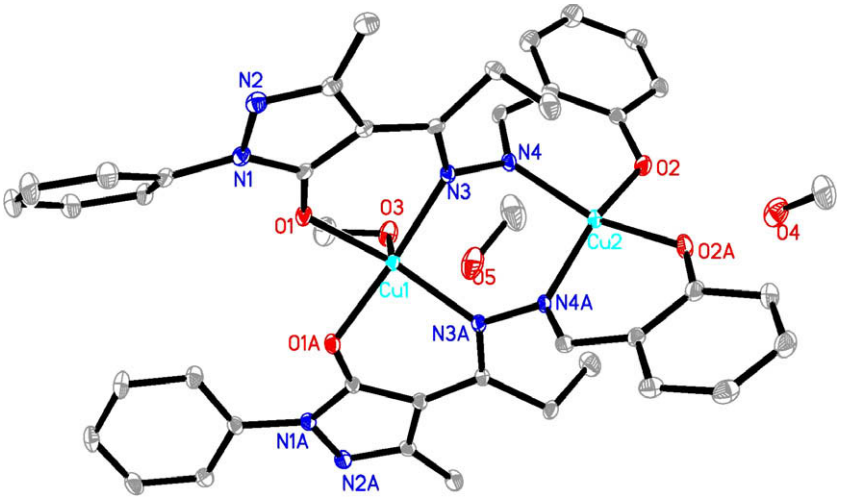


Fig. 1. Crystal structure of the complex $[Cu_2L_2CH_3OH]\cdot 2CH_3OH$ with the non-carbon atomic numbering. Hydrogen atoms are omitted for clarity.

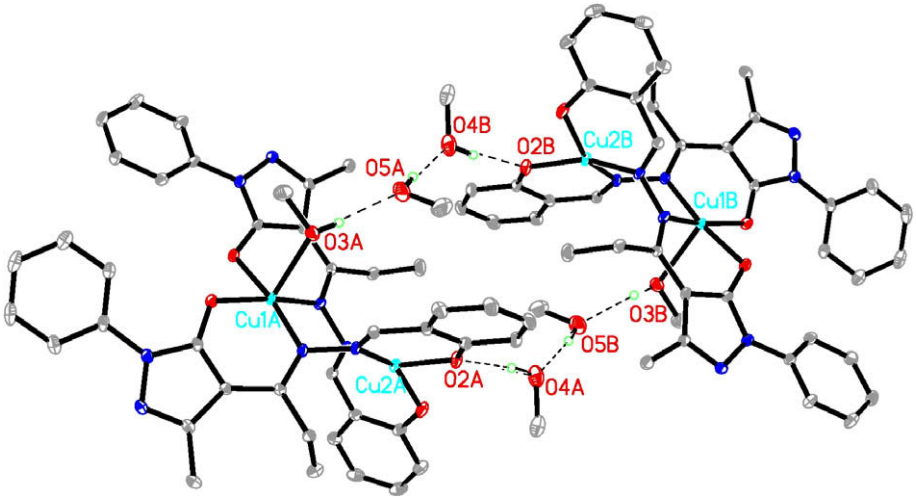


Fig. 2. The hydrogen bonded centrosymmetric dimer in complex.

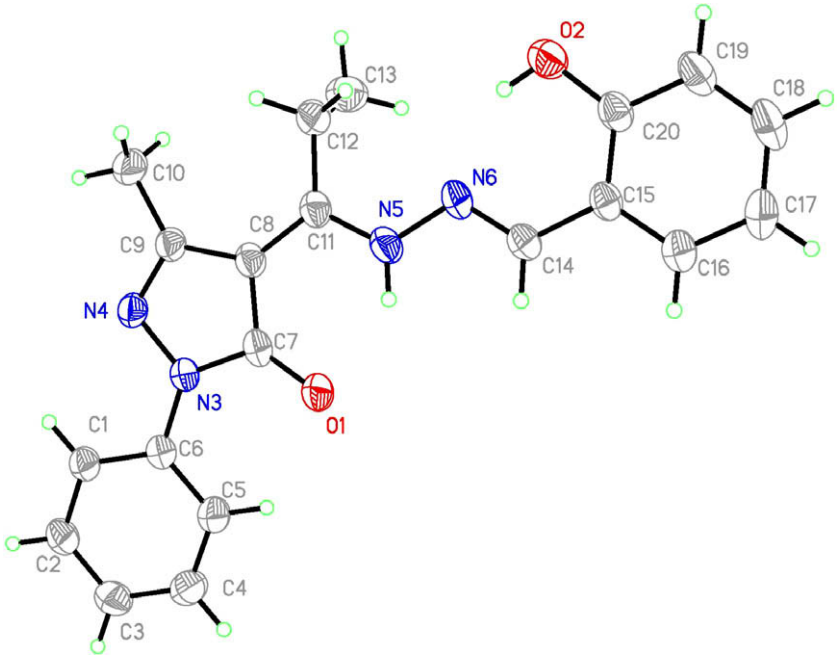
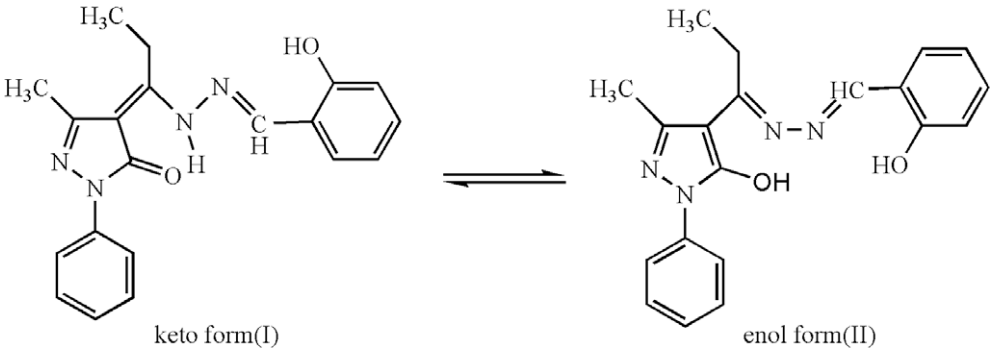


