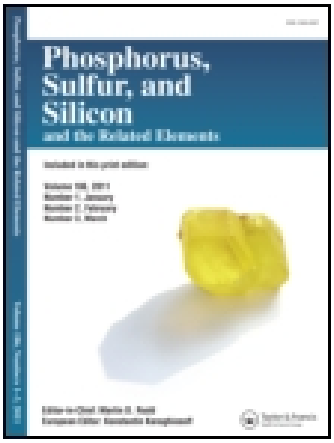

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Bis-Thiourea Bearing Aryl and Amino Acids Side Chains and Their Antibacterial Activities

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BIS-THIOUREA BEARING ARYL AND AMINO ACIDS SIDE CHAINS AND THEIR ANTIBACTERIAL ACTIVITIES

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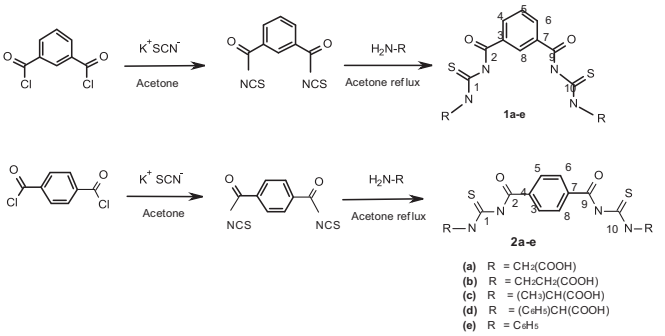
GRAPHICAL ABSTRACT



Abstract *A series of symmetrical 1,3-bis thiourea **1a–e** and 1,4-bis thiourea derivatives **2a–e** have been successfully synthesized from the reactions of amines with 3-acetylbenzoyl isothiocyanate and 4-acetylbenzoyl isothiocyanate, respectively. All the synthesized compounds were characterized by FT-IR spectroscopy and ^{1}H and ^{13}C NMR spectroscopy. The compounds were screened for their antibacterial activity by turbidimetric method using gram-negative bacteria (E. coli ATCC 8739) using turbidimetric method. The newly synthesized bis-thiourea derivatives bearing aryl side chains showed good antibacterial activity against* E. coli. *The effect of the molecular structure of the synthesized compounds on the antibacterial activity is discussed.*

Keywords Antibacterial activity; amino acid; bis-thiourea

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INTRODUCTION

Thiourea is an organic compound that consists of carbon, nitrogen, sulfur, and hydrogen atoms from the synthesis of amino group and thiocyanate. It is well known that thioureas and their derivatives play important roles in many biological processes.[1,2]

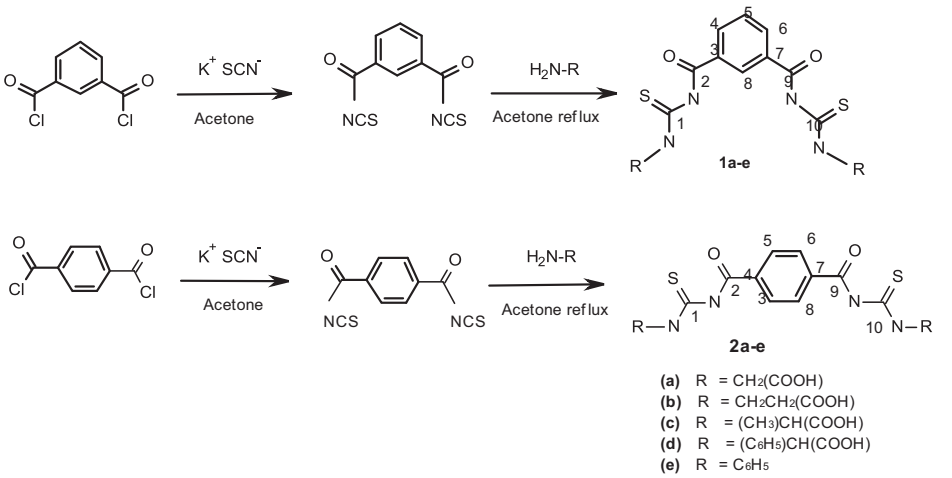
The search for new thiourea derivatives with biological activities has intensified. Most studies reported on compounds with a single thiourea moiety with trivial antimicrobial properties.[3–5] The quantity of thiourea units in the thiourea backbone contributed to enhancement of antimicrobial activity.[6] For example, the unsymmetrical bis-thiourea bearing triazole moieties were reported with excellent antimycobacterial activity against *M. tuberculosis* in monolayers of mouse bone marrow macrophages. The presence of two groups of thiourea moieties inhibited 90% of the mycobacterial growth. Other symmetrical bis-thioureas were also reported for their significant anticancer activities against Calu-6 lung carcinoma cells. The thioureas were able to induce the production of lysine-specific demethylase, thus, able to control the development of cancer cell.[7]

