Synthesis, Biological Properties and Comparative Molecular Docking Evaluation Studies of 1,3 and 1,4 Bis-Thiourea Derivatives as Potential Antimicrobial Resistant Agents

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ABSTRACT

Introduction: Thiourea is one of the promising class of compounds that possess various pharmacological activity including antibacterial properties.

Objective: The rise of microbial resistant drugs has triggered an alarming response among researchers to developed new drugs with effective biological properties. The position of substituents and active pharmacophores play an important role in designing a lead compound with effective biological properties.

Methods: In this study, two series of 1,3-*bis*-thiourea derivatives (3a-l) and 1,4 *bis*-thiourea derivatives (4a-l) were synthesised from the reaction of isophthaloyl diisothiocyanate intermediates with halogenated amines.

Results: The comparative studies of antibacterial properties of 3a-l and 4a-l against the growth of *E. coli* showed the minimum inhibition of 3g (7mm inhibition) and 4g (18mm inhibition) compared to standard drug ampicillin (16mm inhibition). Molecular docking evaluation against *E. coli* DNA gyrase B showed strong binding interaction of 4g with binding affinity -6.7 kcal/mol and more hydrogen bond compared to 3g with binding affinity -6.4 kcal/mol).

Conclusion: The study is significant in drug design in particular for the development of potential drugs with antimicrobial resistant properties.

Key Words: Anilines, Carbamothioyl, *Escherichia coliform*, Halogens

INTRODUCTION

Discovering new drugs has become a major concern nowadays due to the egression of pathogens with drug-resistant properties. The emergence of new drugs with improved antimicrobial properties has become a phenomenal alternative to overcome the issue. Drugs with active pharmacophores able to form strong interaction with the pathogen receptors and retard the growth of the microorganism¹. Interest has grown rapidly in the preparation and studies of the critical effects of compounds containing an O-N-S set of atoms that have an energetic effect against different types of microbes and viruses.¹⁻³

Thiourea is one of the promising class of compounds that possess good antibacterial, antifungal, anticancer, and other

useful pharmaceutical properties.³⁻⁵ The presence of N-H and C=S groups in the thiourea moieties act as active binding sites between the compounds with the protein receptor on the surface of microorganism.¹ The number of thiourea moieties in a molecular network plays a significant role in antibacterial properties. The ratio of binding sites in a compound with the surface of microorganism receptors increases as the number of thiourea moieties increases.^{6,7} We have reported on series of mono-substituted thiourea⁴, di-substituted thiourea derivatives bearing halogenated aryl groups (4a-l)⁵, bearing amino acids⁸ and bearing alkyl chains^{8,9} with excellent antibacterial activities against *Escherichia coliform* (*E. coli*). The presence of two or more substituted thiourea moieties in the molecular network is believed to increase the biological

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binding interaction with the target receptors thus enhancing the biological properties.^{6,7}

The type of substituents incorporated in the molecular frame of the thiourea derivative is yet another factor affecting the biological activities. The presence of halogens such as F, Cl and Br was reported to enhance the antibacterial activities of thiourea derivatives.^{10,11} Different position of halogens attached to the aromatic groups could further attenuate the bacterial inhibition. Zullkiplee et al.⁷ reported on the excellent antibacterial properties for thiourea derivative featuring halogen at the *ortho*-position compared to *meta* and *para* halogenated position.

Herein, we report on the synthesis of N1, N3-*bis*((halophenyl)carbamothioyl)benzene-1,3-bicarboxamide derivatives (3a-l) and N1, N3-*bis*((halophenyl)carbamothioyl)benzene-1,4-bicarboxamide derivatives (4a-l) from the reaction of diacetylbenzoyl isothiocyanato intermediate with two equivalents of halo-substituted anilines at the *ortho*, *meta* and *para* position. The 1,3 bis-thiourea derivatives (3a-l) were evaluated for their antibacterial activities against *E. coli* in comparison to 1,4 bis-thiourea derivatives (4a-l). Structure-activity relationship of 1,3- and 1,4-bis-thiourea was studied via molecular docking interaction analysis against *E. coli* DNA replication protein of gyrase B.

MATERIALS AND METHODS

Materials: Isophthaloyl dichloride and potassium thiocyanate were purchased from Acros Organics and used without purification. Halogenated amines (fluoro, chloro, bromo and iodo) were purchased from Merck and used without purification. Acetonitrile was dried over calcium hydrate. Dimethyl sulfoxide (DMSO) was used without further purification. Other solvents such as ethyl acetate and hexane were used as received.