Fig. 3. Crystal structure of the ligand.



Scheme 1. Tautomerism between enol form and keto form.

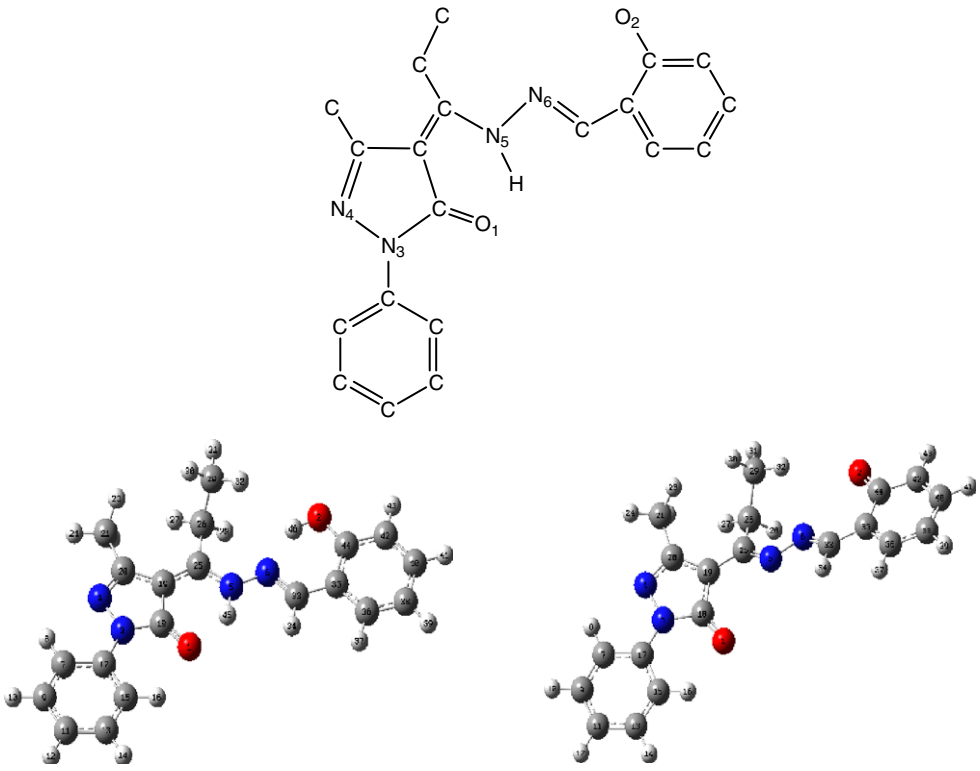


Fig. 4. Optimized geometries of H_2L and L^{2-} formed from the complex.

In IR spectrum of the complex, it can be obviously observed that the C=O and N–H stretching vibrations disappear, and new bands attributed to ν(C–O) and ν(–C=N–N=C–) appear at 1463 and 1605 cm^{-1} [25], respectively. There are other changes that the absorption peaks of C–OH and C=N slightly shift to lower wavenumber, indicating the coordination of phenolic oxygen atom and azomethine nitrogen to the central metal atom [26]. In addition, the new bands at about 473 and 512 cm^{-1} are assigned to Cu–N and Cu–O stretching vibrations [26]. These results indicate that ligand undergoes isomerization from the keto form to the enol form during the coordination, and then loses two protons to coordinate with Cu(II) atom as a double negative charged tetradentate ligand (Scheme 1 enol (II)). Furthermore, the lateral chain of the ligand occurs torsion nearly 180° going with losing protons. This perhaps resulted by the coordination effect of the copper ion.

