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CRYSTAL STRUCTURE AND ANTITUMOR ACTIVITIES OF A DINUCLEAR COBALT(II) COMPLEX BASED ON *MESO*-1,2,3,4-TETRA(1H-BENZO[*d*]IMIDAZOL-2-YL)BUTANE

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The $[Co_2(tbb)Cl_4]\cdot 4DMF$ complex, where tbb is *meso*-1,2,3,4-tetra(1H-benzo[*d*]imidazol-2-yl)butane, is synthesized and characterized by single crystal X-ray diffraction. For the complex: $C_{44}H_{54}Co_2C_{l4}N_{12}O_4$, $M_r = 1074.65$, monoclinic crystal system, space group $P21/n$, $a = 9.2350(13)$ Å, $b = 11.3566(15)$ Å, $c = 23.879(3)$ Å, $\beta = 90.547(2)°$, $V = 2504.3(6)$ Å^3, $Z = 2$, $D_c = 1.425$ g/cm^3, $\lambda = 0.71073$ Å, $\mu(MoK_\alpha) = = 0.929$ mm^{-1}, $F(000) = 1112$, $S = 1.047$, $R = 0.0765$, and $wR = 0.2110$ for 13668 observed reflections with $I > 2\sigma(I)$. It is a neutral dinuclear complex. One *meso*-1,2,3,4-tetra(1H-benzo[*d*]imidazol-2-yl)butane coordinates two cobalt(II) ions. Each cobalt(II) ion is formed by two tbb nitrogen atoms and two chloride ions. The antiproliferative activities of the complex are screened by MTT assay against Eca109 cancer cells. The complex exhibits inhibition on the growth of Eca109 cancer cells with IC_{50} of 22.1±6.7 μM after 48 h treatment. The cobalt complex has potential application in treatment of Eca109 cancer. CCDC 1015791.

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Keywords: benzimidazole, cobalt complex, crystal structure, antitumor activities.

INTRODUCTION

The widespread success of cisplatin in the clinical treatment of various types of neoplasias has placed metal-based drugs in the frontline against cancer [1, 2]. The cure with cisplatin is still limited by dose-limiting side effects and inherited or acquired resistance phenomena, only partially amended by the employment of new platinum drugs [3-5]. Intensive efforts have been made to develop new metal complexes that are effective against cancer cells either by changing the metal or the ligand. Various transition metals combined with a variety of ligand classes, such as Schiff bases, amino acids, and extended polypyridine have been proposed to improve the efficacy of cancer treatment [6-10].

We take interest in searching for non-platinum complexes with higher activities and lower toxicity. Benzimidazole derivatives are important pharmacophores in drugs that display a diversity of pharmacological activities, such as anti-

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inflammatory, antioxidant, gastroprotective, and antiparasitic activities [11-14]. In our recent study, some zinc complexes based on the organic containing benzimidazole group have been evaluated for in vitro anticancer activities [15-17]. Cobalt is an essential element present in biological systems either as a metal center in vitamin B_{12} and other cobalamines or as an ion involved in cellular oxidative stress through mitochondrial mediated apoptosis [18]. Herein we synthesized a new *meso*-1,2,3,4-tetra(1H-benzo[*d*]imidazol-2-yl)butane cobalt(II) complex based on *meso*-1,2,3,4-tetra(1H-benzo[*d*]imidazol-2-yl)butane and investigated its antitumor activities. The result demonstrates that the dinuclear cobalt(II) complex based on (2R,3S)-1,2,3,4-tetra(1H-benzo[*d*]imidazol-2-yl)butane has high proliferation inhibition toward Eca109 cancer cells *in vitro*.

EXPERIMENTAL

Materials and instrument. *o*-Phenylenediamine and *meso*-1,2,3,4-butane tetracarboxylic acid were purchased from ACROS Company. All the chemicals and solvents were analytically pure and used without further purification. The analyses (C, H and N) were made on a Perkin-Elmer 240C elemental analyzer. ^{1}H NMR spectroscopic measurements were carried out on a Bruker AM-300 NMR spectrometer, using TMS ($SiMe_4$) as the internal reference. The infrared (IR) spectra were obtained from KBr pellets on a Bruker IFS66V vacuum-type FT-IR spectrophotometer. The UV absorption spectra were recorded on a model UV-240 spectrophotometer (Shimadzu, Japan). Thermogravimetric analysis (TGA) was carried out up to 600° with a heating rate of 10.0 K/min in atmosphere on an NETZSCH TG-209 thermogravimetric analyzer.