Instrument: The synthesized compounds (3a-l and 4a-l) were elucidated using Perkin Elmer Spectra GX Fourier Transform Spectrometer (FTIR). ¹H NMR and ¹³C NMR spectra were recorded using JOEL ECA500 spectrometer at 500 MHz (¹H) and 125MHz (¹³C) respectively. CHNS microanalyses were performed with a CHNS analyser (FLASHEA 1112). The spectroscopy characterization of compounds (4a-l) has corresponded to the reported literature.⁵

General Procedure for the synthesis of 3a-l

Isophthaloyl dichloride 1a (0.10 g, 0.5 mmol) in 10 mL of dry acetonitrile was added into a stirring suspension of potassium thiocyanate, KSCN (0.10 g, 1.0 mmol) in 10 mL of dry acetonitrile. The mixture was stirred for 1 hour at room temperature to form intermediate 2a. The precipitate of potassium chloride, KCl was formed and filtered. The filtrate was

added into halogenated aniline (1.0 mmol) in dry acetonitrile and left stirred for 16 hours at room temperature. Completion of the reaction was determined via TLC analysis with eluent ethyl acetate: hexane (1:3). The mixture was cooled to room temperature and filtered. The filtrate was evaporated in vacuum to form a yellowish powder. The crude was recrystallized in ethyl acetate. The general procedure for the preparation of 3a-l, utilized different type of amines (g, mmol) and yields are as given below.

N1, N3-bis((2-fluorophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3a)

2-Fluoroaniline (0.13 g, 1.0 mmol). (39.1% yield) as a yellowish powder, m.p: 200-201 °C; $\nu_{\max}$ (KBr/cm⁻¹) 3017 (NH), 1667 (C=O), 1166 (C-N), 1522 (Ar-C), 1258 (C=S), 1106 (C-F). δ_{H} (500 MHz, DMSO-d₆) 8.63 (1H, s, Ar-H), 8.21 (2H, d, *J*= 6.10 Hz, Ar-H), 8.00 (2H, t, *J*= 15.30 Hz, Ar-H), 7.74 (1H, t, *J*= 15.30 Hz, Ar-H), 7.38 (4H, d, *J*= 3.85 Hz, Ar-H), 7.28 (2H, t, *J*= 14.55 Hz, Ar-H), 12.48 (2H, s, 2xNH), 11.81 (2H, s, 2xNH). δ_{C} (125 MHz, DMSO-d₆) 117 (Ar-C), 125 (Ar-C), 125 (Ar-C), 128 (Ar-C), 130 (Ar-C), 134 (Ar-C), 168 (C=O), 181 (C=S). Calculated for C₂₂H₁₆N₄O₂S₂F₂: C, 56.17; H, 3.40; N, 11.91; S, 13.62%, found C, 56.12; H, 3.41; N, 11.71; S, 13.53%.

N1, N3-bis((3-fluorophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3b)

3-Fluoroaniline (0.13 g, 1.0 mmol). (47.8% yield) as yellowish powder, m.p: 196-198 °C; $\nu_{\max}$ (KBr/cm⁻¹) 3028 (NH), 1677 (C=O), 1159 (C-N), 1520 (Ar-C), 1221 (C=S), 1117 (C-F). δ_{H} (500 MHz, DMSO-d₆) 8.57 (1H, s, Ar-H), 8.20 (2H, d, *J*= 7.65 Hz, Ar-H), 7.76 (3H, t, *J*= 13.75 Hz, Ar-H), 7.48 (4H, d, *J*= 4.55 Hz, Ar-H), 7.15 (2H, s, Ar-H), 12.59 (2H, s, 2xNH), 11.66 (2H, s, 2xNH). δ_{C} (125 MHz, DMSO-d₆) 112 (Ar-C), 113 (Ar-C), 121 (Ar-C), 129 (Ar-C), 132 (Ar-C), 133 (Ar-C), 140 (Ar-C), 168 (C=O), 180 (C=S). Calculated for C₂₂H₁₆N₄O₂S₂F₂: C, 56.17; H, 3.40; N, 11.91; S, 13.62%, found C, 56.15; H, 3.41; N, 11.72; S, 13.58%.

N1, N3-bis((4-fluorophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3c)

4-Fluoroaniline (0.13 g, 1.0 mmol). (47.8 % yield) as a yellow crystal, m.p: 195-197 °C; $\nu_{\max}$ (KBr/cm⁻¹) 3040 (NH), 1684 (C=O), 1139 (C-N), 1506 (Ar-C), 1254 (C=S), 1102 (C-F). δ_{H} (500 MHz, DMSO-d₆) 8.57 (1H, s, Ar-H), 8.20 (2H, d, *J*= 8.40 Hz, Ar-H), 7.70 (5H, m, Ar-H), 7.28 (4H, t, *J*= 17.55 Hz, Ar-H), 12.42 (2H, s, 2xNH), 11.62 (2H, s, 2xNH). δ_{C} (125 MHz, DMSO-d₆) 115 (Ar-C), 127 (Ar-C), 128 (Ar-C), 129 (Ar-C), 132 (Ar-C), 133 (Ar-C), 134 (Ar-C), 167 (C=O), 180 (C=S). Calculated for C₂₂H₁₆N₄O₂S₂F₂: C, 56.17; H, 3.40; N, 11.91; S, 13.62%, found C, 56.08; H, 3.33; N, 11.72; S, 13.60%.

