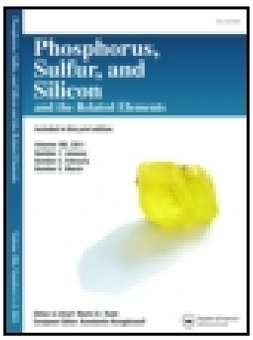

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Synthesis and characterisation of halogenated bis(acylthiourea) derivatives and its antibacterial activities

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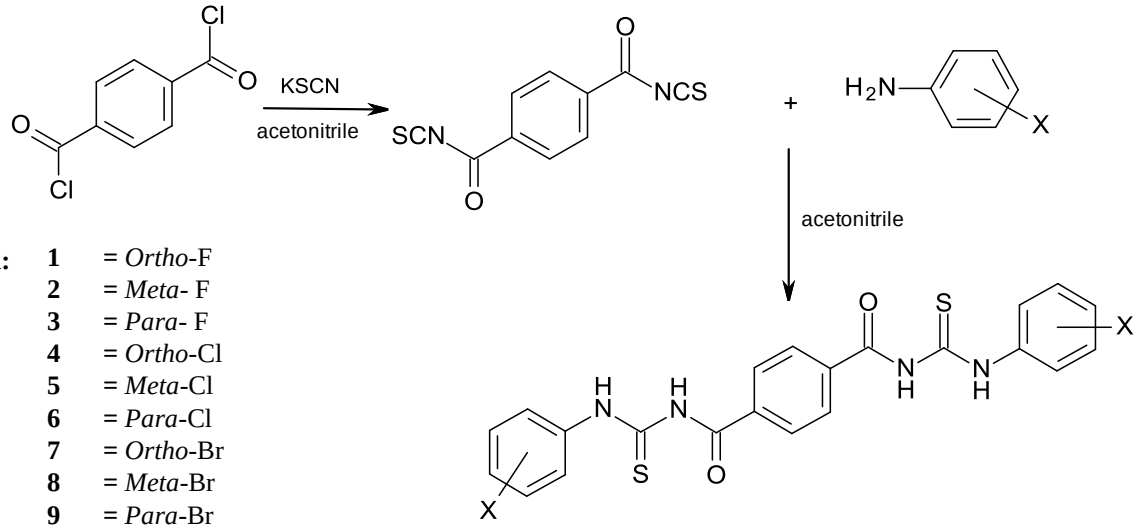
ABSTRACT

A total of 12 bis(acylthiourea) derivatives with different pharmacophores have been synthesized *via* nucleophilic substitution reaction of benzene-1,4-dicarbonyl isothiocyanate intermediate with aromatic amine bearing halogens at the *ortho*, *meta* and *para* positions. The structures of the synthesised compounds were confirmed by CHN elemental analysis, FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopies. Antibacterial studies of the compounds *via* the Kirby Bauer disc diffusion method against *Escherichia coli* (*E.coli*) ATCC 25922 and *Staphylococcus aureus* (*S. aureus*) S48/81 demonstrated that bis(acylthiourea) N^1,N^4-bis[(2-chlorophenyl)carbamothioyl]terephthalamide **(4)** and N^1,N^4-bis[(2-bromophenyl)carbamothioyl]terephthalamide **(7)** bearing Cl and Br at the *ortho* position exhibited excellent activities against both bacteria strains compared to ampicillin standard.



X:
1 = *Ortho*-F
2 = *Meta*- F
3 = *Para*- F
4 = *Ortho*-Cl
5 = *Meta*-Cl
6 = *Para*-Cl
7 = *Ortho*-Br
8 = *Meta*-Br
9 = *Para*-Br
10 = *Ortho*-I
11 = *Meta*-I
12 = *Para*-I

