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Antibacterial Studies of Copper(I) Complexes with Benzoylthiourea Derivatives

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Four copper(I) complexes of *bis*benzoylthiourea derivatives have been synthesized and characterized by elemental analyses as well as IR spectroscopy and TG-DTA analyses. Antibacterial activity assay exhibited MIC_{50} values of these compounds comparable to standard drugs for both gram-positive and gram-negative bacteria, some of them showed great antibacterial activity.

Key Words: Benzoylthiourea, Copper(I) complex, Synthesis, Antibacterial activity.

INTRODUCTION

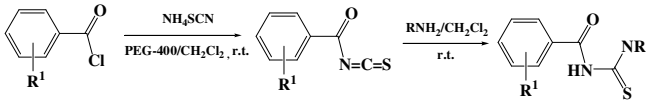
Thiourea compounds are excellent agents of bioactive substances. A number of biological activities are associated with substituted thiourea derivatives[1,2]. Complexes of thiourea derivatives have also been reported in several papers[3-6] and these compounds have been widely used in organic synthesis, such as in the metal-catalyzed asymmetric reduction of carbonyl compounds and carbonylative cyclization of *o*-hydroxyarylacetylenes[7,8]. Of all the thiourea derivatives, the N-substituted N'-acylthiourea compounds have received the most attention, because the existence of acyl and thiocarbonyl groups in these complexes enhances the coordination ability of the ligands, which readily form supramolecular structures via hydrogen bonds[9-12]. In many syntheses of copper complexes, irreversible Cu(II)/Cu(I) systems have been observed[1,2] and there are many reports of the reduction of Cu(II) in the presence of thione derivatives[13-15]. For the synthesis of the title compound, the cuprous complex was obtained by the redox reaction of cupric ions with the thiourea ligand. The reducing agent in this reaction is the thiourea ligand, *viz*., N-benzoyl-N'-(3-pyridyl)thiourea or N-(*o*-chloro)benzoyl-N'-(3-pyridyl)thiourea, according to previous publications[13]. This reaction is similar to those reported[9,16-18]. Herein, four copper(I) complexes of benzoylthiourea derivatives have been synthesized and characterized by elemental analyses, IR and ^{1}H NMR spectroscopy. In the present studies, several new complexes have been synthesized to evaluate its bioactivities. The antibacterial activities are also reported.

EXPERIMENTAL

Benzoyl chloride, *o*-chlorobenzoyl chloride, 1,2-dibromoethane, 1,3-dibromopropane and polyethylene glycol-400 were purchased and used without further purification. The other reagents and solvents were analytical grade reagents from Tianjin Chemical Reagent Factory. Elemental analyses for Cu was detected by an IRIS ER/S·WP-1 ICP atomic emission spectrometer. C, H and N analyses were carried out with a GmbH VariuoEL V3.00 automatic elemental analyzer. IR spectra in the range 4000-400 cm^{-1} were recorded on a VERTEX70 FT-IR spectrophotometer using KBr pellets. The ^{1}H NMR spectra were recorded on a Mercury-400BB spectrometer at room temperature using $CDCl_3$ as solvent. Melting points was measured by the use of a microscopic melting point apparatus made in Beijing Taike Instrument Limited Company and the thermometer was uncorrected. The microbiology stains were obtained from China Center of Industrial Culture Collection, including 4 species of gram-negative bacteria (*Salmonella typhimurium* CCTCCM91098, *Escherichia coli* ACCC11864, *Pseudomonas aeruginosa* NKCCMRNK10.PAO1ΔrhlI and *Shigella flexneri* CICC21534) and two gram-positive bacteria (*Staphylococcus aureus* ACCC01331 and *Mycobacterium tuberculosis* CVCC343), Streptomycin and Ampicillin were served as standard antibacterial agents, respectively. The absorbance was measured using Synergy HT BioTekR USA microplate reader.

General procedure: Benzoyl chloride or 2-chlorobenzoyl chloride was treated with ammonium thiocyanate under the condition of solid-liquid phase transfer catalysis using PEG-400 as the catalyst to give the corresponding benzoyl isothiocyanates, without isolation, the obtained benzoyl isothiocyanates was treated with 3-pyridine to afford the ligands L^1-L^2 in good-to-excellent yield[19,20]. Synthetic route to benzoylthiourea ligands L^1-L^2 is shown in Fig. 1. All the synthesized complexes have been characterized by elemental

analyses as well as IR spectroscopy and TG-DTA analyses (Tables 1 and 2)[21,22].



