



CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF FIVE SUBSTITUTED BIS-THIOUREAS

(Pencirian dan Kajian Antimikrobia Terhadap Lima Sebatian Bis-Tiourea)

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Abstract

Thioureas play an important role in medicinal chemistry and agricultures due to their biological activity such as antibacterial, antifungal, antiviral, herbicides, rodenticides, phenoloxidase enzymatic inhibitors, anti-HIV and anti-tumor agents. In this study, five substituted bis-thioureas have been synthesized. The isophthaloyl chloride and 2,6- pyridinedicarbonyl dichloride were easily converted to bis-isothiocyanate compound via the reaction with ammonium thiocyanate by solid-liquid phase transfer catalysis of polyethylene glycol-400 (PEG-400). Bis-isothiocyanate compound was reacted with aniline derivatives to produce substituted bis-thioureas in good yield at room temperature. All the novel compounds were obtained as yellow solid after recrystallization using DMF/EtOH/H₂O. Their chemical structures were confirmed by Infrared spectroscopy (IR), Nuclear Magnetic Resonance (NMR) ¹H and ¹³C and mass spectrometry. The five synthesized compounds were screened for antimicrobial activities using disc diffusion method for antimicrobial activity against Gram-positive bacteria (*Bacillus Subtilis* and *Staphylococcus Aureus*), Gram-negative bacteria (*Escherichia Coli* and *Salmonella Typhi*) and a mold (*Aspergillus Niger*). All tested compounds showed low antimicrobial activity since the diameter of inhibition zone (IZ) measure was less than positive control inhibition zone.

Keywords: bis-thiourea, antimicrobial activities, PEG-400

Abstrak

Sebatian tiourea memainkan peranan penting dalam kimia perubatan dan pertanian kerana sebatian ini mempunyai aktiviti biologi seperti antibakteria, antivirus, herbisid, rodentisid, perencat enzim fenoloksidase, anti-HIV dan agen anti-tumor. Lima sebatian bis-tiourea telah disintesis dalam kajian ini. Sebatian bis-isotiosianat disintesis dengan mudah melalui tindak balas di antara sebatian isofatolil klorida atau 2,6-piridinadikarbonil diklorida dengan ammonium tiosianat melalui pemindahan mangkin fasa pepejal-cecair polietilena glikol-400 (PEG-400). Seterusnya, sebatian bis-isotiosianat ditindak balas dengan sebatian amina bagi menghasilkan sebatian bis-tiourea dengan hasil yang baik pada suhu bilik. Semua sebatian diperolehi dalam bentuk pepejal berwarna kuning selepas penghabluran semula menggunakan pelarut DMF/EtOH/H₂O. Pencirian struktur sebatian dibuat melalui kaedah spektroskopi Infra Merah (IR), Resonans Magnet Nukleus (NMR) ¹H dan ¹³C dan spektrometer jisim (MS). Kajian aktiviti antimikrob bagi lima sebatian yang telah disintesis dibuat melalui kaedah resapan cakera melawan bakteria Gram positif (*Bacillus Subtilis* and *Staphylococcus Aureus*), bakteria Gram negatif (*Escherichia Coli* and *Salmonella Typhi*) dan kulat (*Aspergillus Niger*). Semua sebatian menunjukkan aktiviti antimikrob yang diperolehi rendah kerana diameter zon perencatannya (IZ) lebih kecil daripada zon perencatan kawalan positif.

Kata kunci: bis-tiourea, aktiviti antimikrob, PEG-400

Introduction

Antimicrobial agents has received much attention in recent years due to the dramatically increased of bacterial and fungi infectious diseases. Since penicillin was introduced as antibiotics in 1940's and were among the first medications to be effective against many bacterial infections, antibiotics have been used widely in fight against infectious diseases caused by bacteria and fungi. However, the usage and misuse of antibiotics has led to the emergence of antibiotic-resistant pathogens including multidrug-resistant strains [1 – 3]. As consequence, it has caused failure in the treatment of infectious diseases. In that respect, there is growing interest in the discovery of new antibacterial agents.

Thioureas were first synthesized by Neuki in 1873 [4]. Thiourea derivatives exhibit various biological activities such as antiviral [5, 6], antibacterial [7], fungicidal [8, 9], herbicidal [10] and anti-HIV [11]. In 2011, Peng et al. have reported some 1,3-benzenedicarbonyl bithiourea showed inhibition activities against cancer cells, such as human nasopharyngeal cancer cell line, CNE₂ and human oral cavity cancer cell line, KB with IC₅₀ value 10.72 and 9.91 μm respectively [12].