KEYWORD

bis(acylthiourea), antibacterial activity, aromatic amine, halogen

INTRODUCTION

The emergence of new drugs has become a priority in recent decades due to alarming cases of many infectious diseases caused by antimicrobial resistance.[1,2] Concomitant with this phenomenon, thiourea and its derivatives have been studied by researchers to widen the usages particularly for biological purposes.[2] This is due to the ability of C=S and N-H groups in thiourea moieties that are easily protonated under acidic conditions and reacts with the carboxyl and phosphate groups of the bacterial surface for antibacterial activities.[3] Thiourea is a diamide of thiocarbonic acid that resembles urea, with C=S instead of C=O.[4] Thiourea consists of reactive functional groups, namely amino, imino and thiol, and is also an excellent precursor in the synthesis of organic molecules for various biological applications.[5] Several biological activities reported for thiourea derivatives are antioxidant,[6] anti-HIV,[7] antituberculosis agents,[8] anticancer,[9] antibacterial,[10-13] antifungal and antiparasitic activities.[14]

In a previous study, we have reported on a series of symmetrical bis(thiourea) derivatives for their antibacterial activity.[15] The symmetrical position of 1,4-bis(thiourea) showed better activity compared to 1,2-bis(thiourea)[16] and 1,3-bis(thiourea)[15,17] due to less steric hindrances, thus increased the contact between the active site of compound and the bacteria receptor.[18] 1,4-Bis(thiourea) bearing an aryl side chain showed good antibacterial activity against *E.coli* ATCC 8739 due to the lipophilicity of the aromatic group which correlates well with the bioactivity of the compounds.[15,19-20]

A halogen is yet another substituent that is commonly introduced onto thiourea moieties, which may lead to an improvement in the biological activities.[1,10] Structure-activity relationship

studies indicated that halogens (Cl, Br, F) at the *ortho*, *meta* and *para* positions of aromatic groups attributed to excellent activities against microorganisms.[21] Saeed and co-workers reported that thiourea derivatives bearing fluorine atoms at the *ortho* position in an aromatic group demonstrated excellent activities against different fungal strains *R. oryzae*, *F. oxysporum*, *A. niger* and *A. fumigates*.[22] Similar findings were also reported by Wang *et al.* on the influence of halogens to the biological activities.[23]

Herein, we report on the synthesis of halogenated bis(acylthiourea) derivatives **1-12** from the reaction of benzene-1,4-dicarbonyl isothiocyanate with various aromatic amine bearing halogens at *ortho*, *meta* and *para* positions. Compounds **4**, **5**, **6**, **9** and **12** in the series were earlier reported with no spectroscopic analysis and were evaluated for antifungal activities.[24] In this study, the synthesised compounds **1-12** were screened for their antibacterial activity against gram-negative bacteria *E.Coli* (ATCC 25222) and gram-positive bacteria *S.aureus* (S48/81) in which the effect of halogens at different positions was evaluated.

RESULTS AND DISCUSSION

Synthesis

The bis(acylthiourea) derivatives **1–12** were prepared by introducing intermediate benzene-1,4-dicarbonyl isothiocyanate, which was prepared from benzene-1,4-dioylchloride with potassium thiocyanate in acetonitrile, onto aromatic amine bearing halogens at different positions. The preparation of **1-12** is depicted in Scheme 1.

The IR spectra illustrated the formation of **1-12** by the presence of the expected absorption frequency at 3393-3168 cm^{-1} attributed to ν_{NH}. The disappearance of the NCS absorption band at

2000 cm^{-1} and appearance of an NH absorption band indicates the formation of target compounds.[25] The strong absorption band at 1680-1663 cm^{-1} were attributed to $\nu_{C=O}$. The carbonyl absorption observed was lower than the expected value (1700 cm^{-1}) due to intra-molecular hydrogen bond that causes a slight downshift in the frequency.[26,27] The aromatic ring was represented by the absorption band at 1542-1502 cm^{-1}, while $\nu_{C=S}$ and ν_{C-N} bands were observed at 1280-1248 cm^{-1} and 1170-1140 cm^{-1}, respectively.[13,22]

The formations of **1-12** were supported by ^{1}H and ^{13}C NMR spectroscopy analyses. Findings from ^{1}H NMR demonstrated the formation of thiourea moieties in the compound by the existence of broad signals at δ_H 12.60-12.32 and δ_H 12.07-11.83 attributed to CSNH and CONH, respectively.[28] The CSNH signal was represented at higher chemical shift due to the downfield effect caused by possible hydrogen bonds.[25,28] Multiple peaks observed at δ_H 8.17-7.13 were assigned as aromatic protons. The ^{13}C NMR spectra exhibit thiol C=S and C=O peaks that resonated at δ_C 180.5-179.1 and δ_C 167.9-167.3, respectively. The C=S peak appeared at higher chemical shift than C=O due to its lower excitation energy $n \rightarrow \pi$.[29,30] The resonance of unresolved aromatic carbons was represented by series of signals at δ_C 163.1-97.5.

