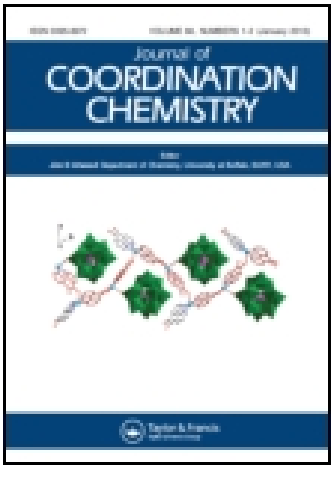

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Jin'an Zhao[a], Kun Peng[b], Yan Guo[b], Jin Zhang[b], Dandan Zhao[b], Shufang Chen[a] & Jiyong Hu[a]

[a] College of Chemistry and Material Engineering, Henan University of Urban Construction, Pingdingshan, PR China

[b] College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou, PR China

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Cytotoxicity towards human alimentary system carcinoma cells resulting from diverse copper(II) complexes

JIN'AN ZHAO*†, KUN PENG‡, YAN GUO‡, JIN ZHANG‡, DANDAN ZHAO‡, SHUFANG CHEN† and JIYONG HU†

†College of Chemistry and Material Engineering, Henan University of Urban Construction, Pingdingshan, PR China
‡College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou, PR China

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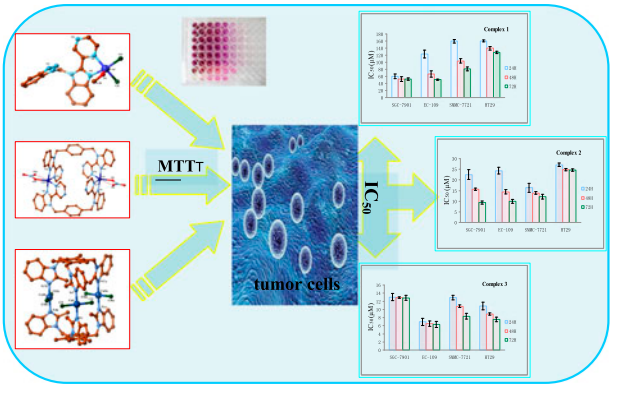

Three coordination compounds with heterocyclic ligands were constructed to investigate the potential cytotoxicity of copper(II)-based complexes. IC_{50} values revealed that complexes **2** and **3** show strong cytotoxicity, whereas complex **1** is weakly cytotoxic after being tested by MTT assay against a panel of several human alimentary system carcinoma cell lines.

To investigate the potential cytotoxicity of copper(II)-based complexes, three coordination compounds with heterocyclic ligands, $Cu(pbmbt)Cl_2(CH_3OH)$ (**1**), $Cu_2(ddbib)_2(NO_3)_4{\cdot}3CH_3OH$ (**2**), and $Cu_3(ttmtmb)_2Cl_6{\cdot}2.5H_2O$ (**3**), which include mononuclear, dinuclear, and trinuclear structures, have been synthesized from reactions of corresponding copper(II) salts with 1-((2-pyrazinyl)-1H-benzoimidazol-1-yl)methyl)-1H-benzotriazole (pbmbt), 2-(2,3-dihydropyrazin-2-yl)-1-((4-((2-(2,3-dihydropyrazin-2-yl)-1H-benzo[d]imidazol-1-yl)methyl)phenyl)methyl)-1H-benzo[d] imidazole (ddbib), and 1,1′,1″-((2,4,6-trimethylbenzene-1,3,5-triyl)tris(methylene)tris(2-methyl-1H-benzoimidazole) (ttmtmb), respectively. IC_{50} values revealed that **2** and **3** show strong cytotoxicity, whereas **1** is weakly cytotoxic after being tested against a panel of several human alimentary system carcinoma cell lines (SGC7901, EC109, SMMC7721, and HT29). The number of copper centers and different structures could make a tremendous difference on their cytotoxicity.

Keywords: Copper(II)-based complexes; Crystal structure; Cytotoxicity

*Corresponding author. Email: zjinan@zzu.edu.cn

1. Introduction

Cisplatin is an effective agent used to treat various types of human cancer [1, 2]; its clinical effectiveness, however, has been limited by high toxicity, severe side-effects, and cross-resistance [3, 4]. For these reasons, exploring modified versions of cisplatin and non-Pt complexes for cytotoxicity is necessary. Some Ru- and Pd-based complexes, such as *trans*-[$RuCl_4$(dmso-S)(Im)] (Im = imidazole, dmso = dimethylsulfoxide) and (NHC)Pd(pyridine)Cl_2 (NHC = N-heterocyclic carbine), have demonstrated excellent antimetastatic activity against tumors [5]. In the past few years, for easy accessibility and less toxicity, complexes based on earlier transition metals have also attracted considerable interest.