Synthesis of *meso*-1,2,3,4-tetra(1H-benzo[*d*]imidazol-2-yl)butane (tbb). *meso*-1,2,3,4-Butane tetracarboxylic acid (2.34 g, 0.01 mol) and *o*-phenylenediamine (4.32 g, 0.04 mol) were added to phosphoric acid (20 ml), and the mixture was stirred at 180°C for 3 h. The resulting dark brown solution was allowed to cool to room temperature and poured into water with vigorous stirring; a white solid formed, then it was filtered off, and the pure product was recrystallized from a DMF solution. It was then filtered off, washed with hot water, dried under vacuum. Yield: 5.2 g, 58.6%. Anal. calcd. (%) for $C_{44}H_{62}N_{12}O_8$ (tbb·4DMF·$4H_2O$): C 59.58, H 7.05, N 18.95. Found (%): C 59.48, H 7.01, N 18.93. Selected IR data (KBr, cm^{-1}): 3593, 3150, 1644, 1531, 1440, 1386 1274, 1030, 839, 746; ^{1}H NMR: (300 MHz, d^6-DMSO):2.50 (water-H), 2.72 (s, 1H), 2.88 (s, 1H), 2.95 (s, 1H), 3.00 (s, 1H), 3.32 (DMSO-CH_3), 3.53 (DMF-CH_3), 4.26 (d,1H), 4.44 (d, 1H), 6.99 (m, 4H), 7.06 (m, 4H), 7.35 (s, 4H), 7.44 (s, 4H), 8.29 (DMF-CHO), 12.16 (s, 2H),12.61(s, 2H).

Synthesis of $[Co_2(tbb)Cl_4]\cdot 4DMF$. $CoCl_2$ (0.02 mol) was added to tbb (886 g, 0.01 mol) dissolved in a DMF solution (30 ml). The reaction mixture was stirred for 20 min at room temperature. Blue block crystals were obtained from air evaporation in three days. Yield: 0.82 g, 67.1%. Anal. calcd. (%) for $C_{44}H_{54}Cl_4Co_2N_{12}O_4$\{$[Co_2(tbb)Cl_4]\cdot 4DMF$\}: C 49.18, H 5.06, N 15.64. Found (%): C 49.27, H 5.01, N 15.54. Selected IR data (KBr, cm^{-1}): 3439, 2919, 1661, 1462, 1368, 1285, 1066, 754, 661.

Structure of the determination. A blue single crystal of the title compound with dimensions of 0.46×0.27×0.19 mm was selected for the X-ray diffraction analysis. Data collection was performed on a Bruker P4 CCD diffractometer equipped with a graphite-monochromatic MoK_α radiation ($\lambda = 0.71073$ Å) using the φ-ω scan mode at 293.15 K. The empirical absorption was applied to the intensity data. A total of 13668 reflections were collected in the range $1.99 < \theta < 27.04°$, out of which 5497 were independent with $R_{int} = 0.0777$, and 5497 were observed with $I > 2\sigma(I)$. The structure was solved by direct methods using SHELXS-97 and refined by full-matrix least squares techniques on F^2 using SHELXL-97 [19, 20]. H atoms were positioned geometrically and constrained with riding model position parameters and fixed isotropic thermal parameters. Final $R_1 = 0.0765$, $wR_2 = 0.2110$ ($w = 1/(\sigma^2(F_0^2) + (0.1291P)^2 + 5.8127P)$, where $P = (F_0^2 + 2F_c^2)/3$. All calculations were performed on a computer using the SHELXTL-97 program package. CIF file containing complete information on the studied structure was deposited with CCDC, deposition number 1015791, and is freely available upon request from the following web site: www.ccdc.cam.ac.uk/data_request/cif. Crystallographic data and experimental details for structural analyses are summarized in Table 1.