Antibacterial activities

The antibacterial activities of bis(acylthiourea) derivatives **1-12** were initially performed against gram-negative bacteria (*E.coli*) and gram-positive bacteria (*S.aureus*) *via* Turbidimetric Kinetic method.[31,32] Compounds **1-12**, however, experienced solubility limitation and dissolution of compounds during administration into the culture media that lead to the precipitation of the vehicle–insoluble particles.[33] This phenomenon has hindered a quantitative determination of the

biological activities. The molecular features of the compounds that highly lipophilic was envisaged to reduce the solubility of the compounds in the culture media.[34]

Alternatively, the antibacterial activities of **1-12** were performed employing Kirby Bauer disc diffusion method following standard methods by the Clinical and Laboratories Standards Institute (CLSI).[35] Ampicillin was used as a standard drug, while dimethyl sulfoxide (DMSO) was used as a negative control. The inhibition zones after incubation are shown in Table S 1 (Supplemental Materials). The results were assigned to three categories, susceptible (6-13mm), intermediate (14-16mm), or resistant (>17mm).[35] The relative inhibition of *E. coli* and *S. aureus* were calculated *via* inhibition zone of **1-12** compared to the inhibition zone of ampicillin.

The average diameter of the inhibition zones varied from 8 to 18 mm for both bacteria. *ortho*-Substituted halogens (**4** and **7**) demonstrated higher antibacterial activities than *meta*- (**5** and **8**) and *para*- (**6**, **9** and **12**) substituted halogens. Compounds **4** and **7** bearing Cl and Br at *ortho* positions exhibited better activities with the relative inhibition zone >100% in comparison to ampicillin (standard drug). The higher antibacterial activity of *ortho*-substituted halogen was due to the strategic position that is favourable for binding process to specific sites of bacterial cells by interaction with outer cellular components.[36,37] The halogen atoms stabilised the non-hydrophobic interactions in bacteria through halogen bond interactions with surrounding amino acids, leading to an improvement of bioavailability.[35] The presence of two thiourea moieties offered more reactive moieties of C=S, N-H and C=O that easily protonated under acidic condition and reacted with the bacterial surfaces, therefore further enhance the biological activity.[3]

CONCLUSION

A series of bis(acylthiourea) derivatives **1-12** were successfully synthesised and fully characterised by typical selected spectroscopic methods. The antibacterial studies of the synthesised bis(acylthiourea) derivatives elucidated that *ortho*-substituted halogens **4** and **7** possess excellent antibacterial activity than reference drug against both bacteria *E. coli* and *S. aureus*. This concludes that substituent at *ortho* position gave better activity compared to *meta* and *para* positions in the molecular framework of the synthesised molecules.

EXPERIMENTAL

All chemicals were purchased from Merck kGaa or Acros Company and used as received without any further purification. The solvent acetonitrile was dried and distilled over calcium hydride.

Physical Measurement: Melting points were determined by using Stuart SMP3 melting point apparatus and uncorrected. The elemental CHNS analyses were provided by Universiti Teknologi MARA (UiTM), Shah Alam, Malaysia using Thermo Scientific™ FLASH 2000 CHNS/O Analyzers. Infra-red (IR) spectra (ν/cm^{-1}) were recorded as KBr pellets on Perkin Elmer Thermoscientific Smart Omni Transmission Nicolet IS10 Fourier Transform Infrared Spectrometer (FTIR). ^{1}H NMR and ^{13}C NMR spectra were recorded using JEOL ECA 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C) with the chemical shifts δ_H/δ_C (ppm) reported relative to DMSO-d_6 as standards.