Copper-based coordination compounds are important in chemistry and biology; copper is an essential bioelement responsible for numerous catalytic processes in living organisms [6] and has the possibility of exploring magneto-structural correlations resulting from the interaction among metal centers [7]. Some mixed chelate copper-based complexes have exhibited greater antineoplastic potency than cisplatin in both *in vitro* and *in vivo* studies of a range of tumor cell lines [8, 9], which might be caused by its unpaired electrons [10]. These copper-based complexes also show superoxide dismutase-like activity [11] and a low potency to induce genomic instability through intrachromosomal recombination [12]. Though copper has a long history of medical applications as an anti-inflammatory or anti-arthritic drug, cuprous or cupric complexes have not been extensively examined as potential anticancer agents until recently [13]. In the group of Carlo Santini, for example, copper(I) phosphine complexes were applied to test their antitumor activity and proved that these complexes are strongly cytotoxic against certain human tumor cell lines [14]. Very recently, Xu and co-workers reported the cytotoxicity of a copper(II) complexes that exhibited greater cytotoxicity towards HeLa cell lines than cisplatin under *in vitro* conditions [15]. In this paper, three copper(II) complexes were assessed for their potential cytotoxic properties.

Certain copper-based complexes catalyze radical formation, while others seem to have antioxidant efficacy [16]; the different behaviors depend upon the chemical environment and the nature of the connecting agent [3, 4, 17]. To look for possible cytotoxic properties of copper(II) complexes as well as the factors influencing their cytotoxicity, mononuclear, dinuclear, and trinuclear structures, Cu(pbmbt)Cl_2(CH_3OH) (**1**), Cu_2(ddbib)$_2$(NO_3)$_4$·3-CH_3OH (**2**), and Cu_3(ttmtmb)$_2Cl_6$·2.5H_2O (**3**) (pbmbt = 1-((2-pyrazinyl)-1H-benzoimidazol-1-yl)methyl)-1H-benzotriazole, ddbib = 2-(2,3-dihydropyrazin-2-yl)-1-((4-((2-(2,3-dihydropyrazin-2-yl)-1H-benzo[d]imidazol-1-yl)methyl)phenyl)methyl)-1H-benzo[d]imidazole, ttmtmb = 1,1′,1″-((2,4,6-trimethylbenzene- 1,3,5-triyl)tris(methylene)tris(2-methyl-1H-benzo [d]imidaz-ole), have been constructed. Four human alimentary system carcinoma cell lines, SGC7901 (gastric tumor cell line), EC109 (esophagus tumor cell line), SMMC7721 (liver tumor cell line), and HT29 (colon tumor cell line), were utilized in our work. These cell lines were chosen to explore the potential selectivity of our copper(II) complexes towards different human alimentary system carcinoma cell lines and all have been previously used in studies with copper complexes that are cytotoxic [18–21]. IC_{50} values indicated that **2** and **3** have promising cytotoxicity. The number of copper centers and different structures may be pivotal influencing factors for their cytotoxicity.

2. Experimental

2.1. *Materials and general methods*

All chemicals were obtained from commercial sources and used without purification. The 1-((2-pyrazinyl)-1H-benzoimidazol-1-yl)methyl)-1H-benzotriazole (pbmbt), 2-(2,3-dihydropyrazin-2-yl)-1-((4-((2-(2,3-dihydropyrazin-2-yl)-1H-benzo[d]imidazol-1-yl)methyl)phenyl)methyl)-1H-benzo[d]imidazole (ddbib), and 1,1′,1″-((2,4,6-trimethylbenzene- 1,3,5-triyl)tris(methylene)tris(2-methyl-1H-benzo[d]imidaz-ole) (ttmtmb) were synthesized according to procedures in the literature [22, 23]. IR spectra were recorded from 400 to 4000 cm^{-1} with KBr pellets on a BRUKER TENSOR 27 spectrophotometer. Elemental analyses for C, H, and N were carried out with a Flash EA 1112 elemental analyzer.

2.2. *Synthesis of Cu(pbmbt)Cl_2(CH_3OH) (1)*

A mixture of $CuCl_2 \cdot 2H_2O$ (0.0034 g, 0.02 mM), pbmbt (0.0065 g, 0.02 mM), methanol (1 mL), and chloroform (1 mL) was placed in a glass reactor (10 mL) which was heated at 80 °C for three days and then gradually cooled to room temperature at 5 °C h^{-1}. Green crystals of **1** were obtained. Yield: 40% (based on Cu). Elemental Anal. Calcd for Cu(pbmbt)Cl_2(CH_3OH) (%): C, 46.26%; H, 3.37%; N, 19.87%. Found (%): C, 46.39%; H, 3.33%; N, 19.78%. IR (KBr/pellet, cm^{-1}): 3424.04(m), 1797.81(w), 1610.81(m), 1592.97(m), 1514.46 (m), 1481.37(s), 1162.58(s), 1076.17(w), 767.18(m), and 545.47(m).

2.3. *Synthesis of Cu_2(ddbib)$_2$(NO_3)$_4$·3CH_3OH (2)*

A methanol solution (2 mL) of $Cu(NO_3)_2 \cdot 3H_2O$ (0.0048 g, 0.02 mM) was added slowly to a solution of ddbib (0.0098 g, 0.02 mM) in chloroform (4 mL). Then DMF (2 mL) was added with constant stirring to the reaction mixture. The resulting mixture was left to stand at room temperature for one week. Blue block crystals were obtained. Yield: 35% (based on Cu). Elemental Anal. Calcd for $Cu_2(ddbib)_2(NO_3^-)_4 \cdot 3CH_3OH$ (%): C, 51.82%; H, 3.87%; N, 19.18%. Found (%): C, 51.70%; H, 3.85%; N, 19.27%. IR (KBr/pellet, cm^{-1}): 3419.95 (m), 1762.38(w), 1614.39(m), 1516.69(m), 1481.61(s), 1383.77(s), 1178.14(w), 1028.30 (m), 761.26(m), and 510.39(w).