N1, N3-bis((2-chlorophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3d)

2-Chloroaniline (0.15 g, 1.0 mmol). (60% yield) as a yellow powder, m.p: 209-211 °C; $\nu_{\max}$ (KBr/cm-1) 3052 (NH), 1675 (C=O), 1186 (C-N), 1514 (Ar-C), 1245 (C=S), 752 (C-Cl), δ_H (500 MHz, DMSO-d₆) 8.63 (1H, s, Ar-H), 8.21 (2H, d, J = 7.65 Hz, Ar-H), 8.00 (2H, d, J = 7.65 Hz, Ar-H), 7.74 (1H, t, J = 16.05 Hz, Ar-H), 7.60 (2H, d, J = 7.60 Hz, Ar-H), 7.44 (4H, t, J = 15.30 Hz, Ar-H), 12.59 (2H, s, 2xNH), 11.85 (2H, s, 2xNH). δ_c (125 MHz, DMSO-d₆) 128 (Ar-C), 129 (Ar-C), 130 (Ar-C), 132 (Ar-C), 136 (Ar-C), 168 (C=O), 181 (C=S). Calculated for C₂₂H₁₆N₄O₂S₂Cl₂: C, 52.49; H, 3.18; N, 11.13; S, 12.72%, found C, 52.52; H, 3.17; N, 11.08; S, 12.73%.

N1, N3-bis((3-chlorophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3e)

3-Chloroaniline (0.15 g, 1.0 mmol). (52 % yield) as a yellow powder, m.p: 208-210 °C; $\nu_{\max}$ (KBr/cm-1) 3014 (NH), 1674 (C=O), 1180 (C-N), 1518 (Ar-C), 1244 (C=S), 707 (C-Cl). δ_H (500 MHz, DMSO-d₆) 8.57 (1H, s, Ar-H), 8.20 (2H, d, J = 8.40 Hz, Ar-H), 7.94 (2H, s, Ar-H), 7.74 (1H, t, J = 15.30 Hz, Ar-H), 7.58 (2H, d, J = 8.40 Hz, Ar-H), 7.47 (2H, t, J = 16.05 Hz, Ar-H), 7.36 (2H, d, J = 7.60 Hz, Ar-H) 12.54 (2H, s, 2xNH), 11.68 (2H, s, 2xNH). δ_c (125 MHz, DMSO-d₆) 123 (Ar-C), 123 (Ar-C), 126 (Ar-C), 132 (Ar-C), 134 (Ar-C), 140 (Ar-C), 168 (C=O), 180 (C=S). Calculated for C₂₂H₁₆N₄O₂S₂Cl₂: C, 52.49; H, 3.18; N, 11.13; S, 12.72%, found C, 52.50; H, 3.11; N, 11.09; S, 12.63%.

N1, N3-bis((4-chlorophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3f)

4-Chloroaniline (0.15 g, 1.0 mmol). (68.0 % yield) as yellowish powder, m.p: 208-210 °C; $\nu_{\max}$ (KBr/cm-1) 3040 (NH), 1670 (C=O), 1188 (C-N), 1529 (Ar-C), 1260 (C=S), 718 (C-Cl). δ_H (500 MHz, DMSO-d₆) 8.56 (1H, s, Ar-H), 8.19 (2H, t, J = 7.55 Hz, Ar-H), 7.73 (5H, d, J = 8.40 Hz, Ar-H), 7.49 (4H, d, J = 8.45 Hz, Ar-H), 12.49 (2H, s, 2xNH), 11.64 (2H, s, 2xNH). δ_c (125 MHz, DMSO-d₆) 127 (Ar-C), 127 (Ar-C), 129 (Ar-C), 130 (Ar-C), 133 (Ar-C), 168 (C=O), 180 (C=S). Calculated for C₂₂H₁₆N₄O₂S₂Cl₂: C, 52.49; H, 3.18; N, 11.13; S, 12.72%, found C, 52.40; H, 3.14; N, 11.16; S, 12.63%.