General procedure for the preparation of bis(acylthiourea) derivatives 1-12

A solution of benzene-1,4-dioylchloride (2 mmol) in acetonitrile (15 mL) was added to a suspension of potassium thiocyanate (4 mmol) in acetonitrile (15 mL). The mixture was stirred at room temperature to form KCl as a white precipitate. The mixture was filtered and KCl was removed. A series of aromatic amine bearing halogens (4 mmol) in acetonitrile (15 mL) was slowly added to the filtrate. The resulting mixture was stirred at room temperature until a precipitate formed. The mixture was filtered and the solid crude was purified by recrystallisation from hot ethanol to afford yellow or white solids of the title compounds.

N^1,N^4-bis[(2-fluorophenyl)carbamothioyl]terephthalamide **(1)**

Compound **1** was obtained from *o*-fluoroaniline (0.5 mL, 4 mmol). Yield 0.73 g, 77 % as light yellow solids; m.p: 249-250 ˚C; Anal.Calcd for $C_{22}H_{16}F_2N_4O_2S_2$ (470 g) : C, 56.16; H, 3.43; N, 11.91; S, 13.63%. Found: C, 56.66; H, 3.50; N, 12.09; S, 13.36.); IR (KBr/ cm^{-1}): 3246 (NH), 3138 (CH), 1679 (C=O amide), 1530 (Ar-C), 1253 (C=S), 1165 (C-N). ^{1}H NMR (500 MHz, DMSO-d_6) 7.27 (m, 2H, Ar-H), 7.36 (d, 4H, *J* = 8.4 Hz, Ar-H), 8.03 (t, 2H, *J* = 7.6 Hz, Ar-H), 8.10 (s, 4H, Ar-H), 12.01 (s, 2H, 2xNH), 12.46 (s, 2H, 2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 115.7, 124.3, 126.0, 127.2, 128.4, 135.9, 154.5, 156.4 (Ar-C), 167.8 (C=O), 180.1 (C=S).

N^1,N^4-bis[(3-fluorophenyl)carbamothioyl]terephthalamide **(2)**

Compound **2** was obtained from *m*-fluoroaniline (0.5 mL,4 mmol). Yield 0.59 g, 62 % as light yellow solids; m.p: 247-248 ˚C; Anal.Calcd for $C_{22}H_{16}F_2N_4O_2S_2$ (470 g): C, 56.16; H, 3.43; N, 11.91; S, 13.63%.Found: C, 56.45; H, 3.30; N, 11.61; S, 13.56.; IR (KBr/ cm^{-1}) 3168 (NH), 3032 (CH), 1676 (C=O amide), 1532 (Ar-C), 1280 (C=S), 1154 (C-N). ^{1}H NMR (500 MHz,

DMSO-d_6) 7.13 (t, 2H, *J* = 7.6 Hz Ar-H), 7.46 (d, 2H, *J* = 3.8 Hz , Ar-H), 7.48 (s, 2H, Ar-H), 7.81 (d, 2H, *J* = 8.4 Hz , Ar-H), 8.08 (s, 4H, Ar-H), 11.88 (s, 2H,2xNH), 12.55 (s, 2H, 2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 113.5, 120.8, 129.2, 130.9, 136.4, 140.1, 161.2, 163.1 (Ar-C), 167.9 (C=O), 179.6 (C=S).

***N*^{1},*N*^{4}*-bis[(4-fluorophenyl)carbamothioyl]terephthalamide* (3)**

Compound **3** was obtained from *p*-fluoroaniline (0.5 mL 4 mmol). Yield (0.74 g, 78 %) as white solids; m.p: 247-248 ˚C; Anal.Calcd for $C_{22}H_{16}F_2N_4O_2S_2$ (470 g) : C, 56.16; H, 3.43; N, 11.91; S, 13.63%.Found: C, 56.35; H, 3.50; N, 12.00; S, 13.76.; IR (KBr/ cm^{-1}) 3238 (NH), 3150 (CH), 1678 (C=O Amide), 1502 (Ar-C), 1161 (C-N), 1248 (C=S). ^{1}H NMR (500 MHz, DMSO-d_6) 7.27 (t, 4H, *J* = 8.8 Hz, Ar-H), 7.67 (m, 4H, Ar-H), 8.08 (s, 4H, Ar-H), 11.83 (s, 2H, 2xNH), 12.38 (s, 2H,2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 115.8, 127.5, 129.2, 134.8, 136.5, 159.3 (Ar-C), 167.9 (C=O), 179.9 (C=S).