L^1: R^1=H, R=3-pyridine;

L^2: R^1=2-Cl, R=3-pyridine;

Fig. 1. Synthetic route to benzoylthiourea ligands L^1-L^2

Synthesis of N-benzoyl-N'-(3-pyridyl)thiourea (L^1): 4.41 g (0.01 mol) of benzoyl chloride was reacted with 1.15 g (0.015 mol) of ammonium thiocyanate in 15 mL of CH_2Cl_2 under solid-liquid phase transfer catalysis conditions, using 0.19 g of 3 % polyethylene glycol-400 as the catalyst, to give the corresponding benzoyl isothiocyanate, which was reacted with 0.85 g (0.01 mol) of 3-aminopyridine to give the title compound. The solid isolated was separated from the liquid phase by filtration, washed successively with CH_2Cl_2 and H_2O, respectively, the product was dried under reduced pressure and purified with recrystallization from chloroform to obtain white crystalline solid L^1. Yield: 61.8 %. m.p. 431-432 K. Anal. calcd. (%) for $C_{13}H_{11}N_3OS$: C, 60.68; H, 4.31; N, 16.33. Found (%): C, 60.73; H, 4.23; N, 16.05.

Synthesis of *N*-(*o*-chloro)benzoyl-N'-(3-pyridyl)thiourea (L^2): *o*-Chlorobenzoyl chloride (1.80 g, 0.01 mol) was reacted with ammonium thiocyanate (1.14 g, 0.015 mol) in CH_2Cl_2 (15 mL) under solid-liguid phase transfer catalysis, using 3 % polyethylene glycol-400 (0.18 g) as catalyst, to give the corresponding benzoyl isothiocyanate, which was reacted with 3-aminopyridine (0.86 g, 0.01 mol). The title compound **3d** precipitated immediately. The product was filtered, washed with water and CH_2Cl_2 and dried. Yield 76.6 %, m.p. 442-444 K. Anal. calcd. (%) for $C_{13}H_{10}N_3OSCl$: C, 53.52; H, 3.45; N, 14.40. Found (%): C, 53.43; H, 3.46; N, 14.39.

Synthesis of copper(I) complexes of benzoylthiourea L^1: To a methanol solution (8 mL) of the ligand L^1 (0.2574 g, 1.0 mmol) was added a methanol solution (2 mL) of cupric chloride (0.0860 g, 0.0005 mol) (or $Cu(CH_3COO)_2{\cdot}H_2O$ (0.0998 g, 0.0005 mol)). After the solution had been refluxed for 2 h, the mixture was fltered, washed successively with hot methanol and diethyl ether, respectively, the product was dried under reduced pressure to obtain yellow crystalline solid.

Synthesis of copper(I) complexes of benzoylthiourea L^2: To an acetone solution (6 mL) of the ligand L^2 (0.07294 g, 0.00025 mol) was added an methanol solution (2 mL) of cupric chloride (0.0875 g, 0.0003 mol) (or $Cu(CH_3COO)_2{\cdot}H_2O$ (0.0599 g, 0.0003 mol)), a yellow-green pricipatate was formed immediately. After stirring for 4 h, the mixture was fltered, washed successively with hot methanol and diethyl ether, respectively, the product was dried under reduced pressure to obtain green crystalline solid.

The IR spectrum of the complexes show two bands at *ca*. 3115 and 3436 cm^{-1}, due to NH stretchings. Because C=O groups are locked into the hydrogen bonds, the carbonyl stretching bands appears at *ca*. 1676 cm^{-1}. A strong band at *ca*. 1165 cm^{-1} is assigned as the thionyl group, which has a red shift of 12 cm^{-1} compared with 1176 cm^{-1} in free thiourea L^2. This indicates coordination of the thionyl group with Cu(I). In the range below 1000 cm^{-1}, two peaks at *ca*. 590 and 406 cm^{-1} are attributed to Cu-S and Cu-Cl vibrations[23,24].