In this research, we aimed to synthesize bis-thiourea derivatives which consist of two differently positioned thiourea groups and study their antibacterial activities. The bis-thiourea derivatives were synthesized from the reaction of 3-acetylbenzoyl isothiocyanato and 4-acetylbenzoyl isothiocyanato with various amines groups, such as glycine, *beta*-alanine, L-alanine, L-phenylalanine, and aniline. The synthesized thiourea derivatives with the presence of two thiourea groups were also tested against *E. coli (*ATCC 8739) to determine their antibacterial properties.

RESULTS AND DISCUSSION

Chemistry

The preparations of **1a–e** and **2a–e** are shown in Scheme 1.



Scheme 1 The synthesis of **1a–e** and **2a–e**.

The IR spectra showed the successful formation of **1a–e** and **2a–e** by the disappearance of ν(NCS) at 2500–2000 cm^{-1} and appearance of ν(NH) at 3365–3082 cm^{-1}.

Sharp peaks observed at 1281–1220 cm^{-1} were attributed to ν(C=S).[3,8] The peaks at 1602–1580 cm^{-1} and 1730–1691 cm^{-1} were attributed to ν(C—N) and ν(C=O), respectively. ν(COOH) were observed at 1693–1611 cm^{-1} while the aromatic group was represented by the absorption band at 1557–1509 cm^{-1}.

The chemical structures of **1a–e** and **2a–e** were further confirmed by ^{1}H and ^{13}C NMR spectroscopy. CONH and CSNH signals were observed at δ 11.33–11.54 and δ 11.01–11.26, respectively. The CONH signal was shifted to higher frequency due to the downfield effect.[5] The presence of peaks at δ 7.69–8.50 region was attributed to aromatic protons. Four aromatic protons at $C_{3\text{-}8}$ of **2a–e** were observed as a duplet peak due to the symmetrical structures.

The ^{13}C NMR spectrum showed the presence of C=S at δ 180.0–179.7 whereas C=O resonated at δ 167.4–166.9. The COOH groups were represented by more downfield absorption peak at δ 173.1–171.3 due to the presence of more electronegative oxygen. The presence of the aromatic carbons was observed at δ 128.6–133.1.

Antibacterial Activity

The antibacterial activity of bis-thiourea derivatives **1a–e** and **2a–e** were examined at the concentration of 50 ppm, 80 ppm, and 100 ppm against bacteria *E. coli* ATCC 8739 at 37 °C. Of all the synthesized bis-thiourea, **2e** with aryl side chain exhibited good antibacterial activities compared to **1a–e** and **2a–d**. The comparison of the antibacterial activities for **1e** and **2e** is shown in Figure S1.

The different effects of the newly synthesized compounds **1a–e** and **2a–e** at various concentrations can be represented by their minimum inhibitory concentration (MIC). The MIC value for each compound was determined by extrapolating the concentration at the zero growth rate of *E. coli* ($\mu = 0$).[9] The MIC value of **2e** was observed at 175 ppm, while others were observed to exceed 200 ppm. A compound with a minimum inhibition value up to 400 ppm is still considered to have inhibition activity against Gram negative bacteria, however, a compound with the MIC value $>$ 200 ppm is not suitable to be used for clinical purposes.[10] MIC graph for all the synthesized compounds is shown in Figure S2.