***N*^{1},*N*^{4}*-bis[(2-chlorophenyl)carbamothioyl]terephthalamide* (4)** [24]

Compound **4** was obtained from *o*-chloroaniline (0.5 mL, 4 mmol). Yield (0.87 g, 87 %) as white solids; m.p: 230-231 ˚C (lit[24] 215 ºC); Anal.Calcd for $C_{22}H_{16}Cl_2N_4O_2S_2$ (502 g) : C, 52.49; H, 3.20; N, 11.13; S, 12.74%.Found: C, 52.53; H, 3.34; N, 10.97; S, 12.83.; IR (KBr/ cm^{-1}) 3247 (NH), 3140 (CH), 1678 (C=O amide), 1525 (Ar-C), 1250 (C=S), 1170 (C-N). ^{1}H NMR (500 MHz, DMSO-d_6) 7.35 (t, 2H, *J* = 9.2 Hz Ar-H), 7.43 (t, 2H, *J* = 7.3 Hz Ar-H), 7.61 (d, 2H, *J* = 7.6 Hz , Ar-H), 8.04 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.11 (s, 4H, Ar-H), 12.05 (s, 2H, 2xNH), 12.60 (s,

2H, 2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 127.3, 128.0, 128.2, 128.3, 128.7, 129.53, 135.3, 135.8 (Ar-C), 167.6 (C=O), 180.0 (C=S).

***N*^{1},*N*^{4}*-bis[(3-chlorophenyl)carbamothioyl]terephthalamide* (5)**[24]

Compound **5** was obtained from *m*-chloroaniline (0.5 mL, 4 mmol). Yield (0.88 g, 87 %) as white solids; m.p: 256-257 ˚C (lit[24] 240 ºC); Anal.Calcd for $C_{22}H_{16}Cl_2N_4O_2S_2$ (502 g): C, 52.49; H, 3.20; N, 11.13; S, 12.74%.Found: C, 52.79; H, 3.34; N, 11.67; S, 12.63.; IR (KBr/ cm^{-1}) 3225 (NH), 3035 (CH), 1680 (C=O amide), 1529 (Ar-C), 1258 (C=S), 1161 (C-N). ^{1}H NMR (500 MHz, DMSO-d_6) 7.35 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.45 (t, 2H, *J* = 8.0 Hz Ar-H), 7.58 (d, 2H, *J* = 8.4Hz, Ar-H), 7.93 (s, 2H, Ar-H), 8.07 (s, 4H, Ar-H), 11.87 (s, 2H, 2xNH), 12.47 (s, 2H, 2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 123.2, 124.0, 126.2, 128.7, 130.4, 132.7, 136.0, 139.5 (Ar-C), 167.3 (C=O), 179.3 (C=S).

N*^{1},*N*^{4}*-bis[(4-chlorophenyl)carbamothioyl]terephthalamide* (6)[24]

Compound **6** was obtained from *p*-chloroaniline (0.6 mL,4 mmol). Yield (0.77 g, 77 %) as light yellow solids; m.p: 257-258 ˚C (lit[24] 230 ºC); Anal.Calcd for $C_{22}H_{16}Cl_2N_4O_2S_2$ (502 g): C, 52.49; H, 3.20; N, 11.13; S, 12.74%. Found: C, 51.79; H, 3.43; N, 11.23; S, 12.98.; IR (KBr/ cm^{-1}) 3378 (NH), 3054 (CH), 1673 (C=O amide), 1546 (Ar-C), 1261 (C=S), 1148 (C-N). ^{1}H NMR (500 MHz, DMSO-d_6) 7.49 (d, 4H, *J* = 8.4 Hz, Ar-H), 7.73 (d, 4H, *J* = 8.4 Hz, Ar-H), 8.08 (s, 4H, Ar-H), 11.83 (s, 2H, 2xNH), 12.44 (s, 2H, 2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 126.3, 128.6, 128.7, 130.3, 135.9, 137.0 (Ar-C), 167.5 (C=O), 179.1 (C=S).