2.4. *Synthesis of Cu_3(ttmtmb)$_2Cl_6 \cdot 2.5H_2O$ (3)*

A mixture of $CuCl_2 \cdot 2H_2O$ (0.0085 g, 0.05 mM), ttmtmb (0.0112 g, 0.02 mM), methanol (10 mL), acetonitrile (2 mL), and chloroform (3 mL) was placed in a Teflon-lined stainless steel vessel (20 mL). The mixture was sealed and heated at 100 °C and kept for three days, and then the reaction system was gradually cooled to room temperature at 5 °C h^{-1}. Dark green crystals were obtained. Yield: 60% (based on Cu). Elemental Anal. Calcd for $Cu_3(ttmtmb)_2Cl_6 \cdot 2.5H_2O$ (%): C, 55.84%; H, 4.69%; N, 10.85%. Found (%): C, 55.96%; H, 4.66%; N, 10.77%. IR (KBr/pellet, cm^{-1}): 3424.23(m), 2924.57(m), 2406.61 (w), 1686.26(m), 1456.23(s), 1368.75(w), 1160.44(m), 1010.32(w), 748.06(s), and 545.15(s).

2.5. *Structure determination*

Single crystals suitable for X-ray determination were selected and mounted on a glass fiber separately. The data for **1** and **3** were recorded on a Gemini E at room temperature with graphite monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å) for **1** and Cu-Kα ($\lambda = 1.54184$ Å) for **3**. Complex **2** was recorded on a Rigaku MM-OO7/Saturn 70 with graphite monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å) at 113 K. The structures were handled by direct methods and expanded with Fourier techniques. The calculations of **1**, **2,** and **3** were conducted with the SHELXL-97 crystallographic program [24]. All non-hydrogen atoms were refined with anisotropic thermal parameters. The final cycle of full-matrix least-squares refinement was based on observed reflections and variable parameters. Table 1 gives the crystallographic data and structure-processing parameters of the three complexes; selected bond lengths and angles are listed in table 2.

2.6. *Stability determination*

The stability of complexes in solution is important for biological studies. Complexes **1**–**3** are soluble at 3×10^{-5} M concentration in phosphate-buffered saline (PBS, 0.01 M, pH 7.4 at 25 °C) containing 5% dimethylsulfoxide (DMSO). UV–vis absorption spectra of all three complexes were performed on a Specord 200 UV–visible spectrophotometer under this condition. The ESI-MS spectra of **1**–**3** in DMSO were also carried out on an Agilent 1100 LC/MSD Trap SL Electrospray Ionization Mass Spectrometer to further investigate their stability in solution.

2.7. *Cell culture*

Cell lines of SGC7901 (gastric tumor cell line), EC109 (esophagus tumor cell line), SMMC7721 (liver tumor cell line), and HT29 (colon tumor cell line) were maintained in

Table 1. Crystal data and structure refinements for **1–3**.

Complex	**1**	**2**	**3**
Empirical formula	$C_{38}H_{33}Cl_4Cu_2N_{14}O_2$	$C_{63}H_{52}Cu_2N_{20}O_{15}$	$C_{144}H_{144}Cl_{12}Cu_6N_{24}O_5$
Formula weight	986.66	1456.33	3097.47
Temperature (K)	293(2)	113(2)	293(2)
Wavelength (Å)	0.71073	0.71073	1.5418
Crystal system	Orthorhombic	Triclinic	Hexagonal
Space group	*Pna21*	*P-1*	$P6_3/m$
a	16.653(3)	10.5740(10)	16.6323(11)
b	11.351(2)	13.3990(14)	16.6323(11)
c	22.055(4)	14.1160(15)	16.0672(16)
A	90.00	74.680(18)	90.00
B	90.00	72.14(2)	90.00
Γ	90.00	69.871(17)	120.00
Volume (Å^3), *Z*	4169.2(14), 4	1758.9(3), 1	3849.3(5), 1
F (0 0 0)	2004	748	1594
Flack parameter value	0.0(11)		
θ range for data collection (°)	1.85–25.12	1.64–27.90	4.12–70.19
Goodness-of-fit on F^2	1.063	0.962	1.077
Final $R1$[a], $wR2$[b]	0.0589, 0.1654	0.0557, 0.1301	0.0545, 0.1652

[a] $R_1 = [|F_0| - |F_c|]/|F_0|$.
[b] $wR_2 = [w(F_0^2 - F_c^2)^2]/[w(F_0^2)^2]^{1/2}$; $w = 1/[\sigma^2(F_o)^{2+}0.0297\ P^2 + 27.5680\ P]$, where $P = (F^2{}_o + 2\ F^2{}_c)/3$.