The presence of C=S, C=O, and N-H functional groups in thiourea derivatives are reported to give good antibacterial activity as they react with carboxyl and phosphate groups of the bacterial surface.[11] Compounds **1a–e** and **2a–d,** however, showed no biological activity against *E. coli* ATCC 8739. In contrast, **2e** shows better antibacterial activity compare to **1e.** It was believed that 1,3-bis thiourea produced more steric hindrance compared to 1,4-bisthiourea. The occurrence of steric hindrance creates a force that obstruct the contact between active sites in the compound with receptor site of the bacteria.[6]

Lipophilic characteristic is another important behavior for good antibacterial activities as it correlates well with bioactivity of chemicals. However, the presence of some functional groups such as carboxylic acid group might decrease the lipophilic character of the compound.[10] The carboxylic acid groups presence in compounds **1a–d** and **2a–d** might contribute to reduction in lipophilic behavior thus gave poor antibacterial activities. On the other hand, the aryl side chains present in **2e** was envisaged to increase the lipophilicity thus enhance the antibacterial properties.[10]

CONCLUSION

Bis-thiourea derivatives **1a–e** and **2a–e** were successfully synthesized by the reaction of various types of amine groups with acetylbenzoyl isothiocyanate. The antibacterial study of the synthesized compounds discovered that **2e** showed good antibacterial activity against *E. coli* ATCC8739.

EXPERIMENTAL

Isophthaloyl dichloride, terepthaloyl dichloride, potassium thiocyanate, glycine, *beta*-alanine, L-alanine, L-phenylalanine and aniline were obtained from Merck and used without purification. Acetone was distilled over magnesium sulfate anhydrous. All other reagents and solvents used as received.

Measurements: Melting points were determined by the open tube capillary method and are uncorrected. Infra-red (IR) spectra (ν cm^{-1}) were recorded as KBr pellets on a Perkin Elmer 1605 FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECA 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C) with the chemical shifts δ (ppm) reported relative to acetone-d_6 and DMSO-d_6 as standards.

General Procedure for the Preparation of Bis Thiourea Derivatives 1a–e and 2a–e

Isophthaloyl dichloride or terepthaloyl dichloride (1 mmol) in dry acetone (15 mL) was added drop wise to a suspension of potassium thiocyanate (2 mmol) in dry acetone (15 mL). The mixture was stirred for 1 h at room temperature and the white potassium chloride (KCl) was filtered. Amines (2 mmol) in dry acetone (15 mL) was added into the filtrate and heated under reflux for 7 h. The mixture was cooled to room temperature and filtered. The filtrate was poured into ice in a beaker to form a solid. The crude solid was filtered, washed with ethanol and recrystallized from ethanol–acetonitrile (1:1). The general procedure for the preparation of **1a–e** and **2a–e** with different type of amine groups (g, mmol) and yields is shown below.

2-[[3-(carboxymethylcarbamothioylcarbamoyl)benzoyl] carbamothioylamino] Acetic Acid (1a). Glycine (0.150 g, 2 mmol). (0.274 g, 73%) as a yellowish solid, m.p: 226.0-227.0°C; (Found: C, 42.04; H, 3.36; N, 14.05; S, 15.79. $C_{14}H_{14}N_4O_6S_2$ Requires C, 42.20; H, 3.50; N, 14.10; S, 16.10%); ν_{max} (KBr/ cm^{-1}) 3233 (NH), 2922 (CH), 1729 (C=O), 1679 (COOH), 1602(C−N), 1557(Ar−C), 1231(C=S). δ_H (500 MHz, DMSO-d_6) 4.23 (4H, d, 2×CH_2), 7.69 (1H, t, J = 16.0 Hz, Ar−H), 8.14 (2H, d, J = 6.3 Hz, Ar−H), 8.47 (1H, s, Ar−H), 11.10 (2H, t, 2×NH), 11.40 (2H, s, 2×NH). δ_C (125 MHz, DMSO-d_6) 47.8, 128.4, 129.0, 131.9, 133.0, 166.9, 169.4, 179.7.

