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SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL STUDIES OF Cu(II) COMPLEXES THIOUREA

(Sintesis, Pencirian dan Kajian Aktiviti Antibakteria Kompleks Cu(II) Tiourea)

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Abstract

A series of bisthiourea ligands, 1,2-bis(*N*'-2-methoxybenzoylthioureido)-4-chlorobenzene **(TI)**, 1,2-bis(*N*'-3-methoxy benzoylthioureido)-4-chlorobenzene **(TII)** and 1,2-bis(*N*'-4-methoxybenzoylthioureido)-4-chlorobenzene **(TIII)** and their Cu(II) complexes have been successfully synthesised. The products have been characterised by elemental analysis (CHNS), IR spectroscopy, ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR), melting point and magnetic susceptibility determination method. The values of μ_{eff} (B.M) for Cu(II) complexes in the range of 1.23-2.21 B.M reveal that the complexes are square planar geometry. The thiourea ligands along with their respective Cu(II) complexes were screened for their antibacterial activity using disc diffusion method against four strains of bacteria (*Bacillus subtillis, Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus*). Interestingly, compound TIII, Cu(TII) and Cu(TIII) showed the activity against *Staphylococcus aureus*. The antibacterial activity of their metal complexes is higher than the ligands.

Keywords: Bisthiourea, spectroscopy, antibacterial activity

Abstrak

Siri ligan bistiourea, 1,2-bis(*N*'-2-metoksibenzoiltioureido)-4-klorobenzena **(TI)**, 1,2-bis(*N*'-3- metoksibenzoiltioureido)-4-klorobenzena **(TII)** and 1,2-bis(*N*'-4- metoksibenzoiltioureido)-4-klorobenzena **(TIII)** dan komplek Cu(II) telah berjaya disintesis. Sebatian dicirikan melalui analisis unsur (CHNS), spektroskopi infra merah (IR), ^{1}H and ^{13}C resonans magnet nucleus (NMR), takat lebur dan penetapan suseptibiliti magnet. Nilai μ_{eff} (B.M) untuk kompleks Cu(II) berada pada julat 1.23-2.21 B.M menunjukkan bahawa kompleks ini mempunyai geometri satah persegi empat. Kajian aktiviti antibakteria telah dilakukan ke atas terbitan tiourea dan kompleksnya dengan menggunakan ujian disk resapan menentang empat strain bakteria (*Bacillus subtillis, Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus*). Menariknya, sebatian TIII, Cu(II) dan Cu(III) menunjukkan aktiviti terhadap bacteria *Staphylococcus aureus.* Kajian aktiviti antibakteria kompleks logam adalah lebih tinggi daripada ligan.

Kata kunci: Bistiourea, spektroskopi, aktiviti antibakteria

Introduction

Thiourea ligands and their metal complexes exhibit a wide range of biological activity including anticancer [1], antimicrobial [1-3], antibacterial [4], antifungal [5], antimalarial [6] and antituberculosis [7]. There have been many researches of benzoyl-substituted thioureas and related ligands coordinated to metals where the ligand usually coordinated through S and O, giving a six membered ring system [3-5]. They are able to coordinate to arrange metal centres as neutral ligands or as anionic ligands [8,9]. As some reports demonstrated that the antibacterial activity of some compounds becomes enhanced when they are complexed with copper [4]. Only a few of aromatic diamine being reported such as 1,2-Bis[N'-(2,2-dimethylpropionyl)thioureido]cyclohexane [10], and 1,2-bis(N'-2-methoxybenzoylthioureido)-4-nitrobenzene [11].

In this study, we report herein the synthesis, characterization and antibacterial studies a sum of bisthiourea, namely, 1,2-bis(*N*'-2-methoxybenzoylthioureido)-4-chlorobenzene **(TI)**, 1,2-bis(*N*'-3-methoxybenzoylthioureido)-4-chlorobenzene **(TII)**, 1,2-bis(*N*'-4-methoxybenzoylthioureido)-4-chlorobenzene **(TIII)**, as shown in Figure 1 and their Cu(II) complexes. Basically, there are isomers consisting of chlorobenzene as the bridging group between two substituted benzoyl chloride. The only different is the position of methoxy group. The structures of the ligands and their complexes have been characterized by elemental analysis (CHNS), IR spectroscopy, ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR), melting point and magnetic susceptibility determination method.

