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Research Article

Novel Synthetic Monothiourea Aspirin Derivatives Bearing Alkylated Amines as Potential Antimicrobial Agents

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A new series of aspirin bearing alkylated amines moieties **1–12** were synthesised by reacting isothiocyanate with a series of aniline derivatives in overall yield of 16–56%. The proposed structures of all the synthesised compounds were confirmed using elemental analysis, FTIR, and ^{1}H and ^{13}C NMR spectroscopy. All compounds were evaluated for antibacterial activities against *E. coli* and *S. aureus via* turbidimetric kinetic and Kirby Bauer disc diffusion method. Compound **5** bearing *meta* -CH_3 substituent showed the highest relative inhibition zone diameter against tested bacteria compared to *ortho* and *para* substituent. Furthermore, aspirin derivatives bearing shorter chains exhibited better bacterial inhibition than longer alkyl chains.

1. Introduction

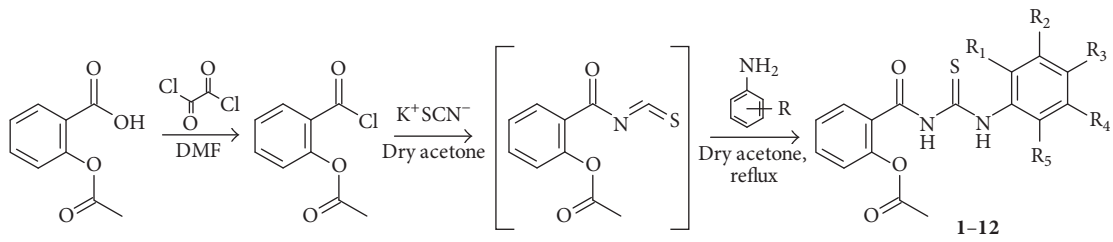
Over the centuries many antibacterial drugs were used to treat bacterial-causing diseases including food poisoning, pneumonia and intestinal infection [1, 2]. The improper usage of these drugs has caused the bacteria to evolve into drug resistant bacteria which reduce the effectiveness of the drugs [3]. The continuing development of new antimicrobial agents therefore remains a priority [1].

Aspirin is a widely used medicine for antipyretic, analgesic, and anti-inflammatory [4]. The demand of aspirin and its derivatives for other biological properties is increasing due to its availability and reactivity as precursor for further modification *via* corresponding carboxyl group [5]. Aspirin derivatives have shown antibacterial activities against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa* [6, 7]. Other significant biological properties of aspirin derivatives are reported for antitumor, anticancer, antifungal, and antimicrobial agents [5, 7–9].

We have previously reported on aspirin with thiourea moieties bearing amino acid and aromatic amines with excellent antibacterial properties. The lipophilicity of the aromatic ring has contributed to the enhancement of the biological activities [5]. Thiourea is a type of reactive precursor due to the presence of C=S, C=O, and NH moieties which are essential in biological activities [10]. Several biological activities reported on thiourea are antibacterial, antifungal, anticancer, antitubercular, antimicrobial, and anti-HIV activities [11–17]. The synthesis of thiourea derivatives has also received great attraction due to their diverse application such as in textile processing and heavy metals extraction as chelating agent and agriculture as plant growth regulator [18–20].

2. Results and Discussion

Aspirin derivatives **1–12** with aryl side chain bearing alkyl substituents were prepared from the reaction of acetoxybenzoyl isothiocyanate with a series of commercially available



Compounds	R_1	R_2	R_3	R_4	R_5
1	$-CH_3$	$-CH_3$	-H	-H	-H
2	$-CH_3$	-H	$-CH_3$	-H	-H
3	$-CH_3$	-H	-H	$-CH_3$	-H
4	$-CH_3$	-H	-H	-H	$-CH_3$
5	-H	$-CH_3$	-H	-H	-H
6	$-OCH_3$	-H	-H	$-OCH_3$	-H
7	-H	$-OCH_3$	$-OCH_3$	-H	-H
8	-H	$-OCH_3$	-H	$-OCH_3$	-H
9	-H	-H	$-OC_6H_{13}$	-H	-H
10	-H	-H	$-OC_{10}H_{21}$	-H	-H
11	-H	-H	$-OC_{12}H_{25}$	-H	-H
12	-H	-H	$-OC_{14}H_{29}$	-H	-H

