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Synthesis, Characterization and Biological Activity of Copper Complex

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A novel dinuclear copper complex was synthesized and characterized by elemental analysis, molar conductance, FT-IR, UV-VIS, TG-DTA and TEM. The antibacterial activity test indicated that the complex exhibited antibacterial ability against *Escherichia coli* and *Staphylococcus aureus* with broad antimicrobial spectrums. (MIC was *ca.* 150 and 120 μg mL^{-1}, respectively). The antitumor activity of the copper complex indicated that the complex exhibited potent antitumor effects against K562 cell lines by MTT method. The IC_{50} value of the complex was *ca.* 0.01 μg mL^{-1}.

Key Words: Dinuclear copper complex, Antibacterial, Antitumor.

INTRODUCTION

In recent years, metal-organic coordination complexes have attracted extensive interest due to their structural diversities and potential applications in the fields of antibacterial, antitumor, catalysis, *etc*.[1-6]. In present study, considering that copper, has an important biological role in all living organisms as an essential trace element, we have investigated the coordination of organic ligand to copper(II) ions. Thiosemicarbazone Cu(II) complex is considered as one of the most potential development of non-platinum anticancer drugs[7,8]. On the other hand, fluorinated compounds, in general, are the focus of much interest in modern medicinal chemistry and ideal for use in drug design because of the good biological activity and low toxicity[9]. 2-Thenoyltrifluoroacetone (TTA) is one of the excellent properties of chelates. Acheampong's studies[10] show that 2-thienyltrifluroacetone has biological activity. At the same time, carboxylates and their derivatives have stimulated interesting research in biology and medicine, due to their potent biological activity[11]. These advantages encourage us to synthesize a novel ternary complex with antibacterial and antitumor properties. The results can provide some basis for the application in antibacterial, antitumor and other related field. Here we mainly report the synthesis, structural characterization, antibacterial and antitumor activity of the complex.

EXPERIMENTAL

RPMI 1640 and tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma; Fetal calf serum (FCS) was purchased from GiBCo, No. 1165723; sodium dodecylsulfate (SDS) was purchased from Sino-American Biotechnology. All other chemicals were AR, used as purchased.

Elemental analysis was carried out on an Elementar Vario EL III elemental analyzer. FT-IR spectra were obtained on a PK-60000 FT-IR spectrometer in KBr pellets (4000-400 cm^{-1}). Thermal analysis was performed on a Q50 thermal analyzer in a flowing air stream at a heating rate of 10 ºC/min. The transmission electron microscope (TEM) image was recorded on a JEM-1200EX transmission electron microscope.

Bacterium and cell: *Staphylococcus aureus (S. aureus, 8099)* and *Escherichia coli (E. coli, ATCC6358P*) were provided by Shanghai Drug Institute, the Chinese Academy of Sciences; cell strain: Human leukemia K562 cells were endowed by Shanghai Institutes for Biological Sciences (Chinese Academy of Sciences).

Synthesis of complex: 2-Thenoyltrifluoroacetone (1.33 g, 6 mmol) was dissolved in 30 mL of ethanol. NaOH (1 N, 6 mL) and a solution of $CuSO_4 \cdot 5H_2O$ (0.5 g, 2 mmol) in 5 mL of water were successively added to the solution of 2-thenoyltrifluoroacetone. The mixture was heated to 60 ºC for 1 h under stirring. After 1 h, 2 mmol sodium *p*-hydroxybenzoate in ethanol solution was added in the mixture dropwise under stirring and the solution was heated to 60 ºC for 4 h[12]. The solvent was removed by rotary evaporation. The product was washed away with water three times and dried *in vacuo*. The colour of the dried complex was dark green.

The compositions of the prepared complex were determined by elemental analysis which exhibited a good agreement with

the stoichiometry of $Na_2[Cu_2(TTA)_3L]SO_4$. (Anal. calcd. (%): C, 34.67; H, 1.86; S, 11.93. Found (%): C, 34.08; H, 1.52; S, 11.69.)

Biological assay

***In vitro* evaluation of antibacterial activity**

Method of paper disc diffusion[13]: 0.005 mol/L aqueous solutions of cupric sulfate, 2-thenoyltrifluoroacetone, sodium *p*-hydroxybenzoate and the complex were prepared respectively and the antibacterial activity of all the compounds against *S. aureus* and *E. coli* were studied. The bacterium suspension concentration was controlled as 5×10^5-5×10^6 cfu/mL; the diameters of filter paper were 5 mm and for the experiments, flat plates were incubated at 37 ºC (bacterium) for 18 h. Their inhibition diameter (including filter paper) was measured with a vernier caliper.