TABLE 1. Crystal Data and Structure Refinement for the Complex

Formula	$C_{44}H_{54}Cl_4Co_2N_{12}O_4$
Formula weight	1074.65
Crystal system	Monoclinic
Space group	$P21/n$
a, b, c, Å; β, deg	9.2350(13), 11.3566(15), 23.879(3); 90.547(2)
V, Å^3	2504.3(6)
Z	2
μ, mm^{-1}	0.929
D_c, g/cm^3	1.425
Index ranges	$-11 \le h \le 11$, $-14 \le k \le 14$, $-30 \le l \le 14$
F(000)	1112
$GOOF$ on F^2	1.047
Reflections collected / unique	13668 / 5497 [R(int) = 0.0320]
Data / restraints / parameters	5465 / 0 / 298
R indices (all data)	$R_1 = 0.1154$, $wR_2 = 0.2437$
Final R indices[$I > 2\sigma(I)$]	$R_1 = 0.0765$, $wR_2 = 0.2110$
Max. and min. transmission	1.22, 0.53
Largest diff. peak and hole, e/Å^3	1.771, –0.564

Cell culture. The cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in the DMEM medium supplemented with fetal bovine serum (10%), penicillin (100 units·ml^{-1}), and streptomycin (50 units·ml^{-1}) at 37°C in a humidified incubator in the 5% CO_2 atmosphere.

MTT assay. The effects of the complex on cell proliferation were determined by MTT assay. Briefly, cells were seeded in 96-well culture plates at different densities. After 24 h, different concentrations of compounds were added and incubated for the indicated time. Then, 20 μl per well of an MTT solution (5 mg·ml^{-1} phosphate buffered saline) was added and incubated for 5 h. The medium was aspirated and replaced with 200 μl per well of DMSO to dissolve the formazan salt formed. The color intensity of the formazan solution, which reflects the cell growth condition, was measured at 570 nm using a microplate spectrophotometer (VERSA max). Each IC50 value represents the mean ±SD of three independent experiments.

RESULTS AND DISCUSSION

Syntheses, IR, and thermal stability for the complex. *meso*-1,2,3,4-Tetra(1H-benzo[*d*]imidazol-2-yl)butane was synthesized by the traditional condensation reaction of *meso*-1,2,3,4-butane tetracarboxylic acid and *o*-phenylenediamine (Fig. 1). The reaction temperature was controlled above 170° so that the four carboxylic acid groups could react completely with *o*-phenylenediamine. *meso*-1,2,3,4-Tetra(1H-benzo[*d*]imidazol-2-yl)butane coordinates easily to $CoCl_2$ to form the complex at room temperature. In the IR spectrum of the complex, the 3439 cm^{-1} (N–H), 1462 cm^{-1} (C=N), 1285 cm^{-1} (C–N) peaks show the absorption bands resulting from skeletal vibrations of the benzimidazole rings. The strong peak at 1661 cm^{-1} is from C=O of the DMF solvent. In the ^{1}H NMR spectrum of the free ligand (Fig. 2) the NH hydrogen atom appears at δ = 12.16 ppm and δ = 12.61 ppm. The signals of δ = 6.99 ppm, 7.06 ppm, 7.35 ppm, 7.44 ppm are from phenyl rings hydrogen atoms. The peaks at δ = 2.72 ppm and 2.95 ppm are attributed to four $-CH_2-$ hydrogen atoms. The double peak of the two hydrogen atoms attached to the tertiary carbon atom appears at δ = 4.26 ppm and 4.44 ppm. The H atom signals of the free ligand are all in good agreement with the assumed structure.

Thermogravimetric analysis (TGA) shows that the complex begins to decompose at 220° (Fig. 3). The weight loss of 27.64% under 220° is due to the loss of crystallized DMF solvents, which is consistent with the theoretical weight-loss value of 27.23%.

