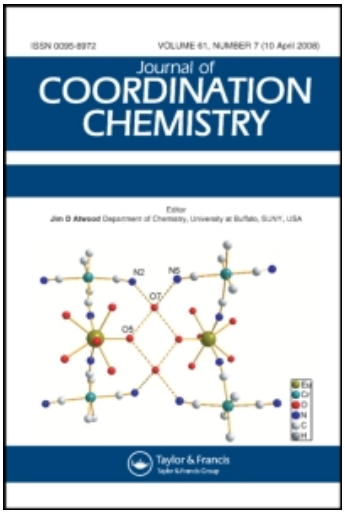

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Synthesis, characterization, and biological activity of a Cu(I) complex with 2-(9H-carbazol-9-yl) acetic acid

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A new dicopper(I) complex with 2-(9H-carbazol-9-yl) acetic acid (HL) of the formula $[Cu_2(dppm)_2L(NO_3)(CH_3OH)]$ [dppm = bis(diphenylphosphino)methane] was prepared. The complex was structurally characterized by IR, ^1H NMR spectra, and elemental analysis. Single crystal X-ray crystallography revealed that this complex is monoclinic, space group $P2_1/c$, with $a = 13.6552(17)$ Å, $b = 23.123(2)$ Å, $c = 19.257(2)$ Å, $\alpha = \gamma = 90.00^\circ$, $\beta = 106.860(2)^\circ$, $V = 5818.8(11)$ Å^3, $Z = 4$, $D_{Calcd} = 1.386\,mg\,m^{-3}$, $F(000) = 2512$, goodness-of-fit = 1.015. The complex was also tested *in vitro* for its cytotoxic activity using human hepatocellular carcinoma cell line (BEL-7402) and human hepatocellular liver carcinoma cell line (Hep-G2); 5-Fluorouracil was used as a positive control substance. The results indicated that the complex exhibited good cytotoxic activity against both human tumor cell lines.

Keywords: Carbazole; Copper(I) complex; dppm; Crystal structure; Antitumor activity

1. Introduction

Metal complexes of phosphines and functionalized phosphines have drawn much attention [1, 2] with diphosphines with general formula $R_1R_2P\text{-}(X)_n\text{-}PR_3R_4$ ($X = CH_2$, NR) extensively studied [3]. Among them, dppm (bis(diphenylphosphino)methane) is a bridging bidentate ligand [4] and its chelating tendency is suitable to lock two metals in close proximity [5]. We have shown that by employing a partly complexed metal–metal bonded unit $[Ag_2(dppm)_2(NO_3)_2]$, instead of mononuclear complexes, some interesting molecular architectures with dimetal units as "corner pieces," such as simple pairs of M_2 units and ladder framework, can be constructed [6].

Currently, complexes based on dicopper(I) units supported by dppm attracted our attention as a convenient source of pre-organized dimetal building blocks. The presence of bridging dppm ligands in complexes of this kind imparts unusual stability to the Cu_2^{2+} framework [7–10]. Furthermore, it has been reported that the reactions of

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$[(\mu\text{-dppm})_2Cu_2(CH_3CN)_2]^{2+}$ with bidentate anionic ligands (6-methyl-pyridin-2-olate, 3,5-di-methyl-pyrazolate, 1,3-di-p-tolyl-triazenide) result in discrete triply bridged dicopper(I) [8]. This shows that the doubly bridged dicopper(I) core can be retained during the course of reaction, while labile acetonitrile molecules can, as expected, be easily substituted by anionic ligands.

In this report, the reaction of $[(\mu\text{-dppm})_2Cu_2(NO_3)_2]^{2+}$ with 2-(9H-carbazol-9-yl) acetic acid, HL is explained. The title complex, which is an example of the simplest type of supramolecular species based on dimetal units in which two M_2 units are joined by a bifunctional linker, has been isolated and its structure has been determined by single-crystal X-ray analysis. Its cytotoxic activities against two tumor cell lines, BEL-7402 and Hep-G2, are also evaluated.

