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Synthesis, Spectroscopic and Antibacterial Studies of Zinc(II) and Copper(II) Complexes Containing Amino Acid Thiourea Ligands

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Abstract

The study on thiourea derivatives and its coordination complexes have been well documented with majority of the synthesized compounds have high potential to be used as antibacterial agents. In line with this interest, two thiourea ligands derived from amino acids, namely 2-(3-dodecanoyl-thioureido)-3-methylbutyric acid (L1) and 2-(3-dodecanoylthioureido)-3-phenylpropionic acid (L2) have been reacted with $ZnCl_2$ and $CuCl_2$, to produce three new complexes with general formula of $[Zn(L1)Cl_2]$ (1), $[Zn(L2)Cl_2]$ (2) and $[Cu(L2)Cl_2]$ (3) as suggested by elemental analysis. The complexes were characterized by spectroscopic techniques such as Fourier Transform Infrared (FTIR) and Ultraviolet-Visible (UV-Vis). The antibacterial activities of the complexes were evaluated against three common Gram-positive strains *(S. aureus, S. epidermidis* and *B. subtilis)* and two Gram-negative strains *(E. coli* and *S. typhimurium).* This study revealed that complex 3 had a good antibacterial activity towards Gram-positive strain namely *S. epidermidis* while complexes 1 and 2 showed moderate activities against all bacteria.

Keywords thiourea, amino acid, metal complexes, antibacterial activity, *S. Epidermidis*

INTRODUCTION

Thiourea are versatile ligand with ability to coordinate various metal centres in different coordination modes to form complexes with good stability (Binzet *et al*., 2012; Liu *et al*., 2013; Waheed, 2012; Yang *et al*., 2012). Thiourea derivatives can act as monodentate, bidentate or polydentate ligands due to the presence of multi-donor atoms such as oxygen, nitrogen and sulfur (Lipowska *et al*., 1996; Smith *et al*., 2013). Meanwhile, thiourea complexes have been successfully evaluated as antifungal, antitumor, antimicrobial, anticancer (Campo *et al*., 2002; Plutin *et al*., 2014; Saeed *et al*., 2010; Lachkova *et al*., 2002). Various reports proposed that chelation enhance the biological activity of a compound and this has thus led to the designation and development of new coordination thiourea compounds with various biological applications (Gokhale *et al*., 2003). In continuation of our previous work, we found that amino acid that combined with alkyl chain displays good biological activity (Kadir *et al*., 2016). Other report on antibacterial study of phenylalanine as antitumor agent has also been reported (Chohan *et al*., 2006). In addition, the antibacterial activity of methionine thiourea derivatives has been investigated and proven to be less toxic and more efficient in the presence of metal ions (Nagesh *et al*., 2015). In a current study reported by Ajibade & Zulu (2011), substituted thiourea that bonded to Cu(II), Zn(II), Co(II) and Fe(III) shown great potential as therapeutic agents (Ajibade & Zulu, 2011). This is supported by few studies that demonstrated the enhancement in antibacterial activity when ligands are attached to metal (Gokhale *et al*., 2011; Nagesh *et al*., 2015). By having the attention to expand the investigation on biological activities of thiourea complexes, three new dodecanoylthioureido complexes were synthesized and evaluated against five bacterial strains. At this initial stage, copper(II) and zinc(II) metal ions were selected as the metal salts due to their common use in antibacterial products.

MATERIALS AND METHODS

Instrumentations

All chemicals or reagents ($CuCl_2$, $ZnCl_2$, dodecanoyl chloride, ammonium thiocyanate, DL-valine, L-phenylalanine, acetone, methanol, sodium hydroxide, hydrochloric acid) used were purchased from standard supplier (Merck, Sigma Aldrich and R&M) and used as received without further purification. Melting points were measured using BÜCHI melting point B-545. The infrared spectra were recorded on a Fourier Transform-Infrared Spectrometer, Perkin Elmer spectrum 100 using potassium bromide (KBr) as the matrix in the range of 4000-400 cm^{-1}. For UV-Visible analysis, all compounds were recorded by using Spectrophotometer Shimadzu UV-1800 in 1 cm^3 cuvette in methanolic solution for absorbance analysis. Elemental analysis (CHNS) was conducted using CHNS Analyzer Flashea 1112 series to determine the purity of the complexes. Ligands L1 and L2 were prepared based on the literature procedures reported by us (Kadir *et al.*, 2016).