Antimicrobial activity: Antibacterial activity was performed in sterile 96-wells microplates under aseptic environments[25]. The method is based on the principle that microbial cell number increases as the microbial growth proceeds in the log phase of growth which results in increased absorbance of broth medium. The organisms were maintained on stock culture agar. The test samples with suitable solvent and dilution were pipetted into wells (20 µg/well). Overnight maintained fresh bacterial cultures after suitable dilution with fresh nutrient broth were poured into wells (180 µL). The initial absorbance of the culture was strictly maintained between 0.12-0.19 at 540 nm. The total volume in each well was kept to 200 µL. The incubation was done at 37 ºC for 16-24 h with lid on the microplate. The absorbance was measured at 540 nm, before and after incubation and the difference was noted as an index of bacterial growth. The percent inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{(X-Y)}{X} \times 100$$

TABLE-1
COLOUR, YIELDS AND ANALYTICALDATA OF SYNTHESIZED COMPLEXES

Complex	Colour	Yield (%)	m.f. (m.w.)	Elemental analysis (%): found (calcd.)			
				C	H	N	Cu
Cu(L^1)Cl	Yellow	82.3	$C_{13}H_{11}ClCuN_3OS$ (356.31)	43.58 (43.82)	2.90 (3.11)	11.45 (11.79)	17.56 (17.83)
Cu(L^2)Cl·H_2O	Yellow	81.8	$C_{13}H_{12}C_{12}CuN_3O_2S$ (408.77)	38.31 (38.20)	2.81 (2.96)	10.01 (10.28)	15.23 (15.55)
Cu(L^1)(CH_3COO)	Green	90.6	$C_{15}H_{14}CuN_3O_3S$ (379.90)	47.13 (47.42)	3.59 (3.71)	10.86 (11.06)	16.52 (16.73)
Cu(L^2)(CH_3COO)	Green	65.6	$C_{15}H_{13}ClCuN_3O_3S$ (414.35)	43.63 (43.48)	2.93 (3.16)	10.56 (10.14)	15.10 (15.34)

TABLE-2
IR SPECTRAL DATA AND TG-DTA DATA OF SYNTHESIZED COMPLEXES

Complex	IR (cm^{-1})	Endothermic peak	Exothermic peak	Residual rate found (calcd.)(%)	Finalproduct
Cu(L^1)Cl	3435, 3119 (NH); 1676 (C=O); 1167 (C=S); 590 (Cu-S); 406 (Cu-Cl)	61, 142	238, 340	22.6(23.4)	CuO,CuS
Cu(L^2)Cl·H_2O	3443, 3115 (NH); 1690 (C=O); 1165 (C=S);592 (Cu-S); 405 (Cu-Cl)	69, 163	296, 329	20.9(21.4)	CuO,CuS
Cu(L^1)(CH_3COO)	3433,3114 (NH); 1684 (C=O); 595 (Cu-S)	135	242, 365	22.5(23.1)	CuO,CuS
Cu(L^2)(CH_3COO)	3427,3117 (NH); 1674 (C=O); 590 (Cu-S)	159	230, 390	20.3(21.1)	CuO,CuS

TABLE-3
ANTIBACTERIAL ACTIVITIES OF COPPER(I) COMPLEXS (MEAN±SEM, n=3)

Compound	Antibacterial activity MIC_{50} (μg/mL)					
	S. typhi	*E. coli*	*P. aeruginosa*	*S. flexneri*	*M. tuberculosis*	*S. aureus*
3a	13.35±0.09	13.15±0.3	–	–	11.8±0.24	12.8±0.44
3b	11.9±0.44	11.12±0.21	11.1±0.45	10.85±0.26	9.25±0.31	9.8±0.34
3c	–	14.77±0.55	–	–	13.5±0.11	14.98±0.3
3d	12.7±0.24	12.1±0.35	11.61±0.24	12.1±0.3	10.75±0.2	10.84±0.31
Ampicillin	9.33±0.26	9.18±0.31	10.12±0.2	10.56±0.35	11.1±0.33	11.43±0.42
Streptomycin	11.2±0.35	9.41±0.27	10.26±0.41	9.74±0.15	7.95±0.27	9.85±0.39

–: Indicates no activity.

where X is absorbance in control with bacterial culture and Y is absorbance in test sample. Results are mean of triplicate (n = 3, mean ± sem). Streptomycin and Ampicilin were taken as standard drugs. Minimum inhibitory concentration (MIC) was measured with suitable dilutions and results were calculated using EZF it 5 Perrella Scientific Inc. Amherst USA software and data expressed as MIC_{50}.