N1, N3-bis((2-bromophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3g)

2-Bromoaniline (0.17 g, 1.0 mmol). (77.7 % yield) as a yellow powder, m.p: 214-215 °C; $\nu_{\max}$ (KBr/cm-1) 3043 (NH), 1533 (Ar-C), 1251 (C=S), 1193 (C-N), 1659 (C=O), 687 (C-Br). δ_H (500 MHz, DMSO-d₆) 8.70 (1H, s, Ar-H), 8.23 (2H, d, J = 7.65 Hz, Ar-H), 7.86 (3H, d, J = 9.95 Hz, Ar-H), 7.77 (2H, d, J = 7.05 Hz, Ar-H), 7.75 (2H, d, J = 7.65 Hz, Ar-H), 7.47 (2H, t, J = 23.3 Hz, Ar-H), 7.28 (2H, t, J = 6.65 Hz, Ar-H), 11.87 (2H, s, 2x NH), 12.51 (2H, s, 2x NH). δ_c (125 MHz, DMSO-d₆) 137 (Ar-H), 134 (Ar-H), 133 (Ar-H),

132 (Ar-H), 129 (Ar-H), 129 (Ar-H), 128 (Ar-H), 120 (Ar-H), 168 (C=O), 180 (C=S). Calculated for C₂₂H₁₆N₄O₂S₂Br₂: C, 44.59; H, 2.70; N, 9.46; S, 10.81%, found C, 44.50; H, 2.70; N, 9.40; S, 10.70%.

N1, N3-bis((3-bromophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3h)

3-Bromoaniline (0.17 g, 1.0 mmol). (57.6 % yield) as a yellow powder, m.p: 213-214 °C; $\nu_{\max}$ (KBr/cm-1) 3033 (NH), 1529 (Ar-C), 1262 (C=S), 1166 (C-N), 1659 (C=O), 677 (C-Br). δ_H (500 MHz, DMSO-d₆) 8.18 (2H, d, J = 6.56 Hz, Ar-H), 8.03 (3H, m, Ar-H), 7.74 (2H, t, J = 8.9 Hz, Ar-H), 7.60 (1H, t, J = 9.85 Hz, Ar-H), 7.39 (3H, t, J = 7.6 Hz, Ar-H), 12.49 (2H, s, 2x NH), 11.64 (2H, s, 2x NH). δ_c (125 MHz, DMSO-d₆) 144 (Ar-H), 137 (Ar-H), 136 (Ar-H), 131 (Ar-H), 126 (Ar-H), 172 (C=O), 184 (C=S). Calculated for C₂₂H₁₆N₄O₂S₂Br₂: C, 44.59; H, 2.70; N, 9.46; S, 10.81%, found C, 44.55; H, 2.66; N, 9.46; S, 10.72%.

N1, N3-bis((4-bromophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3i)

4-Bromoaniline (0.17 g, 1.0 mmol). (67.6 % yield) as a yellow crystal, m.p: 219-221 °C; $\nu_{\max}$ (KBr/cm-1) 3040 (NH), 1535 (Ar-C), 1253 (C=S), 1173 (C-N), 1660 (C=O), 691 (C-Br). δ_H (500 MHz, DMSO-d₆) 8.53 (1H, s, Ar-H), 8.17 (2H, d, J = 7.65 Hz, Ar-H), 7.72 (9H, m, Ar-H), 12.45 (2H, s, 2x NH), 11.60 (2H, s, 2x NH). δ_c (125 MHz, DMSO-d₆) 137.4 (Ar-H), 132 (Ar-H), 118 (Ar-H), 166 (C=O), 179 (C=S). Calculated for C₂₂H₁₆N₄O₂S₂Br₂: C, 44.59; H, 2.70; N, 9.46; S, 10.81%, found C, 44.44; H, 2.68; N, 9.35; S, 10.82%.

N1, N3-bis((2-iodophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3j)

2-Iodoaniline (0.22 g, 1.0 mmol). (61.8 % yield) as a yellow crystal, m.p: 232-235 °C; $\nu_{\max}$ (KBr/cm-1) 3054 (NH), 1526 (Ar-C), 1236 (C=S), 1150 (C-N), 1682 (C=O), 627 (C-I). δ_H (500 MHz, DMSO-d₆) 8.53 (1H, s, Ar-H), 8.17 (4H, t, J = 19.1 Hz, Ar-H), 7.72 (5H, t, J = 16.05 Hz, Ar-H), 7.22 (2H, t, J = 16.05 Hz, Ar-H), 12.45 (2H, s, 2x NH), 11.61 (2H, s, 2x NH). δ_c (125 MHz, DMSO-d₆) 138 (Ar-C), 135 (Ar-C), 133 (Ar-C), 131 (Ar-C), 129 (Ar-C), 124 (Ar-C), 94 (C-I), 167 (C=O), 179 (C=S). Calculated for C₂₂H₁₆N₄O₂S₂I₂: C, 38.48; H, 2.33; N, 8.16; S, 9.33%, found C, 38.49; H, 2.31; N, 8.11; S, 9.23%.