Preparation of Dodecanoylthioureido Complexes Derivatives

Synthesis of 1 *[Zn(L1)Cl₂]:* $ZnCl_2$ (0.027 g, 0.2 mmol) was dissolved in aqueous hydrochloric acid (5 mL, 1.0 M) to give colourless solution. Then, L1 (0.036 g, 0.1 mmol) was added as a solid into the above solution and the mixture was heated and stirred for few minutes. pH value of the above solution was adjusted by slow addition of aqueous sodium hydroxide (0.5 M) until colour change was observed and mixture dissolved. White precipitate was obtained at pH 11 with yield of 55.87 %.

Synthesis of 2 *[Zn(L2)Cl₂]:* $ZnCl_2$ (0.027 g, 0.2 mmol) was dissolved in aqueous hydrochloric acid (5 mL, 1.0 M) to give colourless solution. Then, L2 (0.041 g, 0.1 mmol) was added as a solid into the above solution and the mixture was heated and stirred for few minutes. pH value of the above solution was adjusted by slow addition of aqueous sodium hydroxide (0.5 M) until colour change was observed and mixture dissolved. White precipitate was obtained at pH 11 with yield of 72.14 %.

Synthesis of 3 *[Cu(L2)Cl₂]:* $CuCl_2$ (0.013 g, 0.1 mmol) was dissolved in aqueous hydrochloric acid (5 mL, 1.0 M) to give pale brown solution. Then, L2 (0.041 g, 0.1 mmol) was added as a solid into the above solution and the mixture was heated and stirred for few minutes. pH value of the above solution was adjusted by slow addition of aqueous sodium hydroxide (0.5 M) until colour change was observed and mixture dissolved. Green turquoise precipitate was obtained at pH 12 with yield of 64.44 %.

Evaluation of Antibacterial Activities

Antibacterial study of the complexes was carried out utilizing Gram-positive (*Bacillus subtilis* ATCC 11774, *Staphylococcus epidermidis* ATCC 13518 and *Staphylococcus aureus* ATCC 25923) and Gram-negative (*Escherichia coli* ATCC 11775 and *Salmonella typhimurium* ATCC 14128) strains by implementing common agar well diffusion method. The sub-cultured bacterial strains were prepared in Müeller Hinton agar as the basal medium. Then, the media were seeded with bacterial inoculum using cotton swab. Wells of 6.0 mm diameter were bored into the media using sterile cork borer and 30 µL of the diluted compounds with concentrations of 1 mg/mL were added to each well. Streptomycin (Abtek Biologicals Ltd) was used as positive control while methanol served as negative control. All plates were incubated overnight at 37 °C. The antibacterial activities were evaluated by measuring the zones of inhibition (mm). Since several previous works showed that solvent may interfere in the biological screening, methanol (negative control) was also tested and proven to exhibit no activity against the studied microorganisms.

RESULTS AND DISCUSSION

In our previous work, we have reported the synthesis and characterization of five lauroylthiourea amino acid derivatives (Kadir et al., 2016). However, in this present study, two of the synthesized thiourea compounds namely 2-(3-dodecanoyl-thioureido)-3-methylbutyric acid (L1) and 2-(3-

dodecanoylthioureido)-3-phenylpropionic acid (L2) that have the best antibacterial activities were selected and employed as ligands. From the reaction with zinc(II) and copper(II), three new complexes were obtained