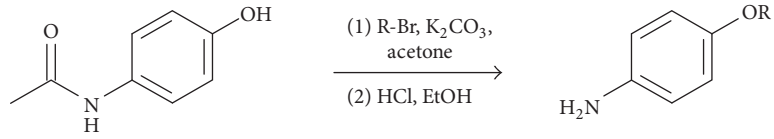
Scheme 1: Synthesis of aspirin derivatives **1–12**.

methyl and methoxy anilines and alkylated anilines prepared *via* Williamson etherification. A general synthesis of **1–12** is depicted in Scheme 1. Characteristic spectroscopic data of the synthesised compounds are provided in the supplementary data in Supplementary Material available online at https://doi.org/10.1155/2017/2378186. Aspirin was added to oxalyl chloride solution in dichloromethane followed by addition of few drops of dimethylformamide [21]. The mixture was stirred for 1 h at room temperature and added dropwise to KSCN in dry acetone to form KCl and filtered [5]. Aniline derivatives in dry acetone were then added to the filtrate and refluxed for 4 h. Crushed ice was added to the mixture to form precipitate. The crude was recrystallised in ethanol to obtain **1–12** in moderate yields (16–56%). The synthesis of thiourea could form urethane as side product which contributed to the low product yield [22]. The structures of all the synthesised compounds were elucidated using elemental analysis, FTIR, and ^{1}H and ^{13}C NMR spectroscopy.

The FTIR spectra of **1–12** showed absorption bands at 3375–3213 cm^{-1} attributed to ν(N-H), while peaks corresponding to long alkyl chain were observed at 2933–2843 cm^{-1}. The successful formations of **1–12** were supported by the disappearance of peak at 2000 cm^{-1} resulting from the conversion of -NCS to -NH. The absorption peaks at 1791–1760 cm^{-1} and 1675–1611 cm^{-1} were attributed to ν(C=O) ester and ν(C=O) amide, respectively. The aryl groups were observed at 1528–1500 cm^{-1}. The presence of ν(C-N) and ν(C=S) was indicated by the absorption band at 1089–1125 cm^{-1} and 890–810 cm^{-1}, respectively [16].

^{1}H NMR spectra of **1–12** showed the presence of methyl groups at 0.83–2.33 ppm, whereas the methoxy groups were observed at 3.72–3.96 ppm due to the electronegativity of oxygen attached to the carbon [23]. The resonance peaks at 6.42–8.50 ppm were assigned to the aryl groups. The peaks at 11.53–11.65 ppm and 11.96–12.90 ppm corresponded to CSNH and CONH, respectively. The deshielding effect of electron-withdrawing carbonyl group and thiocarbonyl group has shifted the signal to downfield region [24]. In ^{13}C NMR, methyl substituents were represented at 13.9–31.9 ppm, while the methoxy group were deshielded at 55.4–68.2 ppm due to the electronegativity of oxygen [23]. The presence of peaks at 98.3 and 160.3 ppm was attributed to aromatic carbons, whereas C=O ester, C=O amide, and C=S were indicated by the signals at 164.2–166.7 ppm, 168.3–169.1 ppm, and 176.9–180.0 ppm, respectively. Thiocarbonyl and carbonyl group appeared at higher chemical shift due to formation of intramolecular hydrogen bonding and different environment and conformations [23, 25].

Prior to the synthesis of **9–12**, **13–16** were prepared as precursors *via* Williamson etherification of 4-hydroxyl acetanilide with bromoalkanes in the presence of K_2CO_3 in dry acetone. The mixture was refluxed for 48 h to obtain white



13–16

13, R = C_6H_{15}
14, R = $C_{10}H_{21}$
15, R = $C_{12}H_{25}$
16, R = $C_{14}H_{29}$

Scheme 2: Preparation of alkylated anilines **13–16**.

Table 1: Antibacterial activities of **1–12** in comparison to aspirin and control.