2-[[3-(2-carboxyethylcarbamothioylcarbamoyl)benzoyl]carbamothioylamino]propanoic Acid (1b). *Beta*-alanine (0.178 g, 2 mmol). (0.183 g, 67%) as a white solid, m.p: 219.1-220.2°C; (Found: C, 45.02; H, 4.00; N, 13.05; S, 15.03. $C_{16}H_{18}N_4O_6S_2$ Requires C, 45.10; H, 4.30; N, 13.10; S, 15.10%); ν_{max} (KBr/ cm^{-1}) 3082 (NH), 2666 (CH), 1691 (C=O), 1611 (COOH), 1580(C−N), 1520 (Ar−C), 1281 (C=S). δ_H (500 MHz, DMSO-d_6) 2.66 (4H, t, 2×CH_2), 3.84 (4H, q, 2×CH_2), 7.63(1H, t, J = 7.7 Hz, Ar−H), 8.15 (2H, d, J = 9.2 Hz, Ar−H), 8.48 (1H, s, Ar−H), 11.01 (2H, t, 2×NH), 11.33 (2H, s, 2×NH). δ_C (125 MHz, DMSO- d_6) 30.1, 32.5, 128.4, 129.0, 131.8, 132.9, 166.9, 173.1, 179.7.

2[[3[(2hydroxy1methyl2oxoethyl)carbamothioylcarbamoyl]benzoyl]carbamothioylamino]propanoic Acid (1c). L-alanine (0.178 g, 2 mmol). (0.282 g, 70%) as a white solid, m.p: 207.5-208.3°C, (Found: C, 45.10; H, 4.27; N, 13.03; S, 15.05. $C_{16}H_{18}N_4O_6S_2$ Requires C, 45.10; H, 4.30; N, 13.10; S, 15.10%); ν_{max} (KBr/ cm^{-1}) 3365 (NH), 2992 (CH), 1730 (C=O), 1693 (COOH), 1600 (C−N), 1514 (Ar−C), 1220 (C=S). δ_H (500 MHz, DMSO-d_6) 1.51 (6H, d, 2×CH_3), 4.85 (2H, m, 2×CH), 7.69 (1H, t, J = 16.0 Hz, Ar−H), 8.14 (2H, d, J = 8.4 Hz, Ar−H), 8.49 (1H, s, Ar−H), 11.26 (2H, d, 2×NH), 11.49 (2H, s, 2×NH). δ_C (125 MHz, DMSO-d_6) 17.1, 53.2, 128.6, 129.0, 131.8, 133.1, 167.4, 172.8, 179.5.

2-[[3-[(2-hydroxy-2-oxo-1 phenylethyl)carbamothioylcarbamoyl]benzoyl]carbamothioylamino]-2-phenyl-acetic Acid (1d). L-phenylalanine (0.302 g, 2 mmol). (0.437 g, 91%) as a yellowish solid, m.p.: 229.0-230.2°C; (Found: C, 57.72; H, 4.22; N, 9.68; S, 11.00. $C_{28}H_{26}N_4O_6S_2$ Requires C, 58.10; H, 4.50; N, 9.70; S, 11.10%); ν_{max} (KBr/ cm^{-1}) 3227(NH),, 2997 (CH), 1718 (C=O), 1690 (COOH), 1600 (C−N), 1509 (Ar−C), 1223 (C=S). δ_H (500 MHz, DMSO-d_6) 5.14 (2H, d, 2×CH), 7.21-7.30 (10H, m, J = 7.7 Hz Ar−H), 7.70 (1H, t, J = 16.1 Hz, Ar−H), 8.10 (2H, d, J = 7.7 Hz, Ar−H), 8.48 (1H, s, Ar−H), 11.17 (2H, d, 2×NH), 11.54 (2H, s, 2×NH). δ_C (125 MHz, DMSO-d_6) 58.7, 126.9, 128.4, 129.2, 131.6, 133.2, 136.1, 167.4, 171.3, 180.0.