***N*^{1},*N*^{4}*-bis[(2-bromophenyl)carbamothioyl]terephthalamide* (7)**

Compound **7** was obtained from *o*-bromoaniline (0.70g, 4 mmol). Yield (0.94 g, 78 %) as white solids; m.p: 254-255 ˚C; Anal.Calcd for $C_{22}H_{16}Br_2N_4O_2S_2$ (591 g) : C, 44.61; H, 2.72; N, 9.46; S, 10.83%. Found: C, 44.53; H, 2.82; N, 9.48; S, 10.35.; IR (KBr/ cm^{-1}) 3242 (NH), 3158 (CH), 1677 (C=O amide), 1576 (Ar-C), 1250 (C=S), 1170 (C-N). ^{1}H NMR (500 MHz, DMSO-d_6) 7.29 (t, 2H,*J* = 8.0 Hz Ar-H), 7.48 (t, 2H,*J* = 7.7 Hz Ar-H), 7.76 (d, 2H, *J* = 7.7 Hz , Ar-H), 7.89 (d, 2H,*J* =6.9 Hz , Ar-H), 8.10 (s, 4H,Ar-H), 12.04 (s, 2H,2xNH), 12.50 (s, 2H,2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 119.5, 128.0, 128.8, 132.8, 135.9, 136.9 (Ar-C), 167.8 (C=O), 180.3 (C=S).

***N*^{1},*N*^{4}*-bis[(3-bromophenyl)carbamothioyl]terephthalamide* (8)**

Compound **8** was obtained from *m*-bromoaniline (0.5 mL, 4 mmol). Yield (0.83g, 70 %) as white solids; m.p: 244-245 ˚C; Anal.Calcd for $C_{22}H_{16}Br_2N_4O_2S_2$ (591 g) : C, 44.61; H, 2.72; N, 9.46; S, 10.83%. Found: C, 44.53; H, 2.91; N, 9.44; S, 10.62.; IR (KBr/ cm^{-1}) 3228 (NH), 3036 (CH), 1677 (C=O amide), 1529 (Ar-C), 1258 (C=S), 1159 (C-N).^{1}H NMR (500 MHz, DMSO-d_6) 7.40 (t, 2H, *J* = 8.0 HzAr-H), 7.48 (d, 2H, *J* = 8.4 Hz , Ar-H), 7.62 (d, 2H, *J* = 7.7 Hz , Ar-H), 8.06 (s, 2H, Ar-H), 8.07 (s, 4H, Ar-H), 11.87 (s, 2H, 2xNH), 12.45 (s, 2H, 2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 121.0, 123.6, 126.8, 128.7, 129.0, 130.6, 136.0, 139.6 (Ar-C), 167.3 (C=O), 179.3 (C=S).

***N*^{1},*N*^{4}*-bis[(4-bromophenyl)carbamothioyl]terephthalamide* (9)**[24]

Compound **9** was obtained from *p*-bromoaniline (0.71 g, 4 mmol). Yield (0.82 g, 69 %) as dusty yellow solids; m.p: 257-258 ˚C (lit[24] 200 ºC); Anal.Calcd for $C_{22}H_{16}Br_2N_4O_2S_2$ (591 g): C, 44.61; H, 2.72; N, 9.46; S, 10.83%. Found: C, 44.43; H, 2.69; N, 9.45; S, 10.61. IR (KBr/ cm^{-1}) 3369 (NH), 3050 (CH), 1663 (C=O amide), 1537 (Ar-C), 1263 (C=S), 1145 (C-N). ^{1}H NMR (500 MHz, DMSO-d_6) 7.63 (d, 4H, *J* = 8.4 Hz, Ar-H), 7.68 (d, 4H, *J* = 9.2 Hz, Ar-H), 8.07 (s, 4H, Ar-H), 11.85 (s, 2H, 2xNH), 12.44 (s, 2H, 2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 118.6, 126.5, 128.6, 131.5, 135.9, 137.3 (Ar-C), 167.3 (C=O), 179.1 (C=S).