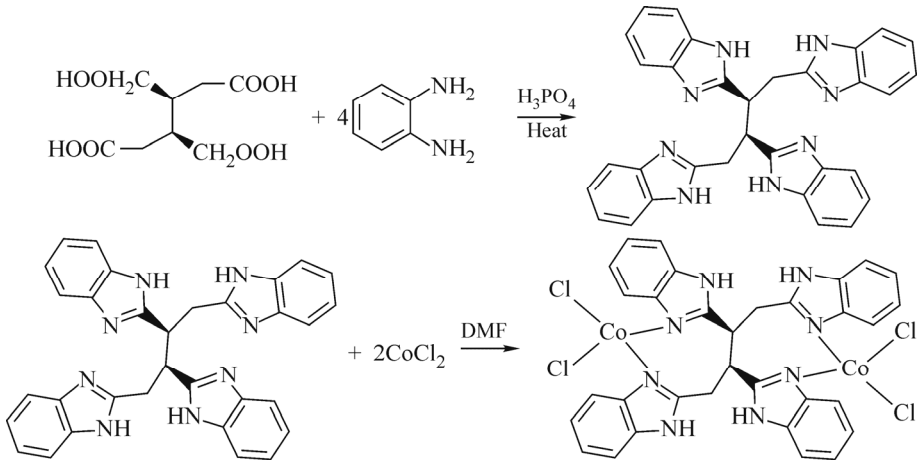

Fig. 1. Synthesis route of the ligand and the cobalt complex.

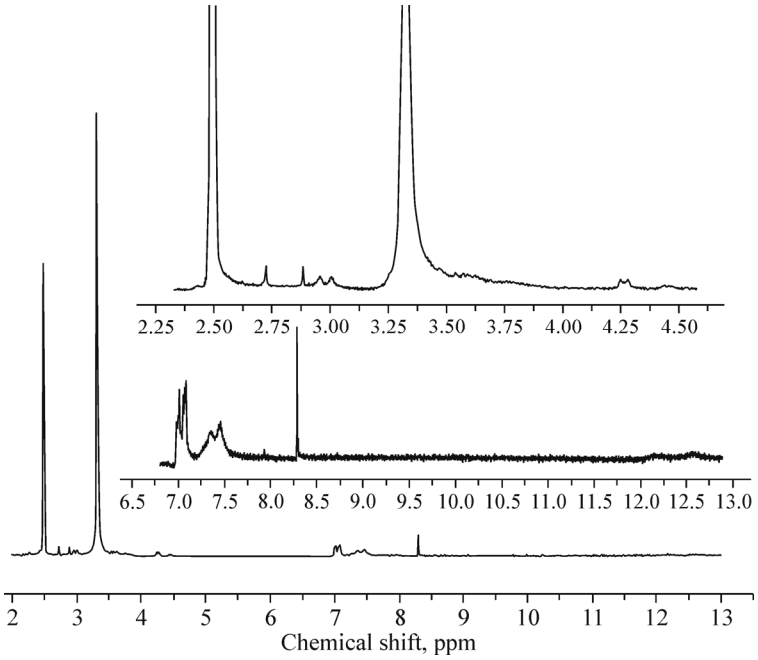

Fig. 2. ^{1}H NMR spectrum of the tbb ligand.

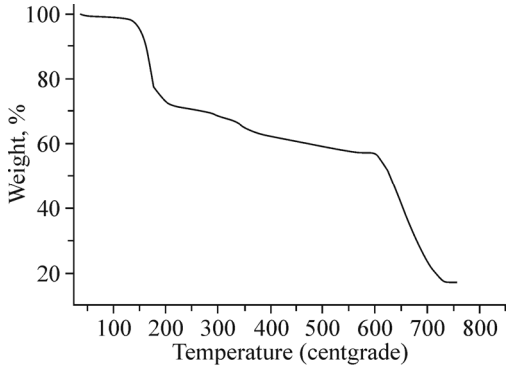

Fig. 3. Thermogravimetric analysis of the cobalt complex.

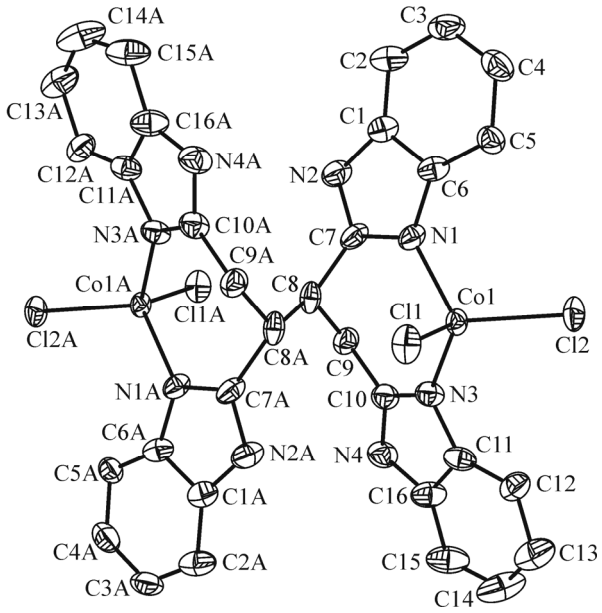

Fig. 4. Molecular structure of the complex.