2. Experimental

2.1. *Materials and instruments*

All chemicals were of reagent grade and used as received. The ligand 2-(9H-carbazol-9-yl) acetic acid (HL) was synthesized as previously reported [11, 12]. The precursor complex $[Cu_2(dppm)_2(NO_3)_2]$ was synthesized by reported methods with some modification [13, 14]. Carbon, hydrogen, and nitrogen assays were carried out with a CHN–O-Rapid instrument and were within ±0.4% of the theoretical values. IR spectra were record on a Nicolet 470 FT-IR spectrophotometer using KBr discs from 4000 to 400 cm^{-1}. ^1H NMR spectra were recorded on a Bruker AV 400 spectrometer with TMS as internal standard. Solid-state fluorescence spectra measurements were performed using a F-2500 fluorescence spectrophotometer.

2.2. *Preparation of $[Cu_2(dppm)_2L(NO_3)(CH_3OH)]$*

To a methanol solution (25 mL) of $[Cu_2(dppm)_2(NO_3)_2]$ (1.018 g, 1 mmol), HL (0.225 g, 1 mmol) was added at room temperature. After addition, the reaction mixture was refluxed for 30 min with stirring and then filtered giving a white solid. Single crystals of the title complex were obtained by slowly evaporating a methanol/ethyl ether solution. Yield: 85.0%, m.p. 220.0°C. ^1H NMR (400 MHz, DMSO-d_6): 3.677 (s, 4H), 5.359 (s, 2H), 7.005–7.563 (m, 46H), 8.241 (d, 2H, $J = 7.6$ Hz). Anal. Calcd for $C_{65}H_{58}Cu_2N_2O_6P_4$: C, 64.30; H, 4.81; N, 2.31. Found (%): C, 62.51; H, 4.57; N, 2.30. IR (KBr): $\nu = 3418$, 3044, 2935, 2878, 1594, 1438, 1456, 1434, 1389, 1323, 1206, 1153, 1096, 1023, 997, 922, 845, 785, 738, 718, 692, 639, 514, 472 cm^{-1}.

2.3. *Crystal structure determination and refinement*

The crystallographic data for $[Cu_2(dppm)_2L(NO_3)(CH_3OH)]$ were collected on a Bruker Smart 1000 CCD area detector diffractometer equipped with Mo-Kα ($\lambda = 0.71073$ Å) radiation using ω-scan mode. Empirical absorption correction was applied to the data. The structures were solved by direct methods and refined by full-matrix least-squares on F^2. All non-hydrogen atoms were located from the trial structure and then refined anisotropically. All hydrogens were generated in

Table 1. Crystallographic and experimental data for $[Cu_2(dppm)_2L(NO_3)(CH_3OH)]$.

Empirical formula	$C_{65}H_{58}Cu_2N_2O_6P_4$
Formula weight	1214.09
Crystal system	Monoclinic
Space group	$P2_1/c$
Crystal size (mm^3)	$0.28 \times 0.21 \times 0.18$
Unit cell dimensions (Å, °)	
a	13.6552(17)
b	23.123(2)
c	19.257(2)
α	90.00
β	106.860(2)
γ	90.00
Volume ($Å^3$), Z	5818.8(11), 4
D_{calcd} ($g\,cm^{-3}$)	1.386
μ (mm^{-1})	0.895
$F(000)$	2512
θ range (°)	1.41–25.00
Reflections collected	28,804
Reflections unique	10,228
Parameters	712
Goodness-of-fit on F^2	1.015
R_1, wR_2 [$I > 2\sigma(I)$]	0.0488, 0.1000
R_1, wR_2 [all data]	0.0969, 0.1250
Max, min $\Delta\rho$ ($e\,Å^{-3}$)	0.579, −0.444

idealized positions. Calculations were performed with SHELXL-97. Other relevant parameters of the crystal structure are listed in table 1.