Method of nutrition broth dilution[13]: Nutrition broth was employed for bacterial growth. The tested cupric sulfate, 2-thenoyltrifluoroacetone, sodium *p*-hydroxybenzoate and the complex were prepared in nutrition broth medium and diluted in concentrations of the range 50-800 μg/mL. Inocula containing 1×10^6 cfu/mL were obtained from broth cultures. The lowest concentration (μg/mL) of compounds, which inhibited the growth of bacteria after 24 h incubation at 37 ºC was taken as the MIC.

All experiments were carried out in duplicate and the results were confirmed in three independent experiments.

Test of antitumor activity: Cell culture the K562 leukemia cells were cultured in RPMI 1640 medium supplemented with FCS (10 %), penicillin (100 U/mL) and streptomycin (100 U/mL) in an incubator (5 % CO_2, 37 ºC). To study the antitumor effect of all the compounds on Leukemia K562 cells, the compounds were added to the other culture of cell culture medium. The culture medium was provided for both the control and the experimental groups and the cells were counted using the leukocytometer.

Antitumor activity[13,14]: The antitumor activities of the now compound on Leukemia K562 cells were tested using the MTT method. Briefly, 1×10^5 mL^{-1} Leukemia K562 cells and each compound at various concentrations were put into 1 mL medium and were then added to each well of 96-well plate. Tests at each concentration were conducted in 8 holes. The plate was incubated at 37 ºC in a humidified atmosphere containing 5 % CO_2 for 44 h. 10 μL of MTT solution (5 mg/mL phosphate buffered saline) was then added to each well. After the plate was further incubated for 4 h, 100 μL of 10 % SDS was added to each well to solubilize formazan dye. The optical density (OD) at 570 nm was read by an enzyme-linked immunosorbent assay (ELISA) reader the next day. The mean and standard deviation of each group were calculated.

The inhibition rate (IR %) =

$$= \left(\frac{1 - OD_{Complex}}{OD_{Blank}} \right) \times 100$$

Morphological observation of apoptosis: Morphological observation of the now compounds induced apoptosis of leukemia K562 cells after staining with acridine orange (AO, 10 %) was carried out on a fluorescence microscope, that is to say, 30 μL of the treated leukemia K562 cells were collected and stained with 10 μL of AO. When the AO entered the cells, the DNA exhibited a green fluorescence and the RNA exhibited an orange fluorescence. Fifteen minutes later, control cells and apoptosis were observed on a fluorescent microscope and photographed.

Morphological observations revealed typical apoptotic features in the infected cells, including cell shrinkage and rounding, chromosome condensation and formation of apoptotic body-like vesicles[15].

RESULTS AND DISCUSSION

FT-IR: The infrared bands in 1544-1405 cm^{-1} region are attributed to the stretching vibrations of the $\nu_s(COO^-)$ and $\nu_{as}(COO^-)$ group of the ligand L (L = sodium *p*-hydroxybenzoate), which shift to 1407 and 1537cm^{-1}, respectively owing to coordination and the new band. The separation values ($\Delta = \nu_{as} - \nu_s$) between $\nu_{as}(COO)$ and $\upsilon_s(COO)$ are about 130 cm^{-1} in the complexes, which are lower than that in L (139 cm^{-1}), which show that the carboxylate is bidendate chelating coordination with copper ions for L.

IR spectra of ligand TTA shows $\nu_s(C=O)$ at 1654 cm^{-1}, which shifts to lower energy 1580 cm^{-1} in the complexes, indicating that the $\nu_s(C=O)$ group is coordinated to Cu(II).

UV-VIS spectra: As seen in Fig. 1, the ligand TTA shows a main absorption band in the UV-VIS in a region of $\pi \rightarrow \pi^*$ transition at 287 nm, which shift to higher energy and a new band at about 342 nm can be observed in the complex. The ligand L shows two strong peaks at 207 and 245 nm that shift to 204 and 271 nm in the complex.