Some example of bis-thiourea derivatives are N³, N^{3'}-bis (disubstituted)isophatalyl-bis(thiourea), bis(thiourea)hydrazide [13], 2,2'-diaminobinaphthalene-bis(thiourea) [14] and (bis)thiourea polyamine [15]. The derivatives of bis (disubstituted)isophthalaly-bis(thiourea) were also reported by Wang et al., Peng et al. and Jamil et al. research groups in 2005, 2011 and 2013, respectively [16, 17, 18]. In 2011, 1,2-Bis(N⁷-benzoylthioureidothioureido)-4-cholorobenzene was reported by Yamin research group [19]. The former compound is similar to 1,2-bis(N⁷-benzoylthioureido)benzene reported by Thiam and co-workers [20] during 2008 except for the presence of 4-chlorobenzene bridge at 1,2 position that connected the two benzoyl thioureido group.

In view of these observations, we have designed 1,3-benzenedicarbonyl and 2,6-pyridinedicarbonyl bithiourea derivatives in order to investigate their antimicrobial activities. Herein, we report the syntheses of five bithioureas compound **NA1-5** as shown in Figure 1 with the aid of solid-liquid phase transfer catalyst, PEG-400 [12]. Their structures of all the compounds were confirmed by IR spectroscopy and ¹H and ¹³C Nuclear Magnetic Resonance (NMR). All compounds were screened for antimicrobial activities against Gram-positive bacteria, Gram- negative bacteria and a mold.

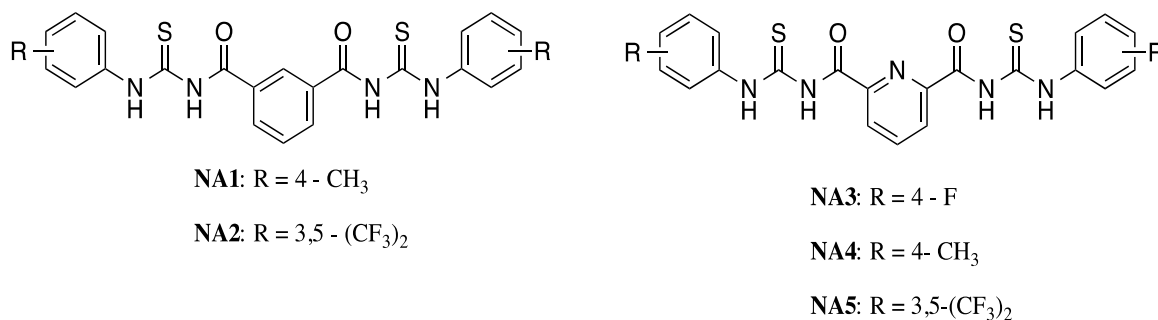


Figure 1. Chemical structures of substituted bis(thioureas)

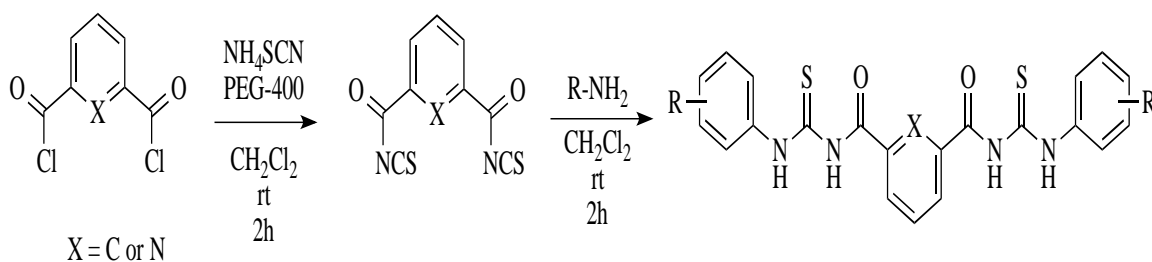
Materials and Methods

Chemicals (isophataloyl chloride, 2,6-pyridinedicarbonyl dichloride, aniline, 4-fluoroaniline, 3,5-bis(trifluoromethyl)aniline, ammonium thiocyanate, and PEG-400) and solvents (dichloromethane, dimethylformamide and ethanol) were purchased from Sigma Aldrich and MERCK and used directly without further purification. Melting points were determined by using BÜCHI Melting Point Model B-545. Infrared spectra were obtained using FTIR Perkin Elmer-1310 Spectrum GX Spectrophotometer in the spectral range of 4000

– 400 cm⁻¹. ¹H and ¹³C were analysed by using Bruker Avance III 600 MHz and Jeol JNM-ECP 400MHz NMR spectrometer.