Table 2. Selected bond distances (Å) and angles (°) for **1–3**.

Complex **1**			
Cu(1)–N(3)	2.015(5)	Cu(1)–N(1)	2.090(5)
Cu(1)–Cl(1)	2.238(2)	Cu(1)–Cl(2)	2.276(2)
Cu(1)–O(1)	2.286(6)		
N(3)–Cu(1)–N(1)	78.5(2)	N(3)–Cu(1)–Cl(1)	170.39(16)
N(3)–Cu(1)–Cl(2)	94.94(18)	N(3)–Cu(1)–O(1)	91.9(3)
N(1)–Cu(1)–Cl(1)	91.89(18)	N(1)–Cu(1)–Cl(2)	156.11(19)
N(1)–Cu(1)–O(1)	101.7(2)	Cl(1)–Cu(1)–Cl(2)	93.66(8)
Cl(1)–Cu(1)–O(1)	90.6(2)	Cl(2)–Cu(1)–O(1)	101.50(19)
Complex **2**			
Cu(1)–O(1)	2.092(2)	Cu(1)–N(1)	1.938(2)
Cu(1)–N(3)	2.118(2)	Cu(1)–N(6)#1	1.948(2)
Cu(1)–N(7)#1	2.175(2)		
O(1)–Cu(1)–N(3)	127.34(9)	O(1)–Cu(1)–N(7)#1	115.82(9)
N(1)–Cu(1)–O(1)	90.71(9)	N(1)–Cu(1)–N(3)	79.47(8)
N(1)–Cu(1)–N(6)#1	175.32(9)	N(1)–Cu(1)–N(7)#1	97.93(9)
N(3)–Cu(1)–N(7)#1	116.75(9)	N(6)#1–Cu(1)–O(1)	93.56(9)
N(6)#1–Cu(1)–N(3)	99.38(9)	N(6)#1–Cu(1)–N(7)#1	78.48(9)
Complex **3**			
Cu(1)–Cl(1)	2.2410(16)	Cu(1)–Cl(2)	2.2604(15)
Cu(1)–N(1)	1.978(3)		
Cl(1)–Cu(1)–Cl(2)	177.46(10)	N(1)–Cu(1)–Cl(1)	91.47(7)
N(1)–Cu(1)–N(1)#1	176.38(16)	N(1)–Cu(1)–Cl(2)	88.58(7)

Note: Symmetry codes #1: $2-x, 1-y, -z$ for **2**; #1:$+x,+y, 1/2-z$ for **3**.

the logarithmic phase at 37 °C in a highly humidified atmosphere of 95% air with 5% carbon dioxide using RPMI1640 medium supplemented with 10% (v/v) heat inactive fetal bovine serum.

2.8. In vitro *cytotoxicity assay*

Complexes **1**, **2**, and **3** were dissolved in DMSO (cell culture reagent) just before the experiment, and a certain amount of complex solution was added to the growth medium containing cells with a final solvent concentration of 3%, which hardly has discernible effect on cell killing [25].

The growth inhibitory effect of **1–3** on human tumor cell lines was evaluated by MTT (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, in which MTT is cut by living cells to produce a DMSO soluble (formazan) that could be detected through colorimetric analysis. Briefly, cells were seeded in 96-well microplates in growth medium (100 μL) and then incubated at 37 °C in a highly humidified atmosphere with 5% CO_2. Amount of cells range from 3×10^3 to 8×10^3 cells/well and the number relies on the growth characteristics of different cell lines to change. The medium was eliminated and replaced with a fresh one (200 μL) containing the complexes at five different concentrations after 24 h, and the range of concentrations used is dependent on each complex (table 3). Three test timescales (24, 48, and 72 h) were established for each treatment. After the time, 20 μL of MTT solution (5 mg mL^{-1}) was added to each well with further incubation for 4 h at 37 °C. Then the medium with MTT was discarded, and 150 μL of DMSO was added to each well to dissolve the formazan crystals at room temperature. The absorbance was measured at 492 nm using a microplate reader. The % cell inhibition was determined as follows:

Table 3. Concentration gradients of **1–3**.

Complex	Concentration ($\mu g\,mL^{-1}$)				
1	2.5	5	10	20	40
2	2.5	5	10	20	40
3	1.8625	3.725	7.5	15	30

% cell inhibition = $(1 - Abs_{treated\ cells}/Abs_{control\ cells}) \times 100\%$. Results of complexes were expressed as IC_{50}, which is the concentration of complexes required to induce 50% inhibition of cell growth. They were calculated on the basis of cell inhibition with modified Karber's method. All tests were independently repeated at least thrice.

3. Results and discussion

3.1. *Crystal structure of Cu(pbmbt)Cl$_2$(CH$_3$OH) (1)*

The mononuclear motif of **1** is depicted in figure 1, crystallizing in orthorhombic with the space group *Pna21*. Complex **1** adopts a slightly distorted square pyramidal geometry. The Cu(II) is five-coordinate, binding two nitrogens (N1, N3) from one pbmbt, one oxygen (O1) of methanol and two chlorides (Cl1, Cl2). The Cu–N distances range from 2.015(5) to 2.090(5) Å and the Cu–Cl bond lengths vary from 2.238(2) to 2.276(2) Å; the distance of Cu–O is 2.286(6) Å.