Compounds	*E. coli*		*S. aureus*	
	Zone of inhibition (mm)	Relative inhibition zone diameter (%)	Zone of inhibition (mm)	Relative inhibition zone diameter (%)
1	7.3	45.6	8.0	72.7
2	7.7	48.1	10.7	97.3
3	10.5	65.6	—	—
4	—	—	—	—
5	10.7	66.9	11.3	>100
6	—	—	—	—
7	8.5	53.1	10.3	93.6
8	9.3	58.1	10.3	93.6
9	8.0	50.0	—	—
10	—	—	—	—
11	—	—	—	—
12	—	—	—	—
Aspirin	—	—	—	—
DMSO	—	—	—	—
Ampicillin	16.0	100	11.0	100

precipitate and hydrolysed in ethanol-HCl (1 : 1) to form **13–16** (Scheme 2) [26–28]. Spectroscopy analysis showed that all peaks corresponded to the proposed structure.

Antibacterial activities of **1–12** were initially performed against *E. coli* ATCC 25922 and *S. aureus* S48/81 *via* turbidimetric kinetic method [5]. All compounds experienced solubility limitation in the assays media which hindered the inhibition studies. Alternatively, disc diffusion method was performed on **1–12** [29]. Ampicillin (positive control) was used as standard drug, while dimethyl sulfoxide (DMSO) was used as negative control with aspirin as a reference. The antibacterial activities of **1–12** are shown in Table 1. The inhibition of *E. coli* and *S. aureus* was calculated *via* inhibition zone of **1–12** in comparison to the inhibition zone of ampicillin. The inhibition zones of all compounds are provided in the supplementary data.

In comparison to aspirin, **1–12** showed moderate to good activities towards the growth of *E. coli*, except **4**, **6**, and **10–12**. The presence of C=O, C=S, and NH groups is envisaged to contribute to the antibacterial activities and formed interaction with the carboxyl and phosphate group of the bacterial surface [10]. The location and type of substituents in the compounds contribute to the inhibition activities. Compound **5** with *meta* substituted -CH_3 gave better inhibition to the bacterial growth with the highest percentage relative inhibition zone diameter (66.9%). Compounds **1–3**, **7**, and **8** with disubstituted methyl and methoxy group showed lower inhibition based on zone diameter percentage (45.6–65.6%). The presence of methyl and methoxy groups has induced steric repulsion and decreased the activities. The steric hindrance at both *ortho* positions in the phenyl group affected the efficacy of antibacterial effect which explained that *E. coli* was insusceptible to **4** [30]. The presence of bulky methoxy substituents at *ortho* position in **6** prevented the compound to act on the active sites of the bacteria [31]. The introduction of shorter alkyl chain, $n = 6$ (**9**), afforded moderate activity, while no activity was observed for **10–12** with $n = 10$–14. The longer alkyl chain, which is similar to the component of the cell wall, decreased the penetration onto the cell membrane [32]. This also suggested that shorter alkyl chain as in **9** is the optimum length for bacterial inhibition [27].

Antibacterial activities were also demonstrated against *S. aureus*. Compound **5** gave better inhibition than that of the standard drug (ampicillin), while **1**, **2**, **7**, and **8** showed 72.7–97.3% inhibition. The presence of a methyl substituent at *meta* position in **5** gave better penetration into the bacterial cell wall *via* suitable binding [33]. On the other hand, no activity was observed for **3**, **4**, **6**, and **9**–**12**. Growth inhibition on *S. aureus* is lesser compared to *E. coli* because *S. aureus* has thicker cell wall that causes ineffectiveness in membrane penetration [34].

3. Experimental

Aspirin, oxalyl chloride, potassium thiocyanate, 2,3-dimethylaniline, 2,4-dimethylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline, 2-toluidine, 2,5-dimethoxyaniline, 3,4-dimethoxyaniline, 3,5-dimethoxyaniline, 4-hydroxyl acetanilide, and 1-bromoalkanes were obtained from Merck. All other reagents were used without further purification; nevertheless the acetone was distilled before being used.