N1,N3-bis(phenylcarbamothioyl)benzene-1,3-dicarboxamide (1e). Aniline (182 μmL, 2 mmol). (0.390 g, 89%) as a yellow crystalline solid, m.p: 202.0-202.6°C; (Found: C, 60.70; H, 4.14; N, 12.75; S, 14.19. $C_{22}H_{18}N_4O_2S_2$ Requires C, 60.80; H, 4.20; N, 12.90; S, 14.80%); ν_{max} (KBr/ cm^{-1}) 3087 (NH), 1672 (C=O), 1597 (C−N), 1520 (Ar−C), 1249 (C=S). δ_H (500 MHz, DMSO- d_6) 7.30 (2H, t, J = 15.3 Hz, Ar−H), 7.44 (4H, t, J = 15.3 Hz, Ar−H), 7.70 (4H, d, J = 16.0 Hz, Ar−H), 7.74 (1H, t, J = 8.7 Hz, Ar−H), 8.19 (2H, d, J = 7.7 Hz, Ar−H), 8.57(1H, s, Ar−H), 11.59 (2H, s, 2xNH), 12.55 (2H, s, 2xNH). δ_C (125 MHz, DMSO-d_6) 17.1, 53.2, 128.6, 129.0, 131.8, 133.1, 167.4, 172.8, 179.5.

2-[[4-(carboxymethylcarbamothioylcarbamoyl)benzoyl] carbamothioylamino] acetic Acid (2a). Glycine (0.150 g, 2 mmol). (0.056 g, 14%) as a yellowish solid, m.p.: 213.2-215.5°C; (Found: C, 42.10; H, 3.41; N, 13.95; S, 16.09. $C_{14}H_{14}N_4O_6S_2$ Requires C, 42.20; H, 3.50; N, 14.10; S, 16.10%); ν_{max} (KBr/ cm^{-1}) 3316 (NH), 2927 (CH), 1719 (C=O), 1672 (COOH), 1642 (C−N), 1552(Ar−C), 1242(C=S). δ_H (500 MHz, DMSO-d_6) 4.48 (4H, d, 2×CH_2), 7.94 (4H, d, J = 13.8 Hz, Ar−H), 9.13 (2H, t, 2×NH), 11.08 (2H, s, 2xNH). δ_C (125 MHz, DMSO-d_6) 39.8, 127.5, 128.6, 134.7, 137.5, 171.20, 169.8, 180.6.

3-[[4-(2-carboxyethylcarbamothioylcarbamoyl)benzoyl]carbamothioylamino]propanoic Acid (2b). *Beta*-alanine (0. 178 g, 2 mmol). (0.064 g, 15%) as a white solid, m.p.: 238.1–245.3°C; (Found: C, 44.61; H, 4.00; N, 12.22; S, 15.00. $C_{16}H_{18}N_4O_6S_2$ Requires C, 45.10; H, 4.30; N, 13.10; S, 15.10%); ν_{max} (KBr/ cm^{-1}) 3293 (NH), 3166 (CH), 1707 (C=O), 1671 (COOH), 1563 (C−N), 1526 (Ar−C), 1265 (C=S). δ_H (500 MHz, DMSO-d_6) 2.66 (4H, t, 2×CH_2), 3.84 (4H, q, 2×CH_2), 7.98 (4H, d, J = 12.3 Hz, Ar−H), 10.98 (2H, t, 2×NH), 11.55 (2H, s, 2×NH). δ_C (125 MHz, DMSO-d_6) 39.2, 39.3, 128.5, 135.9, 167.4, 173.0, 180.1.