N1, N3-bis((3-iodophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3k)

3-iodoaniline (0.22 g, 1.0 mmol). (64.7 % yield) as a yellow crystal, m.p: 231-232 °C; $\nu_{\max}$ (KBr/cm-1) 3180 (NH), 1516 (Ar-C), 1240 (C=S), 1172 (C-N), 1677 (C=O), 605 (C-I). δ_H (500 MHz, DMSO-d₆) 8.51 (1H, s, Ar-H), 8.16 (2H, d, J = 2.0 Hz, Ar-H), 7.76 (2H, d, J = 5.7 Hz, Ar-H), 7.71 (1H, s, Ar-H), 7.70 (1H, s, Ar-H), 7.68 (1H, s, Ar-H), 7.50 (4H, d, J = 7.65 Hz, Ar-H), 12.45 (2H, s, 2xNH), 11.58 (2H, s, 2xNH). δ_c

(125MHz, DMSO- d_6) 140 (Ar-C), 139 (Ar-C), 134 (Ar-C), 132 (Ar-C), 129 (Ar-C), 129 (Ar-C), 129 (Ar-C), 98 (C-I), 167 (C=O), 180.9 (C=S). Calculated for $C_{22}H_{16}N_4O_2S_2I_2$: C, 38.48; H, 2.33; N, 8.16; S, 9.33%, found C, 38.49; H, 2.31; N, 8.10; S, 9.29%.

N1, N3-bis((4-iodophenyl)carbamothioyl)benzene-1,3-tricarboxamide (3l)

4-Iodoaniline (0.22 g, 1.0 mmol). (61.8 % yield) as a yellow crystal, m.p: 230-232°C; ν_{max} (KBr/ cm^{-1}) 3043 (NH), 1533 (Ar-C), 1251 (C=S), 1153 (C-N), 1659 (C=O), 611 (C-I). δ_H (500 MHz, DMSO- d_6) 8.51 (1H, s, Ar-H), 8.16 (2H, d, J = 7.65 Hz, Ar-H), 7.76 (5H, t, J = 16.05 Hz, Ar-H), 7.50 (4H, d, J = 8.4 Hz, Ar-H), 12.45 (2H, s, 2x NH), 11.58 (2H, s, 2x NH). δ_C (125MHz, DMSO- d_6) 137.9 (Ar-H), 134 (Ar-H), 132 (Ar-H), 92 (C-I), 168 (C=S), 180 (C=O). Calculated for $C_{22}H_{16}N_4O_2S_2I_2$: C, 38.48; H, 2.33; N, 8.16; S, 9.33%, found C, 38.41; H, 2.34; N, 8.10; S, 9.23%.

Antibacterial Screening

Turbidimetric kinetic method

The antibacterial activity of 3a-l against *E. coli* ATCC 8739 was analysed by turbidimetric kinetic method. The inoculums have been prepared by letting of *E. coli* to grow on nutrient-containing media Mueller-Hinton broth at 37°C with permanent 18 h stirring at 200 rpm. The compounds were tested with a concentration increase of 50 ppm, 80 ppm and 100 ppm. Transmittances (T) were obtained in spectrophotometers with UV-visibility A 100 mL of culture broth was deposited with compound concentrations of 50, 80 and 100 ppm, inoculated with 0.99 mL of inoculum, and stirred with 200 rpm at 37°C. Negative control (DMSO) was applied to inoculums. For 6 h, aliquots were extracted per 1 h interval and transmittances (T) reported at 560 nm wavelength in a UV-Visible spectrophotometer. T values gained were extrapolated to cfu/mL (colony forming units/mL).

Kirby-Bauer disc diffusion method

The synthesized compounds were screened for their antimicrobial activity via disc diffusion method against *E. coli* and *S. aureus*. Ampicillin and DMSO were used as positive and negative control respectively. All bacteria were grown in a nutrient broth and incubated for 24 h at 37°C. The inoculum was spread on the nutrient agar surface stored in sterilized Petri dishes. Each of the sterilized disks (6 mm diameter) was then impregnated with the test samples (10 μ L/disk) and positioned on the agar media surface. All the agar plates were incubated for 24 h at 37°C. The inhibition zone was measured to assess the antimicrobial activity of the tested sample and compared with Ampicillin.

Molecular Docking Study

The molecular docking study was done using Autodock Vina. 24 kDa Crystal Domain structure of *E. coli* DNA gyrase B

in a small molecule inhibitor complex (PDB entry: 4DUH) was obtained from Protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>). Grid size (x, y, z) was set to 25 x 25 x 25 and grid centre (x, y, z) was set to 33.5, 6.22, 7.65 for the active site pocket.

RESULTS AND DISCUSSION

Figure 1 shows MIC for several 1,3 bis-thiourea derivatives against *E. coli*. Table 1 depicts the inhibition zones of 3a-l in comparison with 4a-l.⁵ Table 2 shows the molecular docking affinity of 3g and 4g with the targeted receptor via Autodock vina docking software. The binding visualizations of 3g and 4g are shown in Table 3.