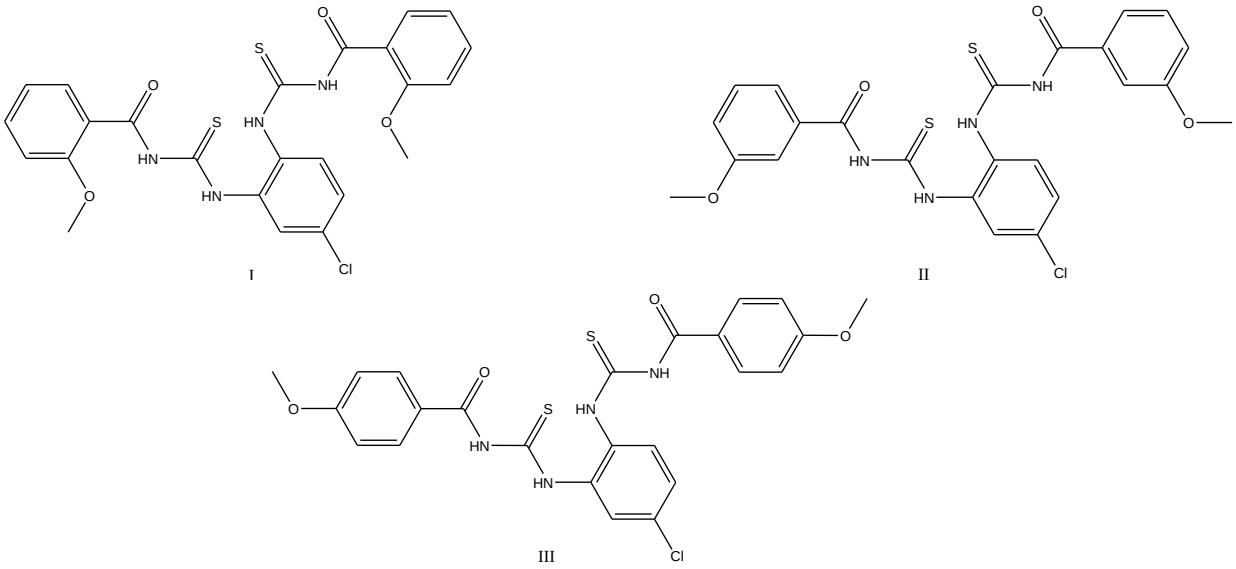

Figure 1: The molecular structures of the 1,2-bis(*N*'-2-methoxybenzoylthioureido)-4-chlorobenzene **(TI)**, 1,2-bis(*N*'-3-methoxybenzoylthioureido)-4-chlorobenzene **(TII)** and 1,2-bis(*N*'-4-methoxy benzoylthioureido)-4-chlorobenzene **(TIII)**

Experimental

Physical measurements

Every part of reactions was performed under an ambient atmosphere and no special precautions were taken to exclude air or moisture. Chemicals and solvents were purchased from Sigma Aldrich or MERCK and used as received without further purification. Melting points were measured using BÜCHI Melting Point B-545 and magnetic susceptibility of compounds was measured on Sherwood Auto Magnetic susceptibility balance. Infrared spectra were obtained using FTIR Perkin Elmer 100 Spectrophotometer in the spectral range of 4000-350 cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded using Bruker Avance III 300 Spectrometer at room temperature. The elemental analyses were conducted using CHNS Analyzer Flash EA 1112 series.

Synthesis of the ligands

Freshly prepared substituted benzoyl chloride (0.013 mol) was added drop wise to a stirring acetone solution (20 ml) of ammonium thiocyanate (0.013 mol). The solution mixture was stirred about 20 minutes. A solution of 4-chloro-1,2-phenylenediamine (0.0065 mol) in acetone was added and the reaction solution was heated under reflux for 3 hours. The solution was poured into a beaker containing some ice cubes. The resulting precipitate was collected by filtration, washed several times with cold ethanol/water and purified by recrystallisation from ethanol/dichloromethane mixture (1:1).