***N*[1],*N*[4]*-bis[(2-iodophenyl)carbamothioyl]terephthalamide* (10)**

Compound **10** was obtained from *o*-iodoaniline (0.9 g, 4 mmol). Yield (0.99 g, 72%) as white solids; m.p: 247-249 ˚C; Anal.Calcd for $C_{22}H_{16}I_2N_4O_2S_2$, (685 g): C, 38.50; H, 2.35; N, 8.16; S, 9.34%. Found: C, 38.95; H, 2.18; N, 8.05; S, 9.57.; IR (KBr/ cm^{-1}) 3163 (NH), 3058 (CH), 1675 (C=O amide), 1522 (Ar-C), 1250 (C=S) 1166 (C-N). ^{1}H NMR (500 MHz, DMSO-d_6) 7.09 (t, 2H, *J* = 7.7 Hz Ar-H), 7.47 (t, 2H,*J* = 6.9 Hz Ar-H), 7.66 (d, 2H, *J* = 7.7 Hz, Ar-H), 7.95 (d, 2H, *J* = 7.7 Hz, Ar-H), 8.11 (s, 4H, Ar-H), 12.07 (s, 2H,2xNH), 12.32 (s, 2H, 2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 97.5, 128.6, 128.7, 128.8, 129.0, 135.9, 138.9, 140.3 (Ar-C), 167.6 (C=O), 180.5 (C=S).

***N*[1],*N*[4]*-bis[(3-iodophenyl)carbamothioyl]terephthalamide* (11)**

Compound **11** was obtained from *m*-iodoaniline (0.5 mL,4 mmol). Yield (1.09 g, 79 %) as dusty brown solids; m.p: 246-247 ˚C; Anal.Calcd for $C_{22}H_{16}I_2N_4O_2S_2$, (685 g): C, 38.50; H, 2.35; N, 8.16; S, 9.34%. Found: C, 38.38; H, 2.27; N, 8.44; S, 9.26.; IR (KBr/ cm^{-1}) 3392 (NH), 3041 (CH), 1670 (C=O amide), 1534 (Ar-C), 1254 (C=S), 1145 (C-N). ^{1}H NMR (500 MHz,

DMSO-d_6) 7.24 (t, 2H, *J* = 8.0 Hz Ar-H), 7.65 (d, 4H, *J* = 7.7 Hz, Ar-H), 8.07 s, 4H, Ar-H), 8.17 (s, 2H, Ar-H), 11.85 (s, 2H, 2xNH), 12.44 (s, 2H, 2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 94.4, 124.5, 129.2, 131.1, 133.2, 135.5, 136.4, 139.8 (Ar-C), 167.5 (C=O), 179.7 (C=S).

***N^1,N^4-bis[(4-iodophenyl)carbamothioyl]terephthalamide* (12)**[24]

Compound **12** was obtained from *p*-iodoaniline (0.91 g, 4 mmol). Yield (1.14 g, 83 %) as yellowish solids; m.p: 257-258 ˚C (lit[24] 225 ºC); Anal.Calcd for $C_{22}H_{16}I_2N_4O_2S_2$, (685 g): C, 38.50; H, 2.35; N, 8.16; S, 9.34%. Found: C, 38.63; H, 2.29; N, 8.41; S, 9.39.; IR (KBr/ cm^{-1}) 3350 (NH), 3047 (CH), 1664 (C=O amide), 1542 (Ar-C), 1262 (C=S), 1147 (C-N). ^{1}H NMR (500 MHz, DMSO-d_6) 7.50 (d, 4H, *J* = 8.4 Hz, Ar-H), 7.73 (d, 4H, *J* = 9.2 Hz, Ar-H), 8.08 (s, 4H,Ar-H), 11.87 (s, 2H,2xNH), 12.45 (s, 2H,2xNH). ^{13}C NMR (125 MHz, DMSO-d_6) 90.9, 126.5, 128.7, , 137.4, 137.6, 137.9 (Ar-C), 170.7 (C=O), 179.0 (C=S).