Melting points were determined on Stuart SMP3 using open tube capillary method. FTIR spectra (ν/cm^{-1}) were recorded as KBr pellets on Perkin Elmer 1605 FTIR Spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on JEOL ECA 500 at 500 MHz (^{1}H) and 125 MHz (^{13}C) with the chemical shift reported relative to DMSO-d_6 and $CDCl_3$ as the standard reference and chemical shift values were expressed in δ ppm.

*3.1. General Procedure for the Preparation of Aspirin Bearing Alkylated Amines (**1**–**12**).* Aspirin (2 mmol) was added to oxalyl chloride solution in dichloromethane followed by addition of few drops of dimethylformamide. The mixture was stirred for 1 h at room temperature and was added dropwise to KSCN (2 mmol) in dry acetone (10 mL). The mixture was stirred at room temperature to form precipitate and it was filtered to remove the solid. Aryl amine (2 mmol) in 10 mL of dry acetone was then added to the filtrate and the reaction mixture was refluxed for 4 h. The mixture was cooled to room temperature and filtered. The filtrate was transferred into a beaker and crushed ice was added to it. The solid formed was recrystallised in ethanol to obtain **1**–**12**. Supplementary data for FTIR, ^{1}H, and ^{13}C NMR spectra of the synthesised compounds are available online.

*[2-[(2,3-Dimethylphenyl)carbamothioylcarbamoyl]phenyl] Acetate (**1**).* Compound **1** was obtained as a white solid. Yield: 0.2786 g (41%); m.p. 133–136°C; (Found: C, 62.88; H, 5.30; N, 7.96. $C_{18}H_{18}O_3N_2S$ Requires C, 63.14; H, 5.30; N, 8.18%); ν 3213 (N-H), 1760 (C=O ester), 1673 (C=O amide), 1525 (Ar-C), 1188 (C-N), 876 (C=S). δ_H (DMSO-d_6) 1.90 (3H, s, CH_3), 2.19 (3H, s, CH_3), 2.26 (3H, s, CH_3), 7.03–7.97 (7H, m, Ar-H), 11.65 (1H, s, NH), 12.22 (1H, s, NH). δ_C (DMSO-d_6) 13.9 (CH_3), 20.1 (CH_3), 20.8 (CH_3), 123.2, 124.9, 125.6, 126.1, 126.8, 128.8, 130.0, 132.5, 133.3, 136.8, 137.4, 148.2 (Ar-C), 166.6 (C=O), 169.0 (C=O) and 180.0 (C=S).

*[2-[(2,4-Dimethylphenyl)carbamothioylcarbamoyl]phenyl] Acetate (**2**).* Compound **2** was obtained as yellowish white solid. Yield: 0. 1117 g (16%); m.p. 115–118°C; (Found: C, 63.00; H, 5.10; N, 7.88. $C_{18}H_{18}O_3N_2S$ Requires C, 63.14; H, 5.30; N, 8.18%); ν 3381 (N-H), 1775 (C=O ester), 1663 (C=O amide), 1523 (Ar-C), 1189 (C-N), 810 (C=S). δ_H (DMSO-d_6) 2.20 (3H, s, CH_3), 2.29 (3H, s, CH_3), 2.31 (3H, s, CH_3), 7.04–7.73 (7H, m, Ar-H), 11.62 (1H, s, NH), 11.96 (1H, s, NH) δ_C (DMSO-d_6) 17.5 (CH_3), 20.6 (CH_3), 20.8 (CH_3), 123.2, 126.0, 126.5, 126.8, 130.0, 131.0, 133.2, 133.3, 134.3, 136.5, 148.2 (Ar-C), 166.6 (C=O), 169.0 (C=O) and 179.7 (C=S).