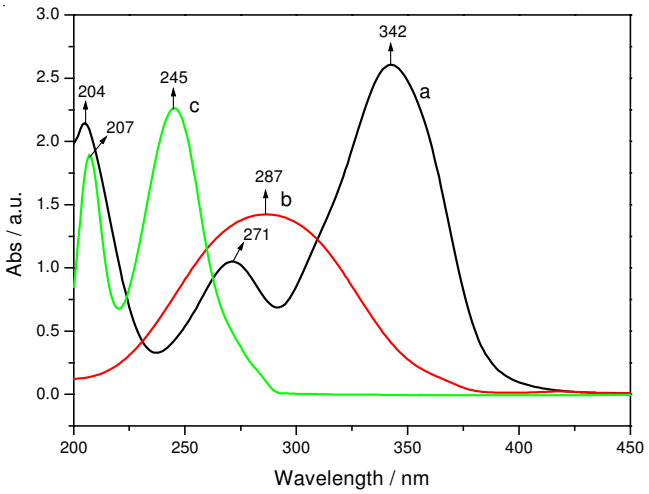


Fig. 1. UV spectra of the copper complex (a); TTA (b) and sodium *p*-hydroxybenzoate (c)

The TTA shows a wide, strong peak at 287 nm which shift to higher energy 342 nm in the complex. Those suggest that both the C=O of HTTA and the COO^- of L involve in metal complexation.

TG-DTA: In order to study the thermal stabilities of the complex, the thermogravimetric analysis (TGA) and differential thermal analysis (DTA) are performed at a heating rate of 10 ºC min^{-1} with air as the static atmosphere in the range 25-900 ºC. The TGA curve of complex exhibits a good agreement with the stoichiometry.

The DTA curve of the present complex shows two distinct steps *i.e.*, the first step is that the complex is melted around 240 ºC accompanied by endothermic effect. The second step is that the complex is decomposed around 410 ºC accompanied by a strong exothermic effect. TGA results show that there is no weight loss in the TG curve around 50-200 ºC suggesting that the complex does not contain crystal water[16]. The complex decomposes completely around 410 ºC and the residues are $CuSO_4$ and Na_2SO_4.

TEM: Fig. 2 depicts the TEM photograph of the complex, it can be known that the particle of complex presents like dendritic shape, which mainly gather through the chain to form. This implies its multi-core structure.

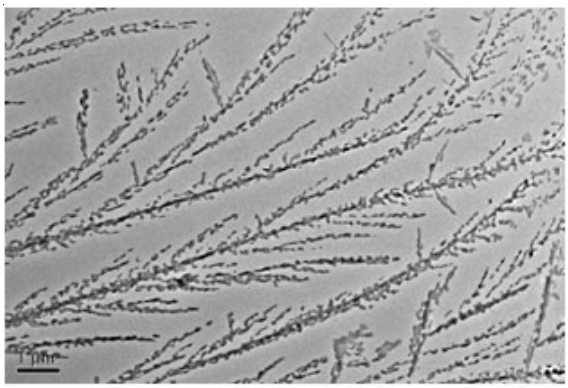
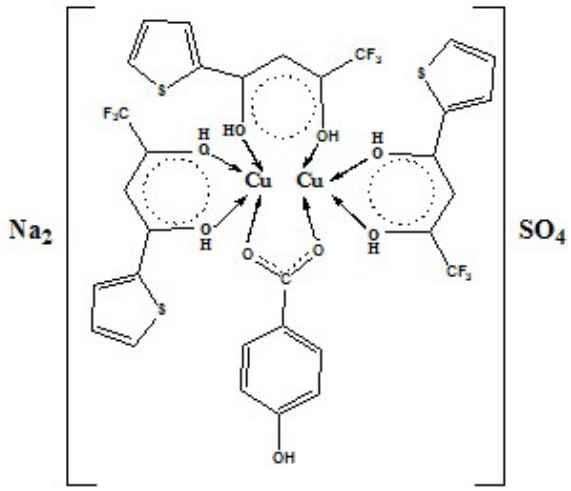


Fig. 2. TEM of of the copper complex

Fig. 2 shows that the complex is dendritic shape, which can easily penetrate the membrane of the cells and react with the cells. So the complex has good biological activity.

Based on the above studies, a tentative coordination structure for the complex is proposed as follows (**Scheme-I**).



Scheme-I: Proposed structure of $Na_2[Cu_2(TTA)_3L]SO_4$

Biological activity

Antibacterial activity: The antibacterial activities are evaluated using the paper disc diffusion method and the nutrition broth dilution method against *E. coli ATCC11229* and *S. aureus ATCC6358P*[13]. Penicillin (North China Pharmaceutical Co. Ltd., D0211107, Hebei 050015, China) is used as standard drug for bacteria. The diameter of growth inhibition area is 17 and 56 mm against *E. coli* and *S. aureus*, respectively. The minimum inhibitory concentration of Penicillin is 150-1.6 μg mL^{-1} against *E. coli* and *S. aureus*, respectively. (Not presented in Table-1). The results in the forms of the diameter of growth inhibition area in mm and the MIC (μg mL^{-1}) are listed in Table-1.