1,2-bis(N'-2-methoxybenzoylthioureido)-4-chlorobenzene, (TI): Yield 59.1%; Dark brown solid, m.p 192.7 ˚C. IR (KBr pellet, cm^{-1}): υ(C=O) 1668.90, υ(N-H) 3320.72, υ(C-N) 1249.18, υ(C=S) 854.76. ^{1}H NMR ($CDCl_3$-d_6, 300.13

MHz): δ 4.06 (s, 3H, OMe); 7.02 to 8.11 (m, Ar-H); 11.19 (d, H, CONH); 12.48, 12.63 (s, H, CSNH). ^{13}H NMR ($CDCI_3$) δ 56.50 (CH_3); 180.16, 180.65 (C=S); 164.92 (C=O); 111.77 – 157.89 (aromatic ring). *Anal.* Calc. For $C_{24}H_{21}ClN_40_4S_2$: C, 54.49; H, 4.00; N, 10.59; S, 12.12. Found: C, 55.22; H, 4.89; N, 9.91; S, 8.70.

1,2-bis(N'-3-methoxybenzoylthioureido)-4-chlorobenzene (TII): Yield 64.0%; Yellowish solid, m.p 203.9 ˚C. IR (KBr pellet, cm^{-1}): υ(C=O) 1674.82, υ(N-H) 3322.14, υ(C-N) 1273.74, υ(C=S) 853.17. ^{1}H NMR ($CDCI_3$-d_6, 300.13 MHz): δ 3.75 (s, 3H, OMe); 7.16 to 8.19 (m, Ar-H); 11.80 (d, H, CONH); 12.45, 12.64 (s, H, CSNH). ^{13}H NMR ($CDCI_3$) δ 55.77 (CH_3); 180.70, 181.20 (C=S); 168.53 (C=O); 113.59 – 159.43 (aromatic ring). *Anal.* Calc. For $C_{24}H_{21}ClN_40_4S_2$: C, 54.49; H, 4.00; N, 10.59; S, 12.12. Found: C, 54.23; H, 4.01; N, 10.49; S, 12.16.

1,2-bis(N'-4-methoxybenzoylthioureido)-4-chlorobenzene, (TIII): Yield 69%; Reddish brown solid, m.p 203.0 ˚C. IR (KBr pellet, cm^{-1}): υ(C=O) 1654.14, υ(N-H) 3286.16, υ(C-N) 1262.71, υ(C=S) 843.40. ^{1}H NMR ($CDCI_3$-d_6, 300.13 MHz): δ 3.82 (s, 3H, OMe); 6.97-8.14 (m, Ar-H); 11.59 (d, H, CONH); 12.49, 12.69 (s, H, CSNH). ^{13}H NMR ($CDCI_3$) δ 56.50 (CH_3); 180.86, 180.36 (C=S); 167.99 (C=O); 114.20 – 163.74 (aromatic ring). *Anal.* Calc. For $C_{24}H_{21}ClN_40_4S_2$: C, 54.49; H, 4.00; N, 10.59; S, 12.12. Found: C, 55.73; H, 4.05; N, 10.50; S, 6.71.

Synthesis of [Cu(TI)]

The complexes were prepared by the template method. A solution of the copper(II) acetate (0.0005 mol) in 20 ml EtOH was added drop wise to a solution of the 1,2-bis(*N*'-2-methoxybenzoylthioureido)-4-chlorobenzene, (I) (0.0003 mol) in DCM (40 ml). The resulting mixture was refluxed for about 6 hours. The resulting precipitate complexes was collected, filtered, and recrystallized from ethanol/dichloromethane mixture (1:1). Yield 80%; Green solid, m.p 173.6 ˚C. IR (KBr pellet, cm^{-1}): υ(C=O) 1609.79, υ(N-H) 3304.34, υ(C-N) 1247.34. *Anal.* Calc. For $C_{24}H_{21}ClCuN_40_4S_2$: C, 48.64; H, 3.57; N, 9.45; S, 10.82. Found: C, 47.84; H, 3.06; N, 9.36; S, 6.24.