Synthesis of compounds

The general procedure for synthesizing compounds **NA1-5** (Scheme 1) was as follows. Isophthaloyl chloride (2.46 mmol) or 2,6-pyridinedicarbonyl dichloride (2.45 mmol) was dissolved in 30 mL dichloromethane. Subsequently, ammonium thiocyanate (7.38 mmol) and PEG-400 (0.22 mmol) were added to the solution and was stirred at room temperature for 2 hours. The resulting mixture was filtered to give isothiocyanate compound which was directly used without further purification. Aniline derivatives (5.15 mmol) was added and stirred for another hour. The yellow precipitate formed was filtered and washed with distilled water and purified by recrystallization using Dimethylformamide/Ethanol/Water.



Scheme 1. Synthesis of bis(thiourea) derivatives

Antibacterial screening

All synthesized compounds were screened for their antimicrobial activity by using disc diffusion method against five microbes (*Bacillus Subtilis*, *Staphylococcus Aureus*, *Escherichia Coli*, *Salmonella Typhi*, and *Aspergillus Niger*). Streptomycin (10µg) and Kinamycin (30µg) were used as positive control and Dimethyl sulfoxide as negative control. All bacteria were cultured in nutrient broth and incubated at 37 °C for 24 hours. After that, the agar media was prepared by pouring the nutrient agar solution into the sterilized petri dishes. Then, the cultures were spread on the surface of Nutrient Agar (NA). The discs (6 mm diameter) were impregnated with (10µL/disc) and (30µL/disc) test samples and then placed on the surface of the agar media. Experimental plates were incubated at 37 °C for 24 hours. Antimicrobial activity was determined by measuring the diameter of inhibition zone (IZ) and compared with positive control.

Results and Discussion

In this study, bis-isothiocyanate compound was not isolated and directly treated with respective aniline derivatives to give the corresponding bis-thiourea (**NA1-5**) in moderate to good yield *via* nucleophilic substitution mechanism. IR studies showed that, strong absorption in region 3250 – 3383 cm⁻¹, which corresponds to ν (N-H) stretching. The ν (C=O) absorption bands were observed in region 1650-1687 cm⁻¹. The medium intensity bands around 1152 – 1127 cm⁻¹ attribute to the stretching of ν (C=S). All absorptions band value was in agreement with standard absorption value for thiourea compounds.

In the ¹H NMR spectra, the CONH protons appear as singlet in the range of δ 12.3 – 12.4 ppm whereas CONH protons appeared at δ 11.1 – 11.5 ppm. The appearance of N-H signals at higher frequency was due to the presence of carbonyl and thiocarbonyl groups which exert strong deshielding effect. The aromatic protons of parent 1,3-benzenedicarbonyl amide appeared around δ 8.5 – 7.8 ppm. The aromatic protons of amine in compound NA1 and NA2 showed peaks in the range of δ 7.5 – 7.2 ppm and δ 8.8 – 7.9 ppm respectively. The protons signals of amine in NA2 were observed at higher frequency because the protons were deshielded by CF₃ groups. Compounds with parent 2,6-pyridinedicarbonyl amide protons signals were found at δ 8.0 – 7.5 ppm while aromatic protons of amine in compound NA3, NA4 and NA5 appeared at δ 8.5 – 6.8 ppm. The aromatic protons signals of amine in compound NA3, NA4 and NA5 were slightly different from each other may be influenced by the nature of substituent group attached to the compound. Electronegative

atom reduced the electron density around those protons and hence the protons were deshielded. The ^{13}C NMR assignments showed that, the carbon signals for (C=S) and (C=O) appeared at δ 180.0 – 178.0 ppm and 167.2 – 162.0 ppm respectively. While aromatic carbon signals were observed at δ 154.1 – 116.9 ppm. All compounds were obtained as yellow solid after recrystallization using DMF/EtOH/H₂O. Their chemical structures were validated by FTIR, ^1H and ^{13}C NMR. The physical and spectroscopic data of the new compounds are shown below.