2.4. *Cytotoxicity*

The cytotoxicity of the prepared compounds against BEL-7402 and Hep-G2 cells was evaluated as described elsewhere [15] with some modifications. Briefly, target tumor cells were grown to log phase in RPMI 1640 medium supplemented with 10% fetal bovine serum. After diluting to 2×10^4 cells mL^{-1} with the complete medium, 100 µL of the obtained cell suspension was added to each well of the 96-well culture plates. The subsequent incubation was at 37°C, 5% CO_2 atmosphere for 24 h before the cytotoxicity assessments. Tested samples at pre-set concentrations were added to six wells with 5-fluorouracil co-assayed as a positive reference. After 48 h exposure, 40 µL of PBS containing 2.5 mg mL^{-1} of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] was added to each well. Four hours later, 100 µL of extraction solution (10% SDS-5% isobutyl alcohol-0.01 mol L^{-1} HCl) was added. After an overnight incubation at 37°C, the optical density was measured at a wavelength of 570 nm on an ELISA microplate reader. In all experiments three replicate wells were used for each drug concentration. Each assay was carried out at least three times.

3. Results and discussion

After coordination, the stretching vibration of O–H of the free ligand disappears. The C=O absorption (1718 cm^{-1}) of HL has been significantly weakened, while the

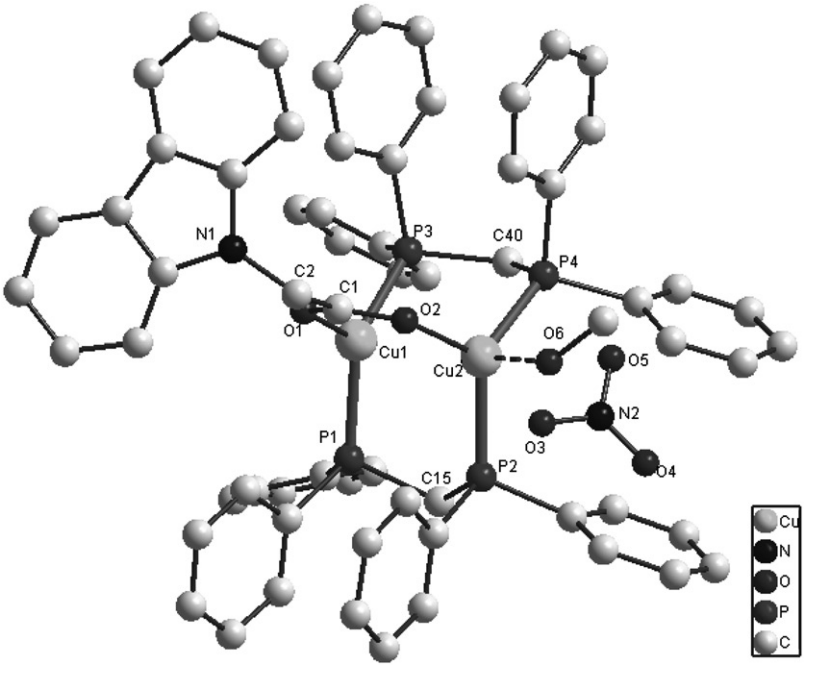

Figure 1. Molecular structure of $[Cu_2(dppm)_2L(NO_3)(CH_3OH)]$ (all hydrogens have been omitted for clarity) with 30% probability ellipsoids.

absorption of C–O at 1245 cm^{-1} vanishes with construction of the conjugation system of O–C–O. Two new absorptions at 1350 and 1096 cm^{-1} in spectra of the complex are NO_3^- and P–Ph vibrations, respectively, confirming the ligand has chelated to $[Cu_2(dppm)_2]$. Evidence for the formation of the complex is the appearance of a new absorption at 472 cm^{-1} for Cu–O vibration.