2-[[4-[(2-hydroxy-1-methyl-2-oxo-ethyl)carbamothioylcarbamoyl]benzoyl] carbamothioyl Amino]propanoic (2c). L-alanine (0. 178 g, 2 mmol). (0.162 g, 38%) as a yellowish solid, m.p: 235.4-246.4°C; (Found: C, 44.80; H, 4.17; N, 12.88; S, 14.80. $C_{16}H_{18}N_4O_6S_2$ Requires C, 45.10; H, 4.30; N, 13.10; S, 15.10%); ν_{max} (KBr/ cm^{-1})

3356 (NH), 3177 (CH), 1728 (C=O), 1675 (COOH), 1451 (C−N), 1406 (Ar−C), 1259 (C=S). δ_H (500 MHz, DMSO-d_6) 3.56 (6H, m, 2×CH_3), 4.85 (2H, d, 2×CH), 8.01 (4H, d, J = 16.3 Hz, Ar−H), 11.23 (2H, d, 2×NH), 11.73 (2H, s, 2×NH). δ_C (125 MHz, DMSO-d_6) 17.2, 40.0, 128.6, 135.9, 167.8, 172.9, 179.8.

2-[[4-[(1-benzyl-2-hydroxy-2-oxoethyl) carbamothioylcarbamoyl]benzoyl] carbamothioyl amino]-3-phenyl-propanoic Acid (2d). L-phenylalanine (0.331g, 2 mmol). (0.335 g, 58%) as a white solid, m.p: 220.4-227.1°C; (Found: C, 57.43; H, 3.97; N, 10.17; S, 11.70. $C_{28}H_{26}N_4O_6S_2$ Requires C, 58.10; H, 4.50; N, 9.70; S, 11.10%); ν_{max} (KBr/ cm^{-1}) 3477 (NH), 3026 (CH), 1714 (C=O), 1666 (COOH), 1549 (C−N), 1515(Ar−C), 1232(C=S). δ_H (500 MHz, DMSO-d_6) 3.36 (4H, d, 2×CH_2), 5.12 (2H, q, 2×CH), 7.30 (10H, t, J = 7.7 Hz, Ar−H), 7.98 (4H, d, J = 13.0 Hz, Ar−H), 11.14 (2H, d, 2×NH), 11.75 (2H, s, 2×NH). δ_C (125 MHz, DMSO-d_6) 36.0,58.8, 126.9, 128.6, 129.3, 135.8, 136.3, 167.7, 171.4., 180.0.

N1,N4-bis(phenylcarbamothioyl)terephthalamide (2e). Aniline (182 μmL, 2 mmol) (0.10 g, 23%) as a light brown solid, m.p: 215.6-220.2°C; (Found: C, 60.70; H, 4.18; N, 12.05; S, 14.59. $C_{22}H_{18}N_4O_2S_2$ Requires C, 60.80; H, 4.20; N, 12.90; S, 14.80%); ν_{max} (KBr/ cm^{-1}) 3409 (NH), 3176 (CH), 1673 (C=O), 1599 (C−N), 1534(Ar−C), 1262(C=S). δ_H (500 MHz, DMSO- d_6) 8.0 (6H, t, J = 10.6 Hz, Ar−H), 8.1(8H, d, J = 7.7 Hz, Ar−H), 11.74 (2H, s, 2×NH), 12.54 (2H, s, 2×NH). δ_C (125 MHz, DMSO-d_6) 120.5, 124.4, 127.5, 128.3, 137.9, 166.9, 179.0.

Antibacterial Screening

The antibacterial activity of synthesized bis-thiourea derivatives were analyzed against *E. coli* ATCC 8739 using turbidimetric kinetic method. The *E. coli* were cultured on a LB plate agar for one day at 37°C. Then the inoculums were transferred and allowed to grow in a media containing nutrient broth at 37°C with permanent stirring at 250 rpm for overnight. And 0.2 mL of inoculums was inoculated with 10 mL of culture medium that was added with increasing concentration of synthesized compounds dissolved in DMSO. The mixture was shaken at 230 rpm at 37°C. Inoculums in the broth medium containing solvent was used as negative control. One mL aliquots of each replicate were taken at every 1 h interval for 6 h. The transmittance (T) was recorded using UV-Visible Spectrophotometer Optima SP-300. The antibacterial activity was determined by plotting a graph of ln N_t versus time. The ln N_t value was represents the number of colony forming units/mL which followed the expression of ln N_t = 27.1–8.56T.[12]

SUPPLEMENTAL MATERIALS

Supplementary data of this article can be accessed on the publisher's website, www.tandfonline.com/gpss

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