1,3-Benzenedicarboxamide, N^1,N^3 -bis[[4-methylphenyl]amino]thioxomethyl] **NA1**: Yield 78%, Yellow solid, m.p 222.0 °C, IR (KBr pellet, cm^{-1}) : ν (N-H) 3250.1, ν (C=O) 1655.9, ν (C-N) 1253.1, ν (C=S) 1151.0, ^1H (DMSO- d_6) δH (ppm): 12.4 (s, 2H, CSNH), 11.5 (s, 2H, CONH), 8.5 (s, 1H, Ar-H), 8.18 (d, J = 5.6 Hz, 2H, Ar-H), 7.8 (t, J = 8 Hz, 1H, Ar-H), 7.56 (d, J = 8.4 Hz, 4H, Ar-H), 7.24 (d, J = 8.0 Hz, 4H, Ar-H), 2.3 (s, 6H, CH₃), ^{13}C (DMSO- d_6) δC (ppm): 179.3 (C=S), 167.7 (C=O), 136.3 (Ar-C), 135.8 (Ar-C), 133.7 (Ar-C), 132.4 (Ar-C), 129.7 (Ar-C), 129.3 (Ar-C), 124.8 (Ar-C), 21.1 (CH₃).

1,3-Benzenedicarboxamide, N^1,N^3 -bis[[3,5-bis(trifluoromethyl)phenyl]amino]thioxomethyl] **NA2**: Yield 72%, Yellow solid, m.p 195.6 °C, IR (KBr pellet, cm^{-1}) : ν (N-H) 3232.8, ν (C=O) 1656.1, ν (C-N) 1245.8, ν (Ar-CF₃) 1280.0, ν (C=S) 1127.0, ^1H (DMSO- d_6) δH (ppm): 8.8 (s, 1H, Ar-H), 8.5 (s, 4H, Ar-H), 8.4 (d, J = 6.6 Hz, 2H, Ar-H), 7.9 (s, 2H, Ar-H), 7.8 (t, J = 7.2 Hz, 7.8Hz, 1H, Ar-H), ^{13}C (DMSO- d_6) δC (ppm): 180.0 (C=S), 167.1 (C=O), 140.1 (Ar-C), 133.4 (Ar-C), 132.5 (Ar-C), 131.7-131.0 (q, $^3J_{\text{CE}} = 33.9$ Hz, C-CF₃), 129.6 (Ar-C), 127.9 (Ar-C), 126.0-120.6 (q, $^1J_{\text{CF}} = 270.1$ Hz, CF₃), 119.5 (Ar-C).

2,6-pyridinedicarboxamide, N^1,N^3 -bis[[4-fluorophenyl]amino]thioxomethyl] **NA3**: Yield 53%, Yellow solid, m.p 210.4 °C, IR (KBr pellet, cm^{-1}) : ν (N-H) 3383.6, ν (C=O) 1679.7, ν (C-N) 1227.8, ν (C-F) 1352.8 ν (C=S) 1151.0, ^1H (DMSO- d_6) δH (ppm): 8.0 (d, J = 7.8 Hz, 2H, Ar-H), 7.94 (t, J = 8.4 Hz, 7.2Hz, 1H, Ar-H), 7.27-7.25 (m, 4H, Ar-H), 6.88-6.83 (m, 4H, Ar-H), ^{13}C (DMSO- d_6) δC (ppm): 178.9 (C=S), 163.7 (C=O), 160.9-159.3 (d, $^1J_{\text{EC}} = 242.8$ Hz, C-F), 149.3 (Ar-C), 140.4 (Ar-C), 134.4 (Ar-C), 127.1-127.0 (d, $^3J_{\text{EC}} = 8.8$ Hz, Ar-C), 126.9 (Ar-C), 115.4-115.5 (d, $^2J_{\text{EC}} = 21.9$ Hz, Ar-C).

2,6-pyridinedicarboxamide, N^1,N^3 -bis[[4-methylphenyl]amino]thioxomethyl] **NA4**: Yield 65%, Yellow solid, m.p 177.0 °C, IR (KBr pellet, cm^{-1}) : ν (N-H) 3280.7, ν (C=O) 1656.0, ν (C-N) 1241.8, ν (C=S) 1144.2, ^1H (DMSO- d_6) δH (ppm): 12.3 (s, 1H, CSNH), 11.7 (s, 1H, CSNH), 11.1 (s, 1H, CONH), 10.9 (s, 1H, CONH), 8.2 (t, J = 8.4 Hz, 7.2 Hz, 1H, Ar-H), 7.7 (d, J = 8.0 Hz, 4H), 7.6 (d, J = 8.4 Hz, 1H, Ar-H), 7.5 (d, J = 1, 8.4 Hz, 1H, Ar-H), 7.2 (d, J = 8.0 Hz, 4H, Ar-H), 2.3 (s, 6H, CH₃), ^{13}C (DMSO- d_6) δC (ppm): 179.0 (C=S), 166.9 (C=O), 154.1 (Ar-C), 140.6 (Ar-C), 138.8 (Ar-C), 134.4 (Ar-C), 130.4 (Ar-C), 129.4 (Ar-C), 126.4 (Ar-C), 25.7 (CH₃).