Synthesis of [Cu(TII)]

The complexes were prepared by the template method. A solution of the copper(II) acetate (0.0005 mol) in 20 ml EtOH was added drop wise to a solution of the 1,2-bis(*N*'-3-methoxybenzoylthioureido)-4-chlorobenzene, (I) (0.0003 mol) in DCM (40 ml). The resulting mixture was refluxed for about 6 hours. The resulting precipitate complexes was collected, filtered, and recrystallized from ethanol/dichloromethane mixture (1:1). Yield 93%; Dark green solid, m.p 169.0 ˚C. IR (KBr pellet, cm^{-1}): υ(C=O) 1650.98, υ(N-H) 3320.96, υ(C-N) 1273.79. *Anal.* Calc. For $C_{24}H_{21}ClCuN_40_4S_2$: C, 48.64; H, 3.57; N, 9.45; S, 10.82. Found: C, 47.61; H, 3.00; N, 9.55; S, 7.24.

Synthesis of [Cu(TIII)]

The complexes were prepared by the template method. A solution of the copper(II) acetate (0.0005 mol) in 20 ml EtOH was added drop wise to a solution of the 1,2-bis(*N*'-4-methoxybenzoylthioureido)-4-chlorobenzene, (I) (0.0003 mol) in DCM (40 ml). The resulting mixture was refluxed for about 6 hours. The resulting precipitate complexes was collected, filtered, and recrystallized from ethanol/dichloromethane mixture (1:1). Yield 64%; Green solid, m.p 343.0 ˚C. IR (KBr pellet, cm^{-1}): υ(C=O) 1663.98, υ(N-H) 3269.62, υ(C-N) 1259.10. *Anal.* Calc. For $C_{24}H_{21}ClCuN_40_4S_2$: C, 48.64; H, 3.57; N, 9.45; S, 10.82. Found: C, 45.63; H, 3.23; N, 8.78; S, 9.76.

Antibacterial screening

All of the synthesized compounds were screened for their antibacterial activity using disc diffusion method against four strains of bacteria (*Bacillus subtillis, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus*). Streptomycin (400 μg mL^{-1}) was used as positive control and DMSO as negative control. These bacteria was cultured in nutrient broth and left for 24 hours to grow. After that, the nutrient broth was added to the sterilized medium before solidification. Then, the media with bacteria was poured into sterilized Petri dishes under aseptic condition. The thiourea ligands along with their respective Cu(II) complexes were dissolved in DMSO solvent at 10mg mL^{-1} and were impregnated on blank disc. The impregnated disc was placed on the surface of the culture and it then incubated at 37ºC for 48 hours. After incubation, the average of the inhibition zones was measured and compared with positive control. The bactericidal tests were performing in triplicate and results are shown in Table 4.

Results and Discussion

Chemical analysis

The microelemental analysis data of the product shows the relevant frequencies with the expected thioureas and their complexes (Table I). The melting point of the ligands were found between 192˚C - 203˚C and for the Cu(II) complexes between 169˚C - 343˚C.

Table 1: Microelemental analysis data of the thiourea ligands and its complexes

Compound	Color	M.P (˚C)	Elemental analysis data found (calculated) (%)			
			C(%)	H(%)	N(%)	S(%)
TI	Dark brown	192.7	55.22 (54.49)	4.89 (4.00)	9.91 (10.59)	8.70 (12.12)
TII	Yellow	203.9	54.23 (54.49)	4.01 (4.00)	10.49 (10.59)	12.16 (12.12)
TIII	Reddish brown	203.0	55.73 (54.49)	4.05 (4.00)	10.50 (10.59)	6.71 (12.12)
Cu(TI)	Green	173.6	48.15 (49.05)	3.18 (3.60)	9.32 (9.53)	8.65 (10.91)
Cu(TII)	Dark Green	169.0	48.25 (49.05)	3.19 (3.60)	9.33 (9.53)	9.79 (10.91)
Cu(TIII)	Green	343.0	47.31 (49.05)	3.14 (3.60)	9.00 (9.53)	8.44 (10.91)