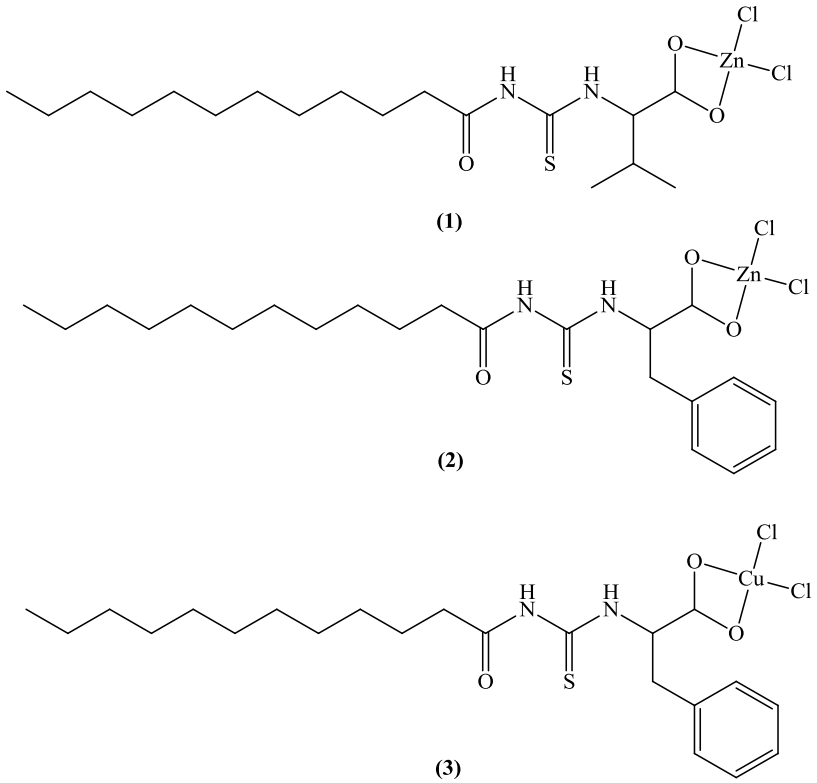

Figure 1 The structure of complexes 1-3

These complexes were prepared by dissolving metal salts, $ZnCl_2$ and $CuCl_2$ in acid solution with ligand to metal ratio of 1:1. Then, ligand was added into the acid solution while the pH of the solution was adjusted until the solid of complex precipitated from the solution. Basically, in basic condition, a carboxylic acid sodium salt of thiourea occurred as intermediate.

The compounds obtained were collected by filtration and left to dry at room temperature. Complexes 1 and 2 are obtained as colourless solid while 3 appeared as green turquoise solid. All compounds were subjected to spectroscopic analysis for structural determination.

Spectroscopic Analysis

In the FTIR spectra, the loss of ν(C=O carboxylic acid) peak at range 1712-1721 cm^{-1} was observed indicating the involvement of carboxylic oxygen atom as donors. Due to the metal-ligand coordination, the stretching peak for (C-OH) at 1700-1708 cm^{-1} was dissapeared and a new vibrational asymmetric stretching and vibrational symmetric stretching modes of COO^- at 1590 and 1385 cm^{-1} that supported the chelation between carboxylate O donors with metal ion were observed (Chohan *et al*., 2006). The formation of symmetric and asymmetric stretching vibrations of COO^- is associated with charged form of carboxyl moiety. Thus, the hydrogen of the COOH was deprotonated and coordinated with the metal atom (Figure 2). In this study, the peaks were observed at 1557-1596 cm^{-1} and 1386-1437 cm^{-1} representing asymmetric and symmetric stretching of COO^-.

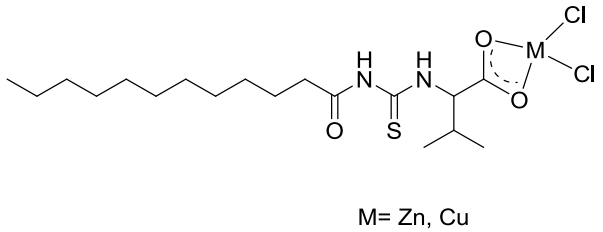

Figure 2 Chelating donors (O,O) from the ligand to metal ions (M)

The ν(C=S) of the complex was not shifted showing no involvement in the coordination, as supported by ElHusseiny *et al*., (2015). It was stated that a peak that remains either unchanged or upward shifted (positive shift) indicate non-bonding nature. In addition, the appearance of new peak at low frequency region (436 cm^{-1}, 422 cm^{-1} and 456 cm^{-1}) in complexes 1-3 were assigned to ν(M-O); suggesting chelation took place at the oxygen donor atom. According to Howlader *et al*., (2008), M-O stretching peak is found in range 428-508 cm^{-1}. Comparison between the IR data for the complexes and ligands is shown in Table 1.