3.3. Theoretical calculation of H_2L

The geometric parameter of ligand was taken from two ways, the neutral form H_2L from the X-ray single crystal diffraction data [20] and the ionic form L^{2-} from the copper(II) complex. The two structures have been both optimized at the DFT/B3LYP level using the 6-31g* basis set. The optimized geometries of H_2L and L^{2-} are shown in Fig. 4. There is a torsion in lateral chain of the ionic form (31°), compared with that of the neutral form.

Since the condensed Fukui function value, f_k^-, represents the relative reactivity of different sites in the ligand, it was frequently used to point to atoms suitable for electrophilic attack [27–29]. The f_k^- values of the neutral and deprotonated form were calculated by Mulliken population analysis (MPA) and Natural Population analysis (NPA). Selected f_k^- values for neutral and deprotonated H_2L are given in Table 3. The calculated condensed Fukui functions predicted that the most probable reactive sites for electrophilic attack are oxygen atoms (O1, O2), which suggested that the metal atoms initially approach to oxygen atoms (O1, O2). And then, the metal atoms coordinate with adjacent reactive sites, N5 and N6. In addition, the much more positive value of f_k^- is found for N4, it should be a stronger coordinated site in theory. Furthermore, N4 atom was demonstrated to coordinate to Cu(II) ion in previously reported compound [21]. Actually, N4 atom

Table 3
Condensed Fukui functions of selected atoms from neutral and ion form of ligand.

Atom[a]	f_k^-			
	The neutral form		The ion form	
	Mulliken	NPA	Mulliken	NPA
O1	0.0877	0.1208	0.0659	0.0855
O2	0.0344	0.0374	0.0944	0.1283
N3	0.0243	0.0546	−0.0091	0.0026
N4	0.0489	0.0619	0.0736	0.1016
N5	0.0170	0.0414	0.0238	0.0361
N6	0.0053	0.0025	0.0232	0.0458

[a] Atom numbering is given in Fig. 4.

Table 4
Lethal concentration 50% of the ligand and complex on OVCAR3 and Hep-G2.

Sample	IC_{50} (μg/ml)	
	OVCAR3	Hep-G2
H_2L	46.8	35.1
$[Cu_2(L)_2CH_3OH]\cdot 2CH_3OH$	17.6	5.0

didn't participate in coordination in the title compound owing to the stereo-hindrance effect of propyl group on the 4-position of pyrazole ring and the result of Cu(II) ion firstly coordinated to O atoms. N3 atom of L^{2-} is the least site with the negative f_k^- value (−0.0091), which is consistent with the structure of ligand. Above all, our calculations showed that the deprotonated form has much more positive values than their corresponding neutral form, and hence the reactive activity is enhanced, which accords with the results of complex structure analyses and IR spectral analyses.

3.4. Pharmacology

The biological activity of the ligand and complex were detected by MTT assay [1]. The pharmacological testing results of H_2L and its copper(II) complex are presented in Table 4.

From the crystal structure, we know that there are several coordination spots, such as N, O atoms in ligand, which are close to biological environment. The pharmacological testing has also proved that the ligand and the complex show excellent efficacy against ovarian cancer cells (OVCAR3) and liver cancer cells (Hep-G2). The results of the effect on the resistant Hep-G2 revealed that the copper complex was an active compound with lethal concentration 50% (IC_{50}) value 5.0 μg/ml. An IC_{50} of 35.1 μg/ml was found for H_2L, which was not as good as the complex. It can conclude that the coordination improve the anticancer activity of the compound. Meanwhile, the anticancer activities of the tested compounds on Hep-G2 were better than that on OVCAR3. While the title complex will be researched on its anticancer mechanism and undergo further thorough pharmacological investigation. What's more, we look forward to synthesizing novel analogous compounds, and try our best to discover their structure–activity relationships.

4. Conclusions

In summary, a novel copper(II) complex coordinated with N-(1-phenyl-3-methyl-4-propyl-pyrazolone-5)-salicylidene hydrazone, has been demonstrated. The pharmacological testing results suggest that the novel copper complex and its ligand, are both potent anticancer agent. Besides, the efficacy on resisting to cancer cell of the copper complex is more powerful than that of the ligand, which shows that the coordination improves the antitumor activity of the compound. The condensed Fukui function has been calculated to predict where the metal atom will initially approach. All the results have been approved by the structure analyses of the copper complex. The findings supply a way for synthesis and design of new anticancer medicine.

Supplementary material

CCDC 713774 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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