Antibacterial Screening

Turbidimetric Kinetic Method

The synthesised bis(acylthiourea) compounds were initially tested for their antibacterial activities against two types of bacteria; *E.coli* ATCC 25922 and *S.aureus* S48/81 using the turbidimetric kinetic method.[31,32] The bacteria were cultured on Luria-Bertani (LB) agar for 1 d at 37 ºC. The inoculums were prepared by allowing bacteria to grow on media containing nutrient broth at 37 ºC with permanent stirring at 250 rpm for 18 h. The inoculums (0.2 mL) were inoculated with 10 mL of culture medium (with increasing concentration of the compounds dissolved in DMSO). The mixture was shaken at 180 rpm at 37 ºC. Inoculums with DMSO were used as a control. Aliquots of each replicate were taken at every 1 h interval for 6 h. The

transmittances (T) were recorded using a UV-visible spectrophotometer (Optima SP-300). The antibacterial activities were determined by plotting a graph of In Nt versus time. The ln Nt, is defined as transmittance value, which represents the number colony forming units (CFU) mL^{-1} for bacteria versus time following expressions: *E. coli* In Nt= 27.1-8.56T and *S. aureus* ln Nt = 27.4 – 10.3T.[31]

Kirby-Bauer Disc Diffusion Method

Kirby-Bauer disc diffusion method was employed due to the dissolution of compounds during administration into the culture media. The bacteria *E.coli* ATCC 25922 and *S.aureus* S48/81 were grown on media with Mueller-Hinton (MH) broth at 37 °C. The inoculums were stirred at 120 rpm for 18 h. The MH agar was poured into a plate and left five minutes to be solid. The surface of the Mueller Hinton plate was inoculated by streaking the swab over the entire agar surface. The plate was rotated approximately 60° each time and swabbed a few times to ensure even inoculums distribution. Filter paper discs were placed on the surface of agar plate by using forceps. Each disc was pressed down softly to make sure complete contact with the surface. The synthesised compounds were impregnated on the discs. The plates were incubated aerobically for 24 h and the diameter of zone inhibition was measured.[35] In this study, ampicillin was used as a control due to its ability to interact with both positive and negative bacteria in comparison to other antibiotic such as penicillin.[39]

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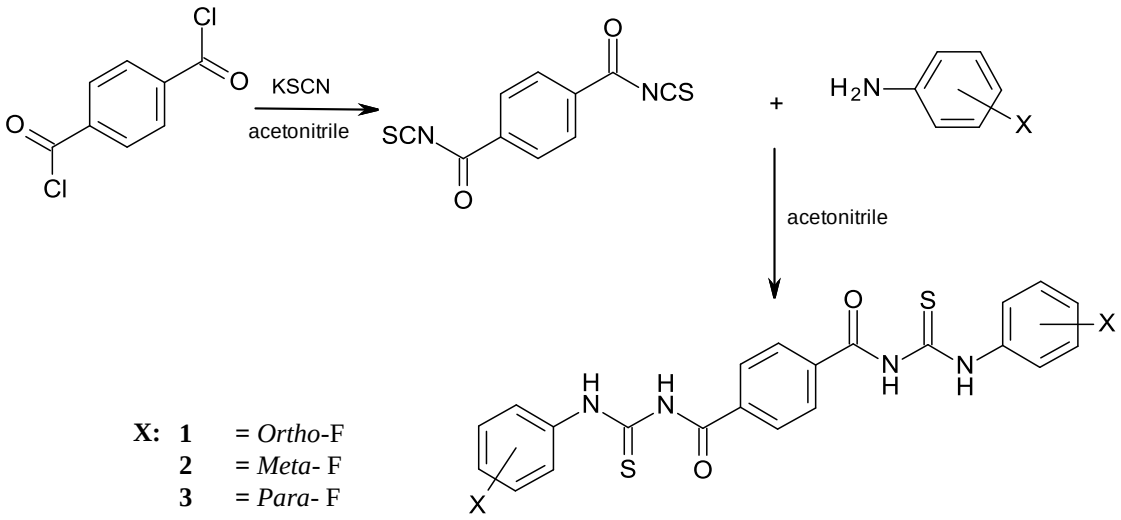

X: **1** = *Ortho*-F
2 = *Meta*- F
3 = *Para*- F
4 = *Ortho*-Cl
5 = *Meta*-Cl
6 = *Para*-Cl
7 = *Ortho*-Br
8 = *Meta*-Br
9 = *Para*-Br
10 = *Ortho*-I
11 = *Meta*-I
12 = *Para*-I

Scheme 1: The synthesis of bis(acylthiourea) 1-12