Table 1 Infrared data of lauroylthiourea amino acid complexes

Compound/ Stretching	ν(N-H) (cm^{-1})	ν(O-H) (cm^{-1})	ν(C=O) $_{COOH}$ (cm^{-1})	ν(C=O) $_{NH}$ (cm^{-1})	ν(C-N) (cm^{-1})	ν(C=S) (cm^{-1})	ν(M-O) (cm^{-1})
L1	NH overlap with OH at 3332 (m)		1712 (s)	1650 (s)	1415 (m)	723 (w)	-
L2	NH overlap with OH at 3314 (m)		1721 (s)	1710 (s)	1467 (s)	698 (m)	-
1	NH overlap with OH at 3436 (br)		-	1623 (s)	1411 (s)	721 (w)	436 (m)
2	NH overlap with OH at 3436 (br)		-	1625 (s)	1466 (m)	700 (w)	422 (w)
3	NH overlap with OH at 3359 (br)		-	1629 (m)	1467 (m)	699 (w)	456 (w)

In the UV-Vis spectra of the complexes, peak at higher energy (254-259 nm) which corresponded to the mixture of n-π* and π-π* transition were assigned to C=S and C=O chromophores. In the case of complex 3 that contains phenylalanine, a broader peak was observed due to the overlapping of π-π* of phenyl functionality with the C=S and C=O chromophores (Figure 3).

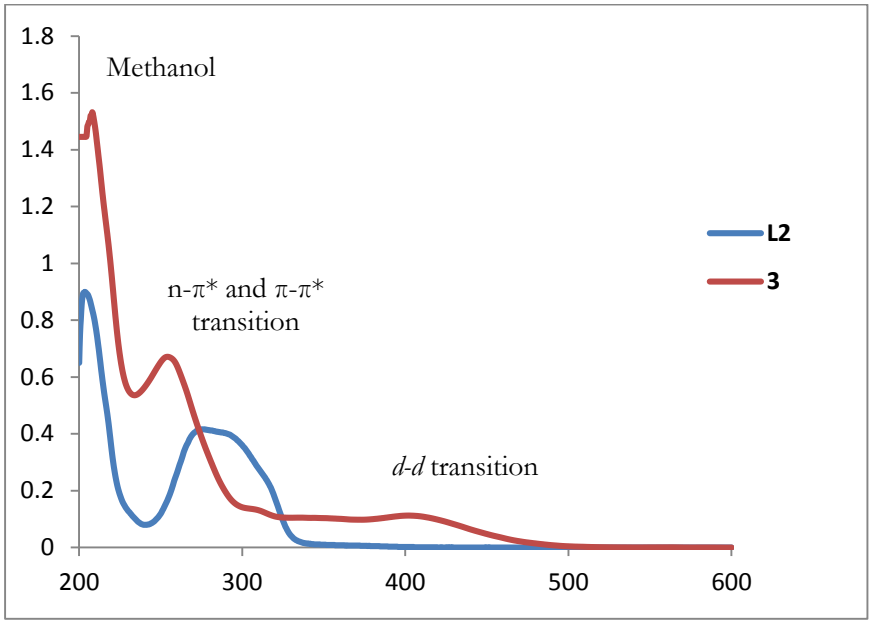

Figure 3 UV-vis spectra of L2 versus complex 3

These peaks undergo blue shift from 272-275.5 nm (ligands) to 254-259 nm (complexes). According to Estévez-Hernández *et al*., (2015) in their mercury complexes, changes happen due to charge redistribution within the molecule and higher stabilization of its n and π energy levels. In addition, prominent bands at range 330-335 nm corresponded to π-π* transition were observed in complex 1 and 2. In contrast, a band at 402 nm that associated to *d-d* transition was identified in complex 3. According to Cakir and Bcer (2010), broad band at 350 nm indicated π-π* transitions of carboxylate group and also due to the interaction between *d* orbital of metal and π system of the ligand.