Spectroscopic studies

The characteristic IR bands of all thiourea ligands showed the expected frequencies of υ(C=O), υ(N-H), υ(C-N) and υ(C=S) at 1654– 1668 cm^{-1}, 3286- 3322 cm^{-1}, 1249-1273 cm^{-1} and 843- 854 cm^{-1}, respectively. After complexation, the υ(C=O) band are shifted to lower frequencies suggesting coordination of the metal. The υ(C=S) band are expected shifted to higher frequency, but this vibration could not be assigned clearly due to appearance of broad peaks.

Table 2: Data of Infrared Spectroscopy

Peaks	TI	Cu(T1)	TII	Cu(TII)	TIII	Cu(TIII)
υ(N-H)	3320.72	3304.34	3322.14	3320.96	3286.16	3269.62
υ(C=O)	1668.90	1609.79	1674.82	1650.98	1654.14	1606.71
υ(C-N)	1249.18	1247.34	1273.73	1273.79	1262.71	1259.10
υ(C=S)	854.76	Broad	853.17	Broad	843.40	Broad

The ^{1}H and ^{13}C NMR chemical shifts of the thiourea ligands are quite similar. ^{1}H NMR data demonstrate the existence of methoxy proton between 3.75-4.05 ppm and aromatic proton in the range 6.97 to 8.19 ppm. The amine protons appear dublet between 11.18 to 11.80 ppm suggesting that the ligands are not planar. There are two signals for thioamide group proton between 12.4 and 12.6 also reflex of non planarity of the ligands. The ^{13}C NMR spectra show that the chemical shifts of the carbon of the CONH group around 164-168 ppm. There are also two signal of thioamide carbon chemical shifts at 180 ppm and 181 ppm.

Magnetic susceptibility determination

The effective magnetic moments of the copper(II) complexes Cu(TI), Cu(TII) and Cu(TIII) are found to be in the range of 1.23-2.21 B.M. These values correspond to the one unpaired electron. The paramagnetism of the complex agrees with the square planar geometry around the copper(II) ion.

Table 3: Magnetic data of Cu(II) complexes

Complex	μ_{eff} (B.M)	d^n	Suggested Geometry
Cu(TI)	2.21	d^9	Square planar
Cu(TII)	1.67	d^9	Square planar
Cu(TIII)	1.23	d^9	Square planar

Antibacterial activities

The antibacterial activities of thioureas and their Cu(II) complexes were tested by the disc diffusion method and the results were showed in Table 4. According to the antibacterial studies, the efficacy of the compounds against Gram positive bacteria is higher than Gram negative bacteria. Interestingly, compound TIII, Cu(TII) and Cu(TIII) showed the activity against *Staphylococcus aureus*. The antibacterial activity of their metal complexes is higher than the ligands. Compound Cu(III) are better antibacterial agents as compared to standard drug Streptomycin.

Table 4: Antibacterial screening of thioureas and their Cu(II) complexes

Compound	Gram positive bacteria Zone of inhibiton (mm)		Gram negative bacteria Zone of inhibiton (mm)	
	Staphylococcus aureus	*Bacillus sp.*	*Pseudomonas aeruginosa*	*Escherichia coli*
TI	-	-	-	-
TII	-	-	-	-
TIII	9	-	-	-
Cu(TI)	-	-	-	-
Cu(TII)	12	-	-	-
Cu(TIII)	19	-	-	-
Streptomycin	12	8	12	12

Concentration of the positive control (Streptomycin) = 400 μg mL^{-1}
Concentration of the sample = 10 mg mL^{-1}
- : No activity