RESULTS AND DISCUSSION

A series of Cu(I) complexes of benzoylthiourea compounds L^1-L^2 have been synthesized and characterized by elemental analyses, IR spectra and TG-DTA analyses.

In the antibacterial experiment, the statistic indicated lowest the MIC_{50} value, highest is the antibacterial activity. These compounds were active againest both gram-positive and gram-negative bacterial. Compounds **3b** possessed the lowest MIC_{50} value, so it has the highest antibacterial activity. Both compounds **3b** and **3d** possessed MIC_{50} value close to the standard drugs streptomycin and ampicillin (Table-3). All compounds have prominent active againest *Mycobacterium tuberculosis.* The results of this study indicated that most of the compounds synthesized above showed their biological importance and could be applied in biological medicine industry.

REFERENCES

1. E. Guillon, I. DeÂchamps-Olivier and J. Barbier, *Polyhedron*, **17**, 3255 (1998).
2. E. Guillon, A. Mohamadou, I. DeÂchamps-Olivier and J. Barbier, *Polyhedron*, **15**, 947 (1996).
3. Y.M. Zhang, T.B. Wei, L, Xian and L.M. Gao, *Phosphorus*, *Sulfur Silicon Rel. Elem.*, **179**, 2007 (2004).
4. R. Campo, J.J. Criado and E. Garcia, *J. Inorg. Biochem.*, **89**, 74 (2002).
5. S.G. Teoh, S.H. Ang and H.K. Fun, *J. Organomet. Chem.*, **580**, 17 (1999).
6. B.H. Abdullah and F.K. Kadir, *Asian J. Chem.*, **23**, 153 (2011)..
7. F. Touchard, F. Fache and M. Lemaire, *Tetrahedron*: *Asymm.*, **8**, 3319 (1997).
8. Y. Nan, H. Miao and Z. Yang, *Org. Lett.*, **2**, 297 (2000).
9. Y.M. Zhang, L. Xian and T.B. Wei, *Acta Cryst.*, **C59**, m473 (2003).
10. Y.M. Zhang, L. Xian, T.B. Wei and K.B. Yu, *J. Chem. Res.*, **12**, 798 (2003).
11. Y.M. Zhang, W.X. Xu and Y.Q. Zhou, *Acta Chim. Sin.*, **64**, 79 (2006).
12. E. Guillon, I. DeÂchamps-Olivier and J. Barbier, *Polyhedron*, **17**, 3255 (1998).
13. S. Jeannin, Y. Jeannin and G. Lavigne, *Inorg. Chem.*, **18**, 3528 (1979).
14. E.S. Raper, *Coord. Chem. Rev.*, **61**, 115 (1985).
15. P. Karagiannidis, P. Aslanidis and S. Papastefanou, *Polyhedron*, **9**, 2833 (1990).
16. X. Shen, T. Wen, Q. Liu, X. Huang, B. Kang, X. Wu, Z. Huang and L. Gu, *Polyhedron*, **16**, 2605 (1997).
17. B.Q. Su, L. Xian, H.B. Song and L. Sheng, *Acta Cryst.*, **C60**, m661 (2004).
18. K.R. Koch, *Coord. Chem. Rev.*, **216-217**, 473 (2001).
19. L.Q. Chai, Y.J. Ding, X.Q. Yang, H.B. Yan and W.K. Dong, *Acta Cryst.*, **E64**, o1407 (2008)
20. W.K. Dong, X.Q. Yang. L.Q. Chai, Y.Q. Tian and J.H. Feng, *Phosphorus, Sulfur Silicon Rel. Elem.*, **183**, 1181 (2008).
21. W.K. Dong, Y.X. Sun, S.J. Xing, Y. Wang and X.H. Gao, *Z. Naturforsch.*, **67b**, 197 (2012).
22. W.K. Dong, J.F. Tong, Y.X. Sun, S.S. Gong and L. Li, *Synth. React. Inorg. Met.-Org. Nano-Met. Chem.*, **41**, 155 (2011).
23. L. Xian, T.B. Wei and Y.M. Zhang, *J. Coord. Chem.*, **57**, 453 (2004).
24. U. Bierbach, T.W. Hambley and J.D. Roberts, *Inorg. Chem.*, **35**, 4865 (1996).
25. M. Kaspady, V.K. Narayanaswamy, M. Raju and G.K. Rao, *Lett. Drug Design Discov.*, **6**, 21 (2009).