Antibacterial Screening

The complexes in this study showed weak antibacterial activity against Gram-negative bacteria with inhibition zone in range of 7-9 mm. However, all complexes showed good antibacterial activity toward *S. epidermidis*, a Gram-positive strain compared to the others bacteria. Complex 3 has the strongest antibacterial activity among the other two complexes with inhibition zone of 17 mm, followed by complex **2** with inhibition diameter of 14 mm (Figure 4).

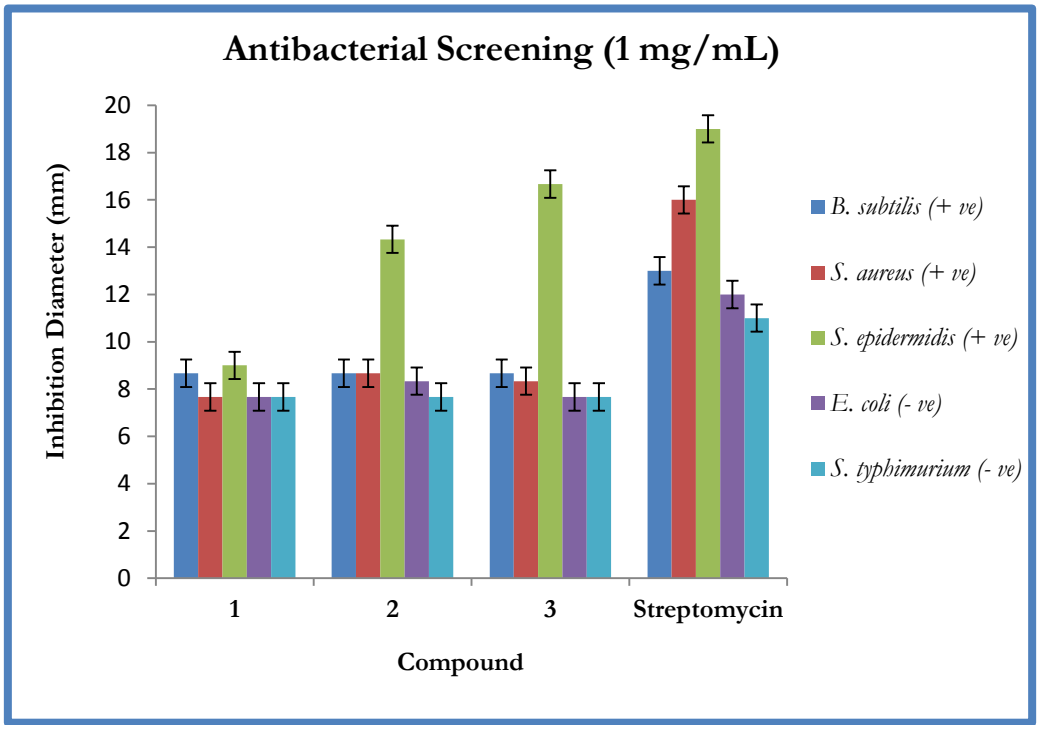

Figure 4 Antibacterial screening of 1-3 complexes

This revealed that complexes containing ligand with phenylalanine have better performance which might be attributed to the delocalized electrons in the phenyl moiety. Evidently, compounds containing phenyl motif is evident to have higher lipophilicity which allows better penetration into the cell wall of the bacteria (Ngaini *et al*., 2012). This result is in agreement with few studies where ligand with phenylalanine moiety has good antibacterial activity (Kadir et al., 2016; Gokhale *et al*., 2011; Nagesh *et al*., 2015). Meannwhile, copper ion can penetrate better into the bacterial cell wall thus enhancing the performance of complex 3 as antibacterial agent.

CONCLUSION

Three new complexes containing dodecanoylthioureido have been successfully synthesized. The complexes exhibited specific antibacterial activity towards Gram-positive strains; *S. epidermidis* with complex **3**, that contains phenylalanine and copper ion, both are good in membrane penetration, displayed the best antibacterial activity. This finding reveals that combination of thiourea with phenylalanine and copper can enhance the performance of the antibacterial activities of the designed compounds.

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