Synthesis

Twelve new compounds 3a-l have been successfully synthesised from the reaction of intermediates isophthaloyl diisothiocyanate 2a with subsequent amines bearing *ortho*, *meta* and *para* halogen substituents to afford yield of 39-77 %. The preparations of 3a-l and 4a-l are shown in Scheme 1. The structural elucidation of 4a-l corresponded to the previously reported literature with yields.⁵

The IR spectra illustrated the successful formation of 3a-l by the disappearance of ν (NCS) at 2500-2000 cm^{-1} and appearance of ν (NH) at 3180-3014 cm^{-1} .¹² All other important functional groups were also observed in the IR spectra. The peaks at 1684-1658 cm^{-1} were attributed to ν (C=O).¹³ The aromatic group was expressed at 1535-1516 cm^{-1} while peaks at 1262-1221 cm^{-1} were represented of ν (C=S). The significant absorbance at 1193-1139 cm^{-1} was attributed to ν (C-N).⁷⁻⁸ The peaks attributed for halogens were observed at region 1117-605 cm^{-1} with the fluoro group.

The chemical structures of 3a-l were further confirmed by 1H and ^{13}C NMR spectroscopy. The existence of thiourea moieties in compounds 3a-l was confirmed by the appearance of two NH resonances at δ 12.59-11.61 ppm and the integral of these signals proved that they consist of two protons which vital to show that 3a-l were bis-substituted thiourea compounds. CSNH signal was observed at δ 11.87-11.61 ppm while CONH signal was observed at higher frequency resonances at δ 12.59-12.42 ppm.¹¹ The ^{13}C NMR spectrum proved the formation of thiourea group by the presence of thiol (C=S) resonances at δ 184-179 ppm. The C=O signals were resonated at δ 172-166 ppm. The multiplet peaks at δ 134-129 ppm were attributed as aromatic carbons in 3a-l.

Antibacterial Activities

The interaction of active NCS groups was evaluated via a turbidimetric kinetic method against *E. coli*. The MIC values of 3a-l were determined by extrapolating the concentration at the zero-growth rate of *E. coli* ($\mu=0$).¹⁴ Figure 1 shows MIC for several synthesized compounds in a range of 200

to 400 ppm. Compound 3g showed the lowest MIC at 228 ppm. Compounds with MIC values less than 200 ppm are suitable for pharmacological purposes as active antibacterial agents.¹⁵

The antibacterial activities of 3a-l were further evaluated *via* Kirby-Bauer disc diffusion method following the standard procedure of the Clinical and Laboratories Standards Institute (CLSI) (CLSI, 2015) for a comparative study with the previously reported 4a-l. Ampicillin was used as a positive control and DMSO as a negative control. The diameter of the inhibition zone of 3a-l was recorded after incubation. Table 1 shows the inhibition zones of 1,3 *bis*-thiourea derivatives 3a-l in comparison with the previously reported of 1,4 *bis*-thiourea derivatives 4a-l.⁷

The presence of multi thiourea moieties with more reactive binding sites of C=S, N-H and C=O able to enhance the biological activities^{6,7} *via* formation of more binding interactions.¹⁶ The presence of halogens is also another factor in enhancing the biopotency, bioavailability and lipophilicity of the compounds.¹⁷ Based on Table 1, the position of substituted thiourea play a significant role to form good binding interaction with the target receptor. In this study, 1,4 *bis*-thiourea derivatives 4a-l exhibited better antibacterial performance against *E. coli* compared to 1,3 *bis*-thiourea derivatives 3a-l. Lack of antibacterial activities of 3a-l was been due to the steric hindrance which caused less binding interaction with receptors as compared to 4a-l.^{8,16} The bulky aromatic groups could also hinder the binding interaction between the active site of the compounds and the bacteria receptor.¹⁸ Interestingly, both 3g and 4g bearing bromine atoms substituted at *ortho* position showed the highest inhibition zone compared to other bis-thiourea molecules in their series. This could be due to the strategic position of *ortho*-substituted halogen that is favourable for a binding process with bacterial cell receptors.¹⁹

Molecular Docking Study

The previous reported of 1,4 *bis*-thiourea derivative 4g showed excellent antibacterial activity against *E. coli*.⁵ Only 1,3 *bis*-thiourea derivative 3g, in the series that showed susceptible inhibition against *E. coli*. To understand the structure-activity relationship of this two *bis*-thiourea, molecular docking study was applied to compare the binding affinity of 3g with 4g against *E. coli* DNA gyrase B. The DNA gyrase is an important enzyme for the formation of nucleic acids and bacterial growth.²⁰ Binding energy measures the strength of the interaction between a ligand to its biological target receptor. The lower binding free energy could form stronger and stable binding interaction²¹ with the target receptor. Table 2 shows the molecular docking affinity of the selected *bis*-thiourea derivatives with the targeted receptor *via* Auto-dock vina docking software.