The structure of **1** is stabilized by face-to-face $\pi\cdots\pi$ interactions between adjacent benzene and pyrazine rings with centroid separation of 3.732(5) Å and dihedral angle of 9.4 (4)°. Further, the mononuclear motif (figure 2) is stabilized by hydrogen bonds (3.714(9) Å for C8–H8···Cl4^{A}, 3.722(8) Å for C12–H12A···Cl2^{B}, 3.655(8) Å for C26–H26···Cl2^{C}, and 3.500(8) Å for C28–H28···Cl2^{D}) [26], and the structure is ultimately connected into a 3-D supramolecular network by those hydrogen bonding as well as $\pi\cdots\pi$ interactions (symmetry codes: $^{A}2-x, 1-y, -1/2+z$; $^{B}-1/2+x, 3/2-y, z$; $^{C}2-x, 1-y, 1/2+z$; $^{D}3/2-x, -1/2+y, 1/2+z$).

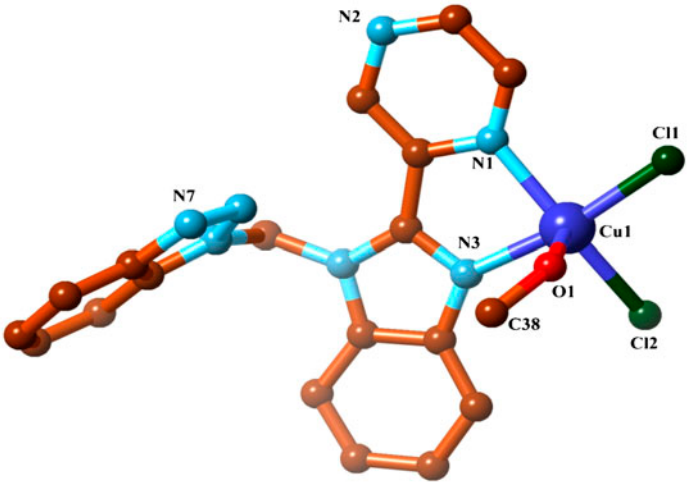

Figure 1. The mononuclear framework of **1** (all hydrogens are omitted for clarity).

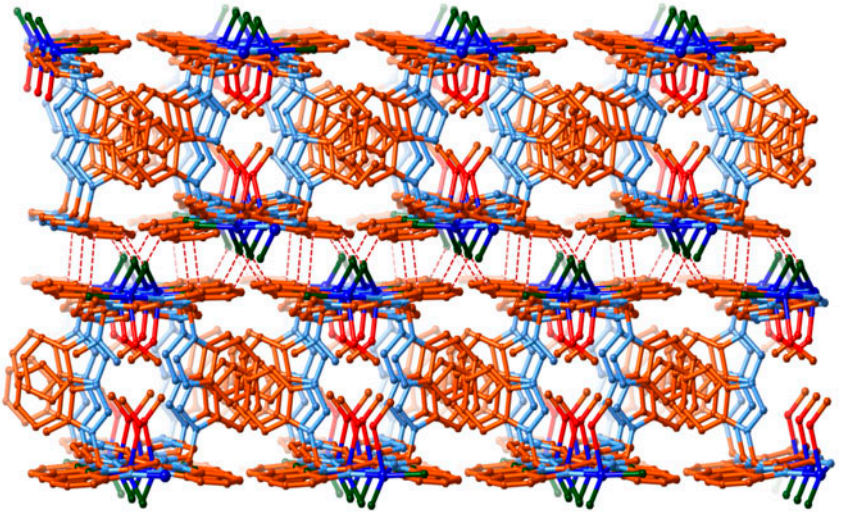

Figure 2. View of the 3-D supramolecular structure of **1** stabilized by hydrogen-bonding and $\pi\cdots\pi$ interactions (Symmetry codes: $^{A}2-x,\ 1-y,\ -1/2+z$; $^{B}-1/2+x,\ 3/2-y,\ z$; $^{C}2-x,\ 1-y,\ 1/2+z$; $^{D}3/2-x,\ -1/2+y,\ 1/2+z$).