*[2-[(2,5-Dimethylphenyl)carbamothioylcarbamoyl]phenyl] Acetate (**3**).* Compound **3** was obtained as yellowish white solid. Yield: 0.2620 g (38%); m.p. 119–122°C; (Found: C, 63.10; H, 5.45; N, 8.02. $C_{18}H_{18}O_3N_2S$ Requires C, 63.14; H, 5.30; N, 8.18%); ν 3352 (N-H), 1773 (C=O ester), 1663 (C=O amide), 1524 (Ar-C), 1150 (C-N), 890 (C=S). δ_H (DMSO-d_6) 1.90 (3H, s, CH_3), 2.19 (3H, s, CH_3), 2.26 (3H, s, CH_3), 7.03–7.97 (7H, m, Ar-H), 11.65 (1H, s, NH), 12.22 (1H, s, NH). δ_C (DMSO-d_6) 17.2 (CH_3), 20.6 (CH_3), 20.8 (CH_3), 123.3, 126.1, 126.8, 127.1, 128.0, 130.1, 130.4, 131.4, 133.4, 135.5, 136.7, 148.3 (Ar-C), 166.7 (C=O), 169.1 (C=O) and 179.6 (C=S).

*[2-[(2,6-Dimethylphenyl)carbamothioylcarbamoyl]phenyl] Acetate (**4**).* Compound **4** was obtained as yellowish white solid. Yield: 0.3230 g (47%); m.p. 166–169°C; (Found: C, 62.77; H, 5.11; N, 8.00. $C_{18}H_{18}O_3N_2S$ Requires C, 63.14; H, 5.30; N, 8.18%); ν 3386 (N-H), 1784 (C=O ester), 1675 (C=O amide), 1504 (Ar-C), 1176 (C-N), 853 (C=S). δ_H (DMSO-d_6) 2.19 (6H, s, $2CH_3$), 2.31 (3H, s, CH_3), 7.12–7.75 (7H, m, Ar-H), 11.64 (2H, s, NH). δ_C (DMSO-d_6) 17.7 (CH_3), 20.7 (CH_3), 123.0, 126.0, 127.0, 127.5, 128.0, 129.9, 133.2, 135.1, 136.2, 148.1 (Ar-C), 166.4 (C=O), 168.9 (C=O) and 179.9 (C=S).

*[2-[(3-Methylphenyl)carbamothioylcarbamoyl]phenyl] Acetate (**5**).* Compound **5** was obtained as yellow solid. Yield: 0.1177 g (46%); m.p 179–181°C; (Found: C, 62.11; H, 4.61; N, 8.28. $C_{17}H_{16}O_3N_2S$ Requires C, 62.18; H, 4.91; N, 8.53%); ν 3382 (N-H), 1778 (C=O ester), 1660 (C=O amide), 1500 (Ar-C), 1079 (C-N), 863 (C=S). δ_H (DMSO-d_6) 2.32 (3H, s, CH_3), 2.33 (3H, s, CH_3), 7.08–7.73 (8H, m, Ar-H), 11.60 (1H, s, NH), 12.34 (1H, s, NH). δ_C (DMSO-d_6) 20.7 (CH_3), 20.9 (CH_3), 121.3, 123.2, 124.6, 126.6, 127.0, 125.8, 128.5, 130.0, 133.1, 137.7, 138.1, 148.2 (Ar-C), 166.5 (C=O), 168.8 (C=O) and 178.5 (C=S).

*[2-[(2,5-Dimethoxyphenyl)carbamothioylcarbamoyl]phenyl] Acetate (**6**).* Compound **6** was obtained as light green solid. Yield: 0.1739 g (23%); m.p. 185-186°C; (Found: C, 57.60; H, 4.40; N, 7.48. $C_{18}H_{18}O_5N_2S$ Requires C, 57.74; H, 4.85; N, 7.48%); ν 3384 (N-H), 1777 (C=O ester), 1611 (C=O amide), 1500 (Ar-C), 1067 (C-N), 821 (C=S). δ_H (DMSO-d_6) 2.31 (3H, s, CH_3), 3.72 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 7.06–8.50 (7H, m, Ar-H), 11.62 (1H, s, NH), 12.90 (1H, s, NH). δ_C (DMSO-d_6) 20.7 (CH_3), 55.5 (OCH_3), 56.6 (OCH_3), 108.9, 110.5, 112.1, 123.1, 125.9, 126.5, 127.6, 130.1, 133.2, 144.3, 148.2, 152.4 (Ar-C), 166.5 (C=O), 168.8 (C=O) and 177.0 (C=S).