Crystal structure. The ORTEP drawing of the complex is shown in Fig. 4. The complex is a dinuclear cobalt(II) complex. One tbb simultaneously coordinates two cobalt(II) ions. The two cobalt(II) ions are at the centrosymmetric site. The central cobalt(II) ion is four coordinated and surrounded by the N_2Cl_2 environment, adopting a distorted tetrahedral geometry. The ligand (tbb) affords two N atoms to coordinate to each cobalt(II) ion. The other sites are occupied by two chloride ions. Selected bond lengths and angles of the complex are listed in Table 2. The Cl(1)–Co(1) and Cl(2)–Co(1) bond lengths are 2.2378(17) Å and 2.2277(14) Å respectively. The average Co–Cl bond length is 2.303 Å. The Co(1)–N(1) and Co(1)–N(3)

TABLE 2. Selected Bond Lengths (Å) and Bond Angles (deg)

Lengths		Angle		Angle	
Co(1)–N(1)	2.006(4)	N(1)–Co(1)–N(3)	103.14(17)	N(3)–Co(1)–Cl(2)	110.41(16)
Co(1)–Cl(1)	2.2378(17)	N(1)–Co(1)–Cl(1)	107.74(15)	Cl(2)–Co(1)–Cl(1)	111.77(6)
Co(1)–N(3)	2.010(4)	N(1)–Co(1)–Cl(2)	113.30(15)		
Co(1)–Cl(2)	2.2277(14)	N(3)–Co(1)–Cl(1)	110.11(15)		

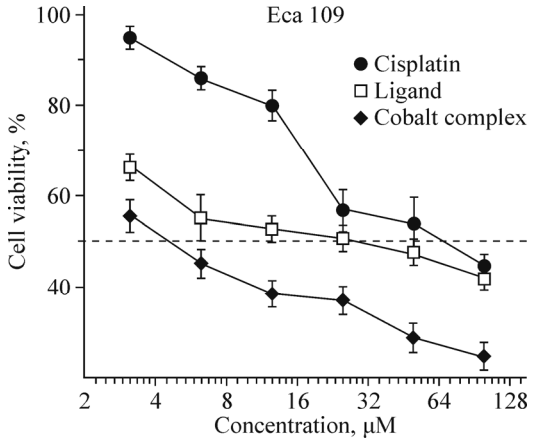

Fig. 5. Inhibition of the ligand (tbb), cobalt complex, and cisplatin on the growth of Eca109 cancer cells.

bond lengths are 2.006(4) Å and 2.010(4) Å, respectively. The average Co–N bond length is 2.008 Å. The bond angles related to Co(1) show that $Co(1)N_2Cl_2$ has a distorted tetrahedral geometry. Each Co^{2+} coordinates to tbb N atoms to form a seven-membered ring.

Antitumor activities. Antiproliferative activities of the ligand, the complex, and cisplatin were screened by MTT assay against Eca109 cancer cells. As is shown in Fig. 5, the cobalt complex exhibits inhibition on Eca109 cancer cells with IC_{50} of 6.1±3.3 μM after 48 h treatment with cisplatin (62.5±12.4 μM) used as a positive control. IC_{50} of this dinuclear cobalt complex also shows a much lower value than the free ligand (tbb) 22.1±6.7 μM. The cobalt complex has potential application in treatment of Eca109 cancer.

CONCLUSIONS

A new dinuclear cobalt(II) complex $Co_2(tbb)Cl_4 \cdot 4DMF$ based on *meso*-1,2,3,4-tetra(1H-benzo[*d*]imidazol-2-yl)butane (tbb) was synthesized and characterized by single crystal X-ray diffraction. In the complex, one tbb molecule coordinated two cobalt(II) ions. Each cobalt(II) ion with a distorted octahedral geometry is coordinated by two nitrogen atoms of *meso*-1,2,3,4-tetra(1H-benzo[*d*]imidazol-2-yl)butane and two chloride ions. The antiproliferative activities of the complex were screened by MTT assay against Eca109 cancer cells. The dinuclear cobalt(II) complex exhibits inhibition on the growth of Eca109 cancer cells.

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