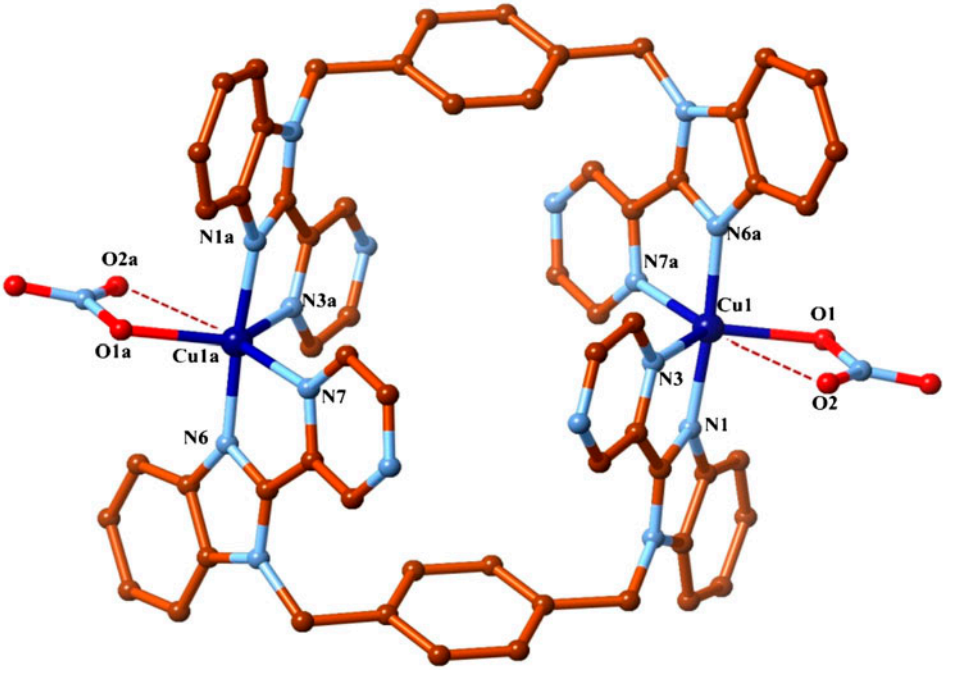

Figure 3. The coordination environment of copper in **2** (all hydrogens, uncoordinated groups and solvent molecules are omitted for clarity).

3.2. *Crystal structure of $Cu_2(ddbib)_2(NO_3)_4{\cdot}3CH_3OH$ (2)*

As presented in figure 3, single-crystal X-ray crystallographic analysis reveals that **2** crystallizes in a triclinic system, space group *P-1*, with a dinuclear motif. The asymmetric unit of **2** contains one Cu(II), one ddbib, a coordinated NO_3^-, an uncoordinated NO_3^-, and one-and-a-half methanol molecules. Each Cu(II) is six-coordinate in a slightly distorted octahedral coordination environment. The basal sites of the coordination sphere are made up of two nitrogens (N3, N7A) from different ddbid ligands and O1 and O2 from a NO_3^-, while the axial sites contain two nitrogens (N1, N6A). The Cu–N distances are 1.938(2)–2.175(2) Å. One Cu–O bond is 2.092(2) Å, whereas another is 2.793(2) Å, reflecting a weak electronic interaction between Cu and oxygen [27].

In **2**, there exist $\pi\cdots\pi$ interactions between imidazole ring and benzene ring (3.98(18) Å). The $\pi\cdots\pi$ interactions and hydrogen bonds (3.384(4) Å for C9–H9$\cdots$O4^{A}, 3.163(4) Å for C10–H10$\cdots$O6^{A}, 3.467(4) Å for C14–H14$\cdots$O7^{B}, 3.109(5) Å for C15–H15$\cdots$O4^{B}, 3.317(3) Å for C19–H19A$\cdots$O1^{C}, 3.217(3) Å for C19–H19A$\cdots$O3^{C}, 3.232(4) Å for C23–H23$\cdots$N4^{B}, 3.201(3) Å for C26–H26$\cdots$O1^{C}, and 3.228(4) Å for C30–H30$\cdots$O7^{A}) extend the binuclear structures (figure S1 see online supplemental material at http://dx.doi.org/10.1080/00958972.2014.938064) into a 3-D supramolecular architecture (symmetry codes: $^{A}1-x$, $1-y$, $-z$; $^{B}1+x$, y, z; $^{C}1+x$, $-1+y$, z).

3.3. *Crystal structure of $Cu_3(ttmtmb)_2Cl_6{\cdot}2.5H_2O$ (3)*

Substitution of pbmbt in **1** by ttmtmb gives trinuclear **3**. Single-crystal structural determination illustrates that **3** crystallizes in a hexagonal system with space group *$P6_3/m$*. As illustrated in figure 4, the structure of **3** has three Cu(II) centers, two ttmtmb ligands, six chlorides, and 2.5 uncoordinated waters. Cu(II) is a distorted tetrahedral geometry coordinating with two nitrogens (N1, N1A) from distinct ttmtmb ligands as well as two

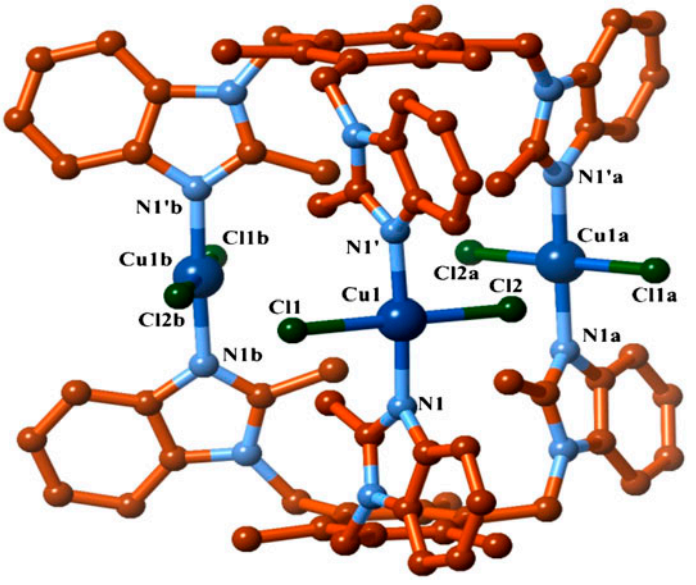

Figure 4. The trinuclear motif of **3** (all hydrogens and solvent molecules are omitted for clarity).