The molecular docking interaction of 1,4 *bis*-thiourea derivative 4g produced strong binding energy of -6.7 kcal/mol compared to 1,3 *bis*-thiourea derivative 3g with -6.4 kcal/mol. The binding visualization of each ligand is analyzed and shown in Table 3. A hydrogen bond is an important specific interaction in biological processes between NH and C=O group, (-OH) with C=O and C-O groups, and hydrogen bonds to aromatic heterocycles.²²⁻²³ These binding interactions provide specificity and stabilisation between the ligands and the active site of enzymes.⁴ The docking simulation only showed one hydrogen bond interaction in 1,3 *bis*-thiourea derivative 3g (Green line) with GLY101 residues *via* C=O in thiourea with the active site of the receptor. The aromatic group in 3g has also formed electrostatic interaction (orange line) with HIS83 and hydrophobic interaction (purple line) with GLY102, ALA100, VAL93, ALA90, ILE94 and PRO79.

On the other hand, 1,4 *bis*-thiourea derivative 4g formed a total of six hydrogen bond interactions (in green line). The C=S group formed three hydrogen bonds interaction with the pocket of HIS99, GLY117 and ASP49.²³ Other H-bonds were formed between NH with LYS103, C=O with ASN46 and aromatic group with VAL120. The electrostatic interaction (orange line) has also formed in 4g *via* NH with GLU42 and ASP49. The aromatic of 4g formed hydrophobic interaction with VAL120 and ILE48, while sulphur interaction was also observed between the C=S group with HIS99. Large numbers of binding interactions between ligand 4g with the pocket of the receptor have supported the relationship of the molecular structure towards excellent biological activity against *E. coli*.

CONCLUSION

A series of 1,3- and 1,4-bis thiourea derivatives 3a-l and 4a-l were successfully synthesised and evaluated against Gram-negative *E. coli*. Bromine at the *ortho*-position of 1,3- and 1,4-bis thiourea demonstrated susceptible inhibition against *E. coli*. The structural activity relationship of 3g and 4g was comparatively evaluated *via* molecular docking studies against *E. coli* DNA replication protein that supporting the differences in antibacterial performance of both compounds. The study could be beneficial in the drugs design process for new drugs with potential antimicrobial-resistant in fewer times and highly economical.

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Authors Contribution:

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Experimental works and data analysis were done by Noratika Ramli, Nur Izma Hani, Ferlicia Rasin, Wan Sharifatun Handayani Wan Zullkiplee, Nur Arif Mortadza and Ainaa Nadiah Abd Halim.