*[2-[(3,4-Dimethoxyphenyl)carbamothioylcarbamoyl]phenyl] Acetate (**7**)*. Compound **7** was obtained as dark green solid. Yield: 0.1801 g (24%); m.p. 182–184°C; (Found: C, 57.25; H, 4.62; N, 7.42. $C_{18}H_{18}O_5N_2S$ Requires C, 57.74; H, 4.85; N, 7.48%); ν 3367 (N-H), 1791 (C=O ester), 1670 (C=O amide), 1500 (Ar-C), 1025 (C-N), 850 (C=S). δ_H (DMSO-d_6) 2.32 (3H, s, CH_3), 3.75 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 6.97–7.41 (7H, m, Ar-H), 11.53 (1H, s, NH), 12.24 (1H, s, NH). δ_C (DMSO-d_6) 20.8 (CH_3), 55.7 (CH_3), 108.9, 111.5, 116.6, 123.4, 126.0, 126.7, 130.1, 130.9, 133.3, 147.2, 148.3, 148.4 (Ar-C), 166.5 (C=O), 169.0 (C=O) and 178.4 (C=S).

*[2-[(3,5-Dimethoxyphenyl)carbamothioylcarbamoyl]phenyl] Acetate (**8**)*. Compound **8** was obtained as yellowish white solid. Yield: 0.4190 g (56%), m.p. 177–179°C; (Found: C, 57.55; H, 4.94; N, 7.61. $C_{18}H_{18}O_5N_2S$ Requires C, 57.74; H, 4.85; N, 7.48%); ν 3382 (N-H), 1778 (C=O ester), 1660 (C=O amide), 1528 (Ar-C), 1080 (C-N), 864 (C=S). δ_H (DMSO-d_6) 2.31 (3H, s, CH_3), 3.75 (6H, s, $2OCH_3$), 6.42–7.74 (7H, m, Ar-H), 11.58 (1H, s, NH), 12.35 (1H, s, NH). δ_C (DMSO-d_6) 20.8 (CH_3), 55.4 ($2OCH_3$), 98.3, 102.1, 123.3, 125.9, 126.6, 130.1, 133.2, 139.4, 148.2, 160.3 (Ar-C), 166.4 (C=O), 168.9 (C=O) and 178.2 (C=S).

*[2-[((4-(Hexyloxy)phenyl)carbamothioyl)carbamoyl]phenyl] Acetate (**9**)*. Compound **9** was obtained as yellowish white solid. Yield: 0.2070 g (55%), m.p. 112–119°C; (Found: C, 63.68; H, 6.15; N, 6.91. $C_{22}H_{26}O_4N_2S$ Requires C, 63.75; H, 6.32; N, 6.76%); ν 3372 (N-H), 2933–2856 (C-H alkyl), 1775 (C=O ester), 1663 (C=O amide), 1527 (Ar-C), 1106 (C-N), 829 (C=S). δ_H (DMSO-d_6) 0.87 (3H, t, J = 7.3 Hz, CH_3), 1.31–1.70 (8H, m, CH_2), 2.31 (3H, s, CH_3), 3.96 (2H, t, J = 6.5 Hz, OCH_2), 6.96–7.73 (8H, m, Ar-H), 11.54 (1H, s, NH), 12.18 (1H, s, NH). δ_C (DMSO-d_6) 14.0, 20.8, 22.1, 25.2, 28.7, 31.0 (C-H alkyl), 67.7 (OCH_2), 114.3, 123.3, 126.0, 126.7, 130.1, 130.6, 133.2, 148.3, 157.0 (Ar-C), 166.5 (C=O), 169.0 (C=O) and 178.7 (C=S).