The antibacterial activities of the complexes are better than that of each free ligand. According to Zhou and He[14], the complex has antibacterial activity against *E. coli* and *S. aureus*. The increase in antibacterial activity of the complex may be due to complexation and π-electron delocalization. Complexation reduces the polarity of the metal ion because of partial sharing of its positive charge with the donor groups. The π-electron delocalization in this chelating ring maybe increases the lipophilic nature of the central metal atom, which subsequently favors its permeation through the lipid layers of cell membrane[17-20].

Antitumour activity: In order to investigate the anticancer activity of the complex and compare it with the ligands, they are all reacted with K562 cancer cell by the method of MTT[12,13,21,22]. When K562 cells are exposed to 0.1-50 ng/mL of complexes for 48 h, a dose-dependent growth inhibition effect is observed (Fig. 3.). The IC_{50} value of the complex is about 0.01 μg mL^{-1} indicate that the complex has a good antitumour activity[23,24]. The results show that the copper complex is rather active against the K562 cells *in vitro* and the inhibitory rate of the complex is higher than that of any ligand. The complex possessing antitumor activities may be attributed to the extended planar structure induced by the conjugation[25].

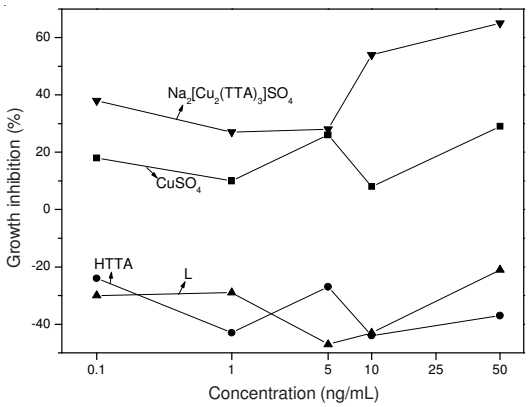


Fig. 3 Inhibiting percentage of K562 tumor cell's growth cycle detected by MTT reduction assay

TABLE- 1
ANTIBACTERIAL *IN VITRO* ACTIVITY EXPRESSED AS DIAMETER OF GROWTH INHIBITION AREA AND MIC

Complex	Diameter of growth inhibition area (mm)			MIC (μg mL^{-1})	
	Concentration (mol/L)	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
TTA	0.005	9	8	>450	>450
L	0.005	8	7	>600	>600
$CuSO_4 \cdot 5H_2O$	0.005	5	5	>500	>500
$Na_2[Cu_2(TTA)_3L]SO_4$	0.005	24	26	150	120

The morphological observation of apoptosis of the complex is shown in Fig. 4[26]. When AO enter the cancer cells, the DNA exhibit a green fluorescence and the RNA exhibit an orange fluorescence. Fifteen minutes later, the control cells and the apoptosis are observed on a fluorescent microscope. We can probably conclude that the complex can induce apoptosis of leukemia K562 cells[27].

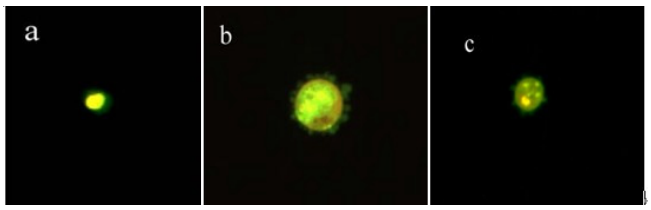


Fig. 4. Morphological observations revealed in the infected cells. (a) Normal cell; (b) apoptotic body-like vesicles; (c) chromosome condensation

Conclusion

In summary, a novel complex containing Cu(II), 2-thenoyltrifluoroacetone and sodium *p*-hydroxybenzoate had been synthesized and structurally characterized. Antibacterial data indicated the copper complex had been taken an antagonistic effect to the antibacterial activities against *E. coli* and *S.aureus*. The antitumor's results showed that the complex exhibited strong inhibitory effect on the leukemia K562 cells. These findings indicated that the copper complex was a promising new targeting complex with potential for management of various bacterial and leukemia, may be also for patients with demonstrated drug resistance[16]. To confirm the potence of the complex, further experiments concerning the anti MDR property of the copper complex were under way.

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