REFERENCES

- Zhong Z, Xing R, Liu S, Wang L, Cai S, Li P. Synthesis of acyl thiourea derivatives of chitosan and their antimicrobial activities in vitro. *Carbohydr Res* 2008;343(3):566-570.
- Alfallous KA, Aburzeza MM. Synthesis, Characterization, Biological Activity and Use of Thiourea Derivative (PhTTU) Studies of dinitrate-1-phenyl-3-(thiazol-2-yl)-2-thiourea to Cd (II) ion Complex. *Int J Sci Res* 2015;350-352.
- Imran S, Taha M, Ismail NH, Khan KM, Naz F, Hussain M, Tauseef S. Synthesis of novel bisindolylmethane Schiff bases and their antibacterial activity. *Molecules* 2014;19(8):11722-11740.
- Nordin NA, Chai TW, Tan BL, Choi CL, Abd Halim AN, Hussain H, et al. Novel synthetic monothiourea aspirin derivatives bearing alkylated amines as potential antimicrobial agents. *J Chem* 2017;2017:1-7.
- Abd Halim AN, Ngaini Z. Synthesis and characterization of halogenated bis (acylthiourea) derivatives and their antibacterial activities. *Phosp Sulf Silicon Relat Elem* 2017;192(9):1012-1017.
- Ngaini Z, Wan Zulkiplee WS, Abd Halim AN. One-pot multi-component synthesis of thiourea derivatives in cyclotriphosphazenes moieties. *J Chem* 2017;2017.
- Wan Zullkiplee WS, Mohd Ariff MA, Hussain H, Khairul WM, Ngaini Z. Bacteriostatic activities of N-substituted tris-thioureas bearing amino acid and aniline substituents. *Phosp Sulf Silicon Relat Elem* 2016;191(10):1329-1333.
- Zullkiplee WS, Abd Halim AN, Ngaini Z, Mohd Ariff MA, Hussain H. Bis-thiourea bearing aryl and amino acids side chains and their antibacterial activities. *Phosp Sulf Silicon Relat Elem* 2014;189(6):832-838.
- Halim AN, Ngaini Z. Synthesis and bacteriostatic activities of bis (thiourea) derivatives with variable chain length. *J Chem* 2016;2016:1-7.
- Abbas SY, El-Sharief MA, Basyouni WM, Fakhr IM, El-Gammal EW. Thiourea derivatives incorporating a hippuric acid moiety: Synthesis and evaluation of antibacterial and antifungal activities. *Eur J Med Chem* 2013;64:111-120.
- Kaswala PB, Chikhalia KH, Shah NK, Patel DP, Patel DH, Mudaliar GV. Design, synthesis and antimicrobial evaluation of s-triazinyl urea and thiourea derivatives. *Arkivoc* 2009;11(9):326-335.
- Ke SY, Xue SJ. Synthesis and herbicidal activity of N-(o-fluorophenoxyacetyl) thioureas derivatives and related fused heterocyclic compounds. *Arkivoc* 2006;10:63-68.
- Ragamathunnisa M, Jasmine VRE, Padmavathy R, Radha N. Ultrasonic and spectroscopic investigation of thiourea in non-aqueous media. *Int J Cur Res Rev* 2012;4(23):30-41.
- Alvarez MD, Zarelli VE, Pappano NB, Debattista NB. Bacteriostatic action of synthetic polyhydroxylated chalcones against *Escherichia coli*. *Biocell* 2004;28(1):31.
- Ngaini Z, Wan Zulkiplee WS, Abd Halim AN. One-pot multi-component synthesis of thiourea derivatives in cyclotriphosphazenes moieties. *J Chem* 2017;2017.
- Pramilla S, Kuldeep S. Synthesis and evaluation of antioxidant activity (In-vitro) of some N-phthalyl-[(3-nitro anilino)-4'' aryl substituted]-1, 3, 4 thiadiazoles. *Int J Cur Res Rev* 2015;7(14):1-6.
- Stefanska J, Nowicka G, Struga M, Szulczyk D, Koziol AE, Augustynowicz-Kopec E, Napiorkowska A, Bielenica A, Filipowski W, Filipowska A, Drzewiecka A. Antimicrobial and antibiofilm activity of thiourea derivatives incorporating a 2-aminothiazole scaffold. *Chem Pharm Bull* 2015;63(3):225-236.
- Rodriguez-Fernandez E, Manzano JL, Benito JJ, Hermosa R, Monte E, Criado JJ. Thiourea, triazole and thiadiazole compounds and their metal complexes as antifungal agents. *J Inorg Biochem* 2005;99(8):1558-1572.
- Li J, Rao X, Shang S, Gao Y, Song J. Synthesis and antibacterial activity of amide derivatives from acrylopimaric acid. *Bio Resources* 2012;7(2):1961-1971.
- Mendoza FHL, Seranno AMT, Aparicio OG, Martinez GG, Suarez COR, Perez RNA, Morales RMS. Synthesis, antimicrobial activity, and molecular docking study of fluorine-substituted indole-based imidazolines. *Med Chem Res* 2018;27(2018):1624-1633.
- Du X, Li Y, Xia YL, Ai SM, Liang J, Sang P, et al. Insights into protein-ligand interactions: mechanisms, models, and methods. *Int J Mol Sci* 2016;17(2):144.
- Bissantz C, Kuhn B, Stahl M. A medicinal chemist's guide to molecular interactions. *J Med Chem* 2010;53(14):5061-5084.
- Firdausiah S, Hasbullah SA, Yamin BM. Synthesis and X-ray crystallographic study of NN'-bis(2-, 3- and 4- methoxybenzamidothiocarbonyl) hydrazines. *Int J Cur Res Rev* 2020;12(24):204-210.

Table 1: Inhibition zone (mm) of 3a-l and 4a-l against *E. coli*

1,3 Bis-Thiourea Derivatives	Inhibition zone (mm)	1,4 Bis-Thiourea Derivatives	Inhibition zone (mm)
3a	-	4a	-
3b	-	4b	-
3c	-	4c	-
3d	-	4d	17
3e	-	4e	10
3f	-	4f	-
3g	7	4g	18
3h	-	4h	8
3i	-	4i	10
3j	-	4j	-
3k	-	4k	-
3l	-	4l	11
Ampicillin	12-16	Ampicillin	16

Table 2: Docking energy results of 3g and 4g

Ligand	Binding affinity (kcal/mol)	H-bond	Electrostatic	Hydrophobic	Sulphur
3g	-6.4	1	1	5	-
4g	-6.7	6	2	2	1

Table 3: Binding interaction visualization

Bis-Thiourea Compound	Docking Image
<p style="text-align: center;">3g</p>

Scheme 1: Synthetic pathway of compounds of 3a-l and 4a-l

Figure 1: MIC of 1,3 *bis*-thiourea derivatives against *E. coli*.