2,6-pyridinedicarboxamide, N^1,N^3 -bis[[3,5-bis(trifluoromethyl)phenyl]amino]thioxomethyl] **NA5**: Yield 62%, Yellow solid, m.p 345.5 °C, IR (KBr pellet, cm^{-1}) : ν (N-H) 3296.0, ν (C=O) 1687.1, ν (Ar-CF₃) 1283.1, ν (C-N) 1246.6, ν (C=S) 1151.8, ^1H (Acetone- d_6) δH (ppm): 11.1 (s, 2H, CONH), 8.7 (s, 4H, Ar-H), 8.5 (d, J = 7.8 Hz, 2H, Ar-H), 8.4 (t, J = 7.2 Hz, 7.8 Hz, 1H, Ar-H), 7.8 (s, 2H, Ar-H), ^{13}C (Acetone- d_6) δC (ppm): 178.8 (C=S), 162.0 (C=O), 148.4 (Ar-C), 140.4 (Ar-C), 140.2 (Ar-C), 140.1 (Ar-C), 132.0-131.4 (q, $^3J_{\text{EC}} = 33.0$ Hz, C-CF₃), 126.0 (Ar-C), 126.2-122.6 (q, $^1J_{\text{EC}} = 270.0$ Hz, CF₃), 120.1 (Ar-C), 116.9 (Ar-C).

Antimicrobial activities

The antibacterial and antifungal activities of five new bithioureas were tested against two Gram-positive bacterias (*Staphylococcus Aureus*, *Bacillus Subtilis*), two Gram-negative bacterias (*Escherichia Coli* and *Salmonella Typhi*) and single fungal strain - *Aspergillus Niger* using disc diffusion method. Streptomycin and Kinamycin were used as positive controls (Table 1).

Table 1. Antimicrobial result for five bis-thiourea compounds

Compound	Bacteria Inhibition Zone (mm)									
	Concentration ($\mu\text{g}/\text{disc}$)									
	<i>A.Niger</i>		<i>B.Subtilis</i>		<i>S.Aureus</i>		<i>E.Coli</i>		<i>S.Typhi</i>	
	10 μg	30 μg	10 μg	30 μg	10 μg	30 μg	10 μg	30 μg	10 μg	30 μg
NA1	-	-	6.5	6.5	7.0	7.0	6.5	6.5	-	-
NA2	-	-	6.5	6.5	7.0	7.0	6.5	6.5	-	-
NA3	-	-	7.5	7.5	7.0	7.0	6.5	6.5	-	-
NA4	-	-	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
NA5	-	-	7.0	7.0	6.5	6.5	6.5	6.5	8.0	9.0
Streptomycin 10 μg	12.0		18.0		16.0		18.0		18.0	
Kinamycin 30 μg	22.0		20.0		20.0		24.0		24.0	

Unfortunately, tested compounds did not display a remarkable activity against both type of bacteria and fungi. Nevertheless, compound NA5 showed valuable inhibitory activity. The activity of these compounds might be attributed to the presence of high electronegative substituent which enhanced the lipophilicity of the compounds. The lipophilicity of the compounds plays an important role in the penetration of solutes into the bacterial cell tissue [13]. The antibacterial activity of compound NA5 was higher than NA3 because NA5 contained multi fluoro group substituents which increase the lipophilicity of this compound. In contrary, all tested compounds had no activity against gram-negative bacteria. This observations showed that, the compounds were generally had better permeability towards gram-negative bacteria cell tissue.

Based on the test results, tested compounds had very low inhibition zone (IZ) values. It is known that thiourea compounds were generally exhibit good antimicrobial activities. However in this study, the results were contrary. This might be due to the solubility of the target compounds on the surface of the agar medium. Thus, the compounds were suspended on the agar surface and cannot reacted against those bacterias

Conclusion

In conclusion, five derivatives of 1,3-benzenedicarboxamide and 2,6-pyridinedicarboxamide bis-thiourea have been synthesized via solid-liquid phase transfer catalysis of PEG-400 and characterized by FTIR, ^1H and ^{13}C NMR. The preliminary studies on the antimicrobial activity via disc diffusion method showed that NA5 exhibit potential activity against *Staphylococcus Aureus*. For further research, we are planning to investigate the effect of transition metal complexation with these compounds toward their antimicrobial activity as some reports demonstrated that bis-thioureas antimicrobial activity were enhanced when complexed with transition metal. In addition, more sensitive antibacterial screening method such as dilution method should be used to evaluate the potential of synthesized compounds.

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