*[2-[((4-(Decyloxy)phenyl)carbamothioyl)carbamoyl]phenyl] Acetate (**10**)*. Compound **10** was obtained as yellowish white solid. Yield: 0.2400 (51%), m.p. 116–121°C; (Found: C, 66.13; H, 7.44; N, 5.90. $C_{26}H_{34}O_4N_2S$ Requires C, 66.35; H, 7.28; N, 5.95%); ν 3374 (N-H), 2923–2849 (C-H alkyl), 1771 (C=O ester), 1664 (C=O amide), 1510 (Ar-C), 1111 (C-N), 826 (C=S). δ_H (DMSO-d_6) 0.85 (3H, t, J = 6.9 Hz, CH_3), 1.31–1.70 (16H, m, CH_2), 2.31 (3H, s, CH_3), 3.96 (2H, t, J = 6.5 Hz, OCH_2), 6.95–7.73 (8H, m, Ar-H), 11.55 (1H, s, NH), 12.19 (1H, s, NH). δ_C (DMSO-d_6) 14.0, 20.8, 22.1, 25.5, 28.8, 31.3 (C-H alkyl), 67.6 (OCH_2), 114.3, 123.3, 125.9, 126.6, 130.1, 130.6, 133.2, 148.2, 156.9 (Ar-C), 166.4 (C=O), 168.9 (C=O) and 178.6 (C=S).

*[2-[((4-(Dodecyloxy)phenyl)carbamothioyl)carbamoyl]phenyl] Acetate (**11**)*. Compound **11** was obtained as yellowish white solid. Yield: 0.2520 (51%), m.p. 121–124°C; (Found: C, 67.21; H, 7.74; N, 5.52. $C_{28}H_{38}O_4N_2S$ Requires C, 67.44; H, 7.68; N, 5.62%); ν 3374 (N-H), 2923–2843 (C-H alkyl), 1771 (C=O ester), 1664 (C=O amide), 1510 (Ar-C), 1114 (C-N), 825 (C=S). δ_H (DMSO-d_6) 0.84 (3H, t, J = 6.9 Hz, CH_3), 1.28–1.68 (20H, m, CH_2), 2.31 (3H, s, CH_3), 3.96 (2H, t, J = 6.5 Hz, OCH_2), 6.94–7.73 (8H, m, Ar-H), 11.55 (1H, s, NH), 12.20 (1H, s, NH). δ_C (DMSO-d_6) 14.0, 20.8, 22.1, 25.5, 28.8, 31.3 (C-H alkyl), 67.6 (OCH_2), 114.3, 123.3, 125.8, 126.6, 130.0, 130.5, 133.1, 148.2, 156.9 (Ar-C), 166.4 (C=O), 168.9 (C=O) and 178.6 (C=S).

*[2-[((4-(Tetradecyloxy)phenyl)carbamothioyl)carbamoyl]phenyl] Acetate (**12**)*. Compound **12** was obtained as yellowish white solid. Yield: 0.1240 (47%), m.p. 125–131°C; (Found: C, 68.08; H, 8.23; N, 5.31. $C_{30}H_{42}O_4N_2S$ Requires C, 68.41; H, 8.04; N, 5.32%); ν 3375 (N-H), 2923–2849 (C-H alkyl), 1771 (C=O ester), 1663 (C=O amide), 1510 (Ar-C), 1111 (C-N), 825 (C=S). δ_H (DMSO-d_6) 0.83 (3H, t, J = 6.9 Hz, CH_3), 1.22–1.69 (24H, m, CH_2), 2.30 (3H, s, CH_3), 3.95 (2H, t, J = 6.5 Hz, OCH_2), 6.94–7.71 (8H, m, Ar-H), 11.53 (1H, s, NH), 12.18 (1H, s, NH). δ_C ($CDCl_3$) 14.1, 21.3, 22.7, 26.0, 29.6, 31.9 (C-H alkyl), 68.2 (OCH_2), 114.6, 123.9, 124.2, 125.7, 126.8, 130.2, 131.4, 134.4, 148.4, 157.9 (Ar-C), 164.2 (C=O), 168.3 (C=O) and 178.3 (C=S).

*3.2. General Procedure for the Preparation of Alkylated Amines (**13**–**16**).* 4-Hydroxyl acetanilide (3 mmol) was added to bromoalkanes (3 mmol) in the presence of K_2CO_3 (3 mmol) in dry acetone (20 mL). The mixture was refluxed for 48 h to obtain white precipitate. Sodium hydroxide (2%, 50 mL) was added and the solid was filtered. The white solid was refluxed for 4 h in solution of ethanol-HCl (1 : 1) and extracted using dichloromethane. The organic layer was separated and dried to form **13**–**16**. Supplementary data for FTIR, ^{1}H, and ^{13}C NMR spectra of the synthesised compounds are available online.