The molecular structure of $[Cu_2(dppm)_2L(NO_3)(CH_3OH)]$ is shown in figure 1 and selected bond lengths and angles are given in table 2. The complex crystallizes as a binuclear complex in the monoclinic space group $P2_1/c$. The molecular structure consists of two copper(I)'s, bridged by a pair of dppm molecules and L. The carbazole plane of L possesses perfect planarity and the carboxylate is bidentate with two coppers. C–O bond lengths are 1.2423 (54) Å (C1–O1) and 1.2535 (70) Å (C1–O2) and Cu–O bond lengths are 2.0084(35) Å (Cu1–O1) and 1.9994(40) (Cu2–O2) Å. X-ray analysis of the complex reveals one methanol in the Cu(2) coordination sphere. Thus, Cu(1) has a three-coordination mode with a O, 2P coordination, while Cu(2) is four-coordinate with 2O, 2P coordination. The Cu(1)–Cu(2) distance of 2.8472(7) Å is shorter than that in $[Cu_2(dppm)_2(NO_3)_2]$ (3.170(4) Å) [14], indicating the existence of weak metal–metal interactions between them [16]. As shown in figure 2 and table 3, intermolecular H-bonds form between adjacent molecules leading to a 1-D chain. Further intermolecular C–H$\cdots\pi$ interactions between 1-D chains generate a 3-D structure. The C–H$\cdots\pi$ separation is 3.1551(3) Å (H33$\cdots\pi$) and 3.2570(2) Å (H57$\cdots\pi$), respectively.

As shown in figure 3, moderate blue shift observed in the emission maxima of the ligand and the title complex in the solid-state arises from metal coordination. In the free

Table 2. Selected bond lengths (Å) and angles (°) for $[Cu_2(dppm)_2L(NO_3)(CH_3OH)]$.

Cu1–Cu2	2.847(7)	Cu1–O1	2.008(4)
Cu2–O2	1.999(4)	C1–O1	1.242(5)
C1–O2	1.254(7)	Cu1–P1	2.245(1)
Cu1–P3	2.253(1)	Cu2–P2	2.243(1)
Cu2–P4	2.234(1)	P1–C15	1.839(5)
P2–C15	1.840(4)	P3–C40	1.842(5)
P4–C40	1.832(4)	Cu2–O6	2.518(5)
P1–Cu1–P3	126.2(5)	P2–Cu2–P4	124.0(5)
Cu1–O1–C1	118.9(3)	Cu2–O1–C1	131.4(4)
O1–C1–O2	127.1(5)	P1–C15–P2	111.0(2)
P3–C40–P4	111.8(2)	P1–Cu1–O1	115.7(0)
P3–Cu1–O1	118.0(9)	P2–Cu2–O2	116.0(9)
P4–Cu2–O2	118.8(1)		

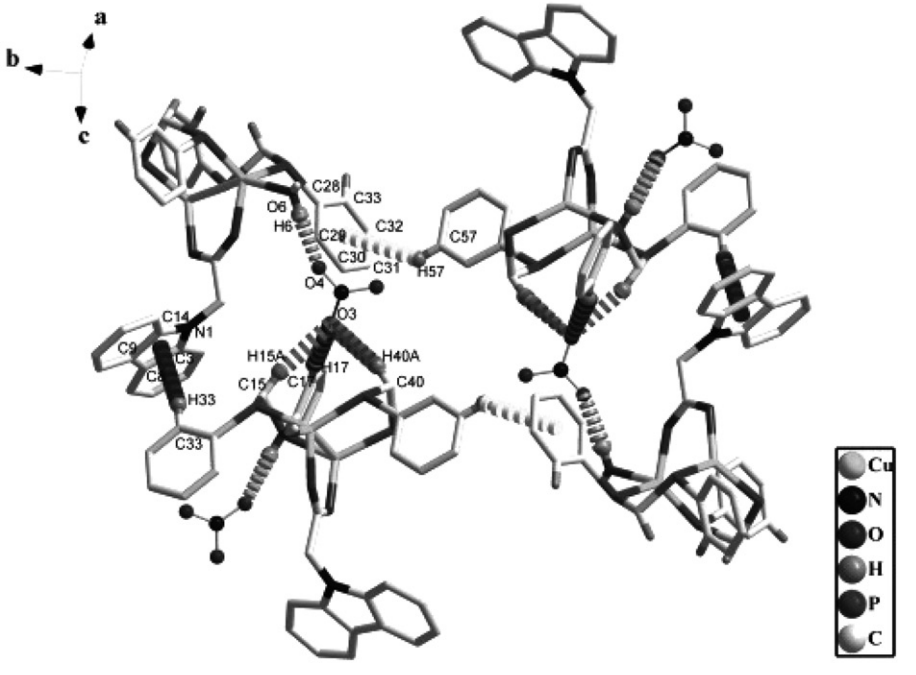

Figure 2. 1-D chains (formed by intermolecular H bonds (C–H···O and O–H···O)) interact with each other through C–H···π stacking interactions between adjacent molecules to generate a 3-D structure.