Cl^- (Cl1, Cl2) with the average Cu–N and Cu–Cl bond distances of 1.978 Å and 2.251 Å, respectively. The bond angles around Cu(II) vary from 88.58(7)° to 177.46(10)°.

In **3**, hydrogen bonds are the main forces to hold the subunits together and sustain the crystal framework. Every two adjacent $[Cu(ttmtmb)]^{2+}$ units are connected by a couple of complementary C4–H4A···Cl^A hydrogen bonds (3.393(3) Å) [26], as displayed in figure S2, thus assembling the trinuclear metallacycle into a 3-D supramolecular structure (symmetry code: $^A1-x, -y, -1/2+z$).

3.4. *Stability of 1–3 in solution*

The UV–vis spectra of **1** at different times are shown in figure 5 (for the spectra of **2** and **3**, see Supporting Information, figures S3 and S4). Over time, the characteristic absorption of each complex displayed hypochromicity but no bathochromic shift. The hypochromicity can be attributed to gradual formation of aggregates of the complexes in solution, which decrease their effective concentration for UV–vis absorption [28]. In a 72 h period, no significant change was observed in the room temperature UV–vis spectrum of each complex, indicating no dissociation. To further prove the stability of these copper complexes in solution, the ESI-MS spectra of **1**–**3** in DMSO were also carried out. The ESI-MS spectra mainly contain species $[Cu(pbmbt)Cl]^+$ (*m/z* 425.2), $[Cu_2(ddbib)_2(NO_3)_2]^{2+}$ (*m/z* 619.3), and $[Cu_3(ttmtmb)_2Cl_4]^{2+}$ (*m/z* 706.3) for **1**–**3**, respectively. The ESI-MS results generally agree well with that of UV–vis spectra analysis, which reveal that **1**–**3** are stable in solution.

3.5. *Cytotoxicity results*

Complexes **1**, **2**, and **3** were assessed with the standard MTT assays for their cytotoxic properties against a panel of four human alimentary system carcinoma cell lines, SGC7901 (gastric tumor cell line), EC109 (esophagus tumor cell line), SMMC7721 (liver tumor cell

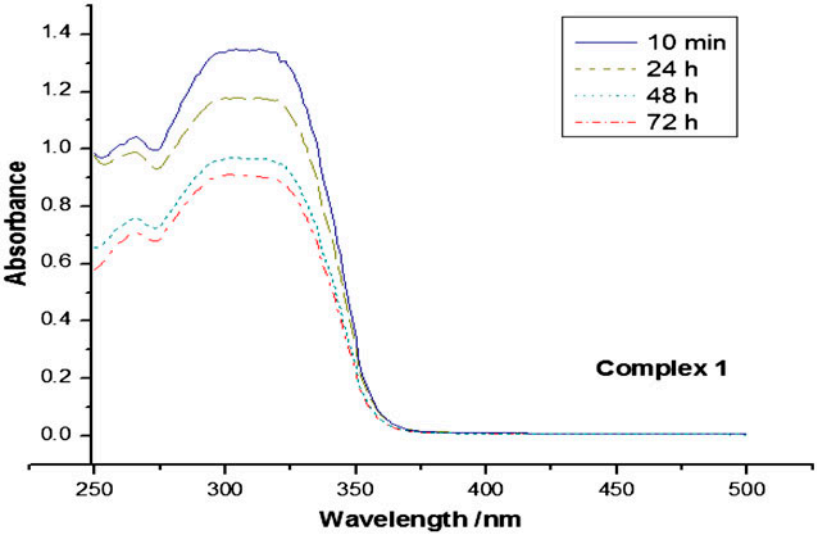

Figure 5. Time-dependent stability studies on **1** in solution monitored by UV–vis absorption spectra.

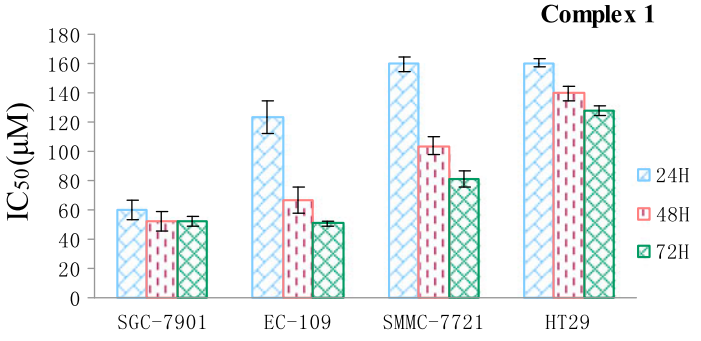

Figure 6. The IC_{50} values of **1** against tumor cell lines.