Conclusion

To conclude, bisthiourea 1,2-bis(*N*'-2-methoxybenzoylthioureido)-4-chlorobenzene (TI), 1,2-bis(*N*'-3-methoxy benzoylthioureido)-4-chlorobenzene (TII) and 1,2-bis(*N*'-4-methoxybenzoylthioureido)-4-chlorobenzene (TIII) and their copper(II) complexes was successfully synthesized and fully characterized by spectroscopic methods. The values of μ_{eff} (B.M) calculated for Cu(II) complexes in the range of 1.23-2.21 B.M reveal that the Cu(II) complexes are square planar geometry. Compound TIII, Cu(TII) and Cu(TIII) showed the ability to inhibit *Staphylococcus aureus*. The antibacterial activity of their metal complexes is higher than the ligands.

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References

1. S. Saeed, N. Rashid, P.G. Jones, M.Ali, R. Hussain. Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. *European Journal of Medicinal Chemistry*. 2010, pp. 1323-1331.
2. A.A. Isab, S. NAwaz, M. Saleem, M. Altaf, M. Monim-al-Mehboob, A.Ahmad, H.S. Evans. Synthesis, characterization and antimicrobial studies of mixed ligand silver(I) complexes of thioureas and triphenylphosphine. *Polyhedron*. 2010, pp. 1251-1256.
3. A. Saeed, U. Shaheen, A. Hameed, S.Z.H. Naqvi. Synthesis, characterization and antimicrobial studies of some new 1-(fluorobenzoyl)-3-(fluorophenyl)thioureas. *Journal of Fluorine Chemistry*. 2009, pp.1028-1034.
4. M.K. Rauf, I. Din, A. Badshah, M. Gielen, M. Ebishara, D.Vos, S. Ahmed. Synthesis, structural characterization and in vitro cytotoxicity and anti-bacterial activity of some copper(I) complexes with N,N'-disubstituted thioureas. *Journal of Inorganic Biochemistry*. 2009, pp.1135-1144.
5. R. Campo, J.J. Criado, R. Gheorghe, F.J. González, M.R. Hermosa, F. Sanz, J.L Manzano, E. Monte, E.R. Fernández. *N*-benzoyl-*N*′-alkylthioureas and their complexes with Ni(II), Co(III) and Pt(II) – crystal structure of 3-benzoyl-1-butyl-1-methyl-thiourea: activity against fungi and yeast. 2004.
6. V.R. Solomon, W. Haq, M. Smilkstein, K. Srivastava, S.K. Puri, S.B. Katti. 4-aminoquinolone derived antimalarials: Synthesis, antiplasmodial activity and heme polymerization inhibition studies. *European Journal of Medicinal Chemistry*. 2010, pp. 4990-4996.
7. S. Karakuş, and S. Rollas. Synthesis and antituberculosis activity of new *N*-phenyl-*N*'-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenyl]thioureas. I1 farmaco. 2002, pp. 577-581.
8. H. Arslan, N. Duran, G. Borekci, C.K. Ozer, C. Akbay. Antimicrobial activity of some thiourea derivatives and their nickel and copper complexes. *Journal of Molecules*. 2009.
9. A. Tadjarodi, F. Adhami, Y. Hanifehpour, M. Yazdi, Z. Moghaddamfard, G. Kickelbick. Structural characterization of a copper(II) complex containing oxidation cyclization of N-2-(4-picolyl)-N'-(4-methoxyphenyl)thiourea, new ligand of 4-picolylthiourea derivatives and the precursor molecular structure of oxidative cyclization of N-(2-pyridyl)-N'-(4-methoxyphenyl) thiourea. *Polyhedron*, 2007, pp. 4609-4618.
10. M.S.M. Yusof, N.A.C. Ayob, M.A. KAdir, B.M. Yamin. 1,2-Bis[N'-(2,2-dimethylpropionyl)thioureido] cyclohexane. *Acta Cryst*. 2008, E64, 937.
11. N.I.M. Halim, K. Kassim, A.H. Fadzil, B.M. Yamin. Synthesis, characterization and antibacterial studies of benzoylthiourea derivatives. IPCBEE, vol. 14, pp 55, 2011.