ligand, there are extensive and strong intermolecular interactions, such as π–π stacking, which lead to luminescence quench and red shift of intramolecular charge transfer [17]. When coordinated to $[Cu_2(dppm)_2]$, these interactions of the free ligand were destroyed, so the emission wavelength of the title complex exhibits blue shift compared with that of free ligand.

The results of cytostatic activity are summarized in table 4. IC_{50} values of the compounds are expressed in microgram per milliliter, together with that of

Table 3. Intermolecular H bonds lengths (Å) and angles (°) in the 1-D chain.

Bond	Lengths	Bond	Angles
H6···O4	1.98(7)	O6–H6···O4	159.8(4)
H15A···O3	2.41(5)	C15–H15A···O3	168.6(7)
H17···O3	2.36(4)	C17–H17···O3	172.0(5)
H40A···O3	2.42(5)	C40–H40A···O3	166.6(3)

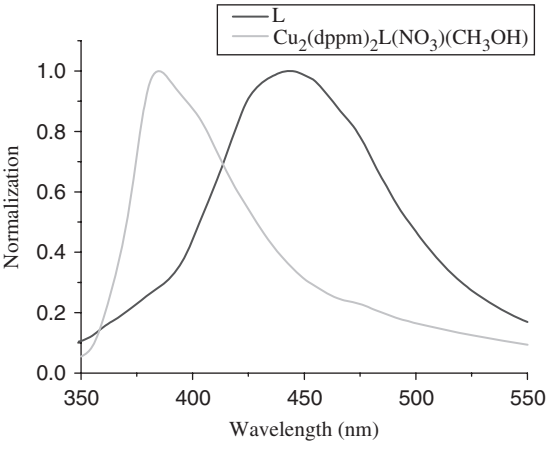

Figure 3. Solid-state luminescence spectra of HL and the title complex.

Table 4. Cytotoxic activities of the synthetic compounds.

	IC_{50} ($\mu g\,mL^{-1}$)	
Compounds	BEL-7402	Hep-G2
HL	40.3 ± 0.4	38.1 ± 0.3
$[Cu_2(dppm)_2(NO_3)_2]$	21.4 ± 0.1	18.1 ± 0.1
$[Cu_2(dppm)_2L(NO_3)(CH_3OH)]$	3.8 ± 0.3	2.0 ± 0.4
5-Fluorouracil[a]	19.2 ± 0.4	17.4 ± 0.2

Antitumor activities are expressed as IC_{50} (50% inhibitory concentration) toward the cell lines BEL-740 2 and Hep-G2. Data are average data of triplicate assay.
[a]Used as a positive control.

5-fluorouracil for comparison. $[Cu_2(dppm)_2L(NO_3)(CH_3OH)]$ exhibits lower IC_{50} values than those of 5-fluorouracil, indicating high cytotoxicity against the tumor cell lines evaluated. Moreover, $[Cu_2(dppm)_2L(NO_3)(CH_3OH)]$ also exhibits higher cytotoxic activity than the ligand and the precursor $[Cu_2(dppm)_2(NO_3)_2]$, which may be due to the presence of cytotoxic Cu(I) species [18, 19] and the ability of HL to form unobstructed H-bonds and/or $\pi\cdots\pi$ stacking that facilitate intracellular uptake of the complex. Further work is in progress to elucidate the detailed mechanisms of antitumor activity of the title complex.

Supplementary material

Crystallographic data for the structure reported in this article have been deposited with the Cambridge Crystallographic Data Center, CCDC reference number 704788. Copies of these information may be obtained free of charge from: The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1223-336033; Email: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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