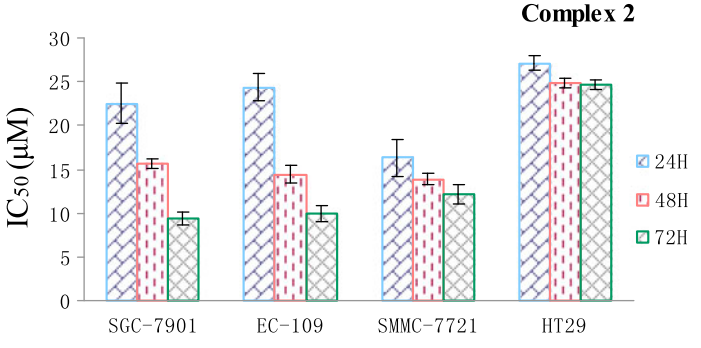

Figure 7. The IC_{50} values of **2** against tumor cell lines.

line), and HT29 (colon tumor cell line), after different times. Complexes **1**, **2**, and **3** were dissolved in DMSO with certain concentrations and a blank sample with the same volume of DMSO was provided as a control. IC_{50} values, which can be seen in figures 6–8, were calculated from the dose-survival curves obtained after 24, 48, and 72 h drug treatment from the MTT test.

Among these complexes, **3** displayed more potent cytotoxicity than others on EC109, SMMC7721, and HT29 with the IC_{50} values (μM) being 6.37, 8.32, and 7.53 for 72 h, respectively, whereas, **2** is most effective on SGC7901 with IC_{50} value of 9.39 μM, lower than that of cisplatin [29]. In particular, the trinuclear **3** exhibited fast-acting, broad spectrum cytotoxicity against EC109, with IC_{50} values being reached at 6.93 μM after 24 h, which is better than cisplatin [30]. The cytotoxic effects of **2** against SGC7901 and EC109 are time-dependent. After 24 h, **2** had weak cytotoxicity with IC_{50} values of 22.54 and 24.35 μM, and the values decreased to 15.68 and 14.41 μM for 48 h, then 9.39 and 10.00 μM another 24 h later, which suggested that it should take a relatively long time before **2** shows highly cytotoxicity to SGC7901 and EC109. The identical situation also appeared for **1** against EC109 and SMMC7721, but the effects are much worse. Significantly, on SMMC7721, resistant to cisplatin [31], cytotoxicities shown by **2** and **3** are very strong; this result is encouraging as we could consider these derivatives to develop new potential agents against liver tumors.

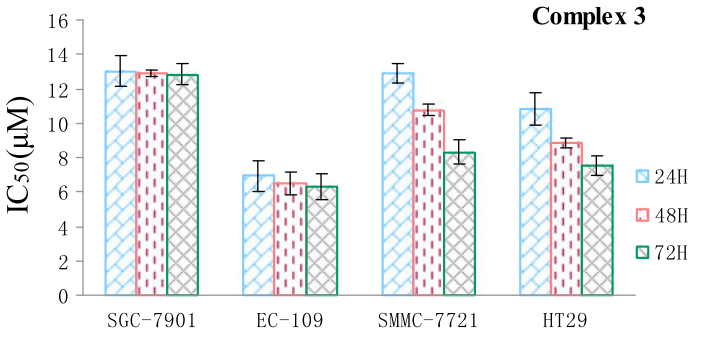

Figure 8. The IC_{50} values of **3** against tumor cell lines.

In parallel with these findings, **2** and **3** exhibited potent cytotoxicity in selected human alimentary system carcinoma cell lines (SGC7901, EC109, SMMC7721, HT29), generally comparable to the one presented by cisplatin under similar conditions [29, 30, 32], with the exception of the SMMC7721 cell line where **2** and **3** are more active. IC_{50} values of **2** and **3** reach about 10 μM, lower than that of some previously reported analogous copper(II) complexes against these human alimentary system carcinoma cell lines [18–20, 33–36], indicating that **2** and **3** have potent cytotoxicity.

The remarkable cytotoxicity of **2** and **3** might be related to the larger number of copper centers (tricopper > dicopper > monocopper) and different structures compared to that of **1**. Similar phenomena are also found in the literature [37, 38]. One interesting trend about how metal-based drugs function for metals such as copper and ruthenium is that each one utilizes multiple biological mechanisms and can work by a variety of different routes. This multifaceted approach could perhaps contribute to their enhanced activity in contrast to other metals such as rhodium and low occurrence of resistance towards them compared with platinum [5]. Work is in progress to comprehensively elucidate the mechanistic pathways employed by **2** and **3** against each selected tumor cell line.

4. Conclusion

Three copper(II) complexes with heterocyclic ligands, forming mononuclear, dinuclear, and trinuclear structures, were constructed and their cytotoxic properties were investigated by MTT assay. IC_{50} values of **2** and **3** against four human alimentary system carcinoma cell lines are comparable to cisplatin and even lower than that of some previously reported copper(II) complexes, indicating that the present copper(II) complexes **2** and **3** demonstrated prominent *in vitro* cytotoxicity. The number of copper centers and different structures could have a profound influence on their cytotoxicity.

Supplementary material

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Center, CCDC reference numbers 961040–961042. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.htm (or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK; Fax:+ 44 1223 336033).

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