*4-Hexoxyaniline (**13**)*. Compound **13** was obtained as a white crystals. Yield: 1.828 g (75%); m.p. 230–237°C; ν 3408 (N-H), 2934–2866 (-CH), 1517 (Ar-C), 1129 (C-N). δ_H (DMSO-d_6) 0.86 (3H, t, J = 6.9 Hz, -CH_3), 1.29–1.69 (8H, m, -CH_2), 3.87 (2H, t, J = 6.5 Hz, -OCH_2), 6.84 (2H, d, J = 9.2 Hz, Ar-H), 6.91 (2H, d, J = 8.4 Hz, Ar-H). δ_C (DMSO-d_6) 13.9, 22.1, 25.2, 28.7, 31.0 (C-H alkyl), 67.9 (OCH_2), 115.4, 120.0, 132.4 and 154.4 (Ar-C).

*4-Decoxyaniline (**14**)*. Compound **14** was obtained as a white crystals. Yield: 2.235 g (80%); m.p. 235–244°C; ν 3427 (N-H), 2921–2849 (-CH), 1513 (Ar-C), 1171 (C-N). δ_H (DMSO-d_6) 0.84 (3H, t, J = 6.9 Hz, -CH_3), 1.29–1.70 (16H, m, -CH_2), 3.93 (2H, t, J = 6.6 Hz, -OCH_2), 6.99 (2H, d, J = 9.2 Hz, Ar-H), 7.27 (2H, d, J = 9.2 Hz, Ar-H), 10.15 (2H, s, -NH_2). δ_C (DMSO-d_6) 14.1, 22.7, 26.0, 29.5, 31.9 (C-H alkyl), 68.2 (OCH_2), 115.2, 122.3, 124.5 and 159.0 (Ar-C).

*4-Dodecoxyaniline (**15**)*. Compound **15** was obtained as a white crystals. Yield: 3.050 g (87%); m.p. 237–247°C; ν 3434 (N-H), 2920–2853 (-CH), 1511 (Ar-C), 1171 (C-N). δ_H (DMSO-d_6) 0.83 (3H, t, J = 6.9 Hz, -CH_3), 1.23–1.71 (20H, m, -CH_2), 3.93 (2H, t, J = 6.5 Hz, -OCH_2), 6.99 (2H, d, J = 9.2 Hz, Ar-H), 7.30 (2H, d, J = 9.2 Hz, Ar-H), 10.27 (2H, s, -NH_2). δ_C (DMSO-d_6) 14.0, 22.1, 25.5, 28.8, 31.3 (C-H alkyl), 67.8 (OCH_2), 115.3, 124.1, 124.4 and 158.1 (Ar-C).

*4-Tetradecoxyaniline (**16**)*. Compound **16** was obtained as a white crystals. Yield: 2.137 g (50%); m.p. 239–248°C; ν 3410 (N-H), 2919–2853 (-CH), 1512 (Ar-C), 1171 (C-N). δ_H (DMSO-d_6) 0.83 (3H, t, J = 6.9 Hz, -CH_3), 1.23–1.71 (24H, m, -CH_2), 3.94 (2H, t, J = 6.3 Hz, -OCH_2), 7.00 (2H, d, J = 9.2 Hz, Ar-H), 7.30 (2H, d, J = 9.8 Hz, Ar-H), 10.21 (2H, s, -NH_2). δ_C (DMSO-d_6) 13.9, 22.1, 25.2, 28.7, 31.0 (C-H alkyl), 67.9 (OCH_2), 115.4, 120.0, 132.4 and 154.4 (Ar-C).

3.3. Antibacterial Studies

3.3.1. Turbidimetric Kinetic Method. The antibacterial activities of the synthesised compounds were initially studied using turbidimetric kinetic method against *E. coli* bacteria ATCC 25922 and *S. aureus* S48/81. The inoculums were allowed to grow at 37°C with permanent stirring at 150 rpm overnight in Luria-Bertani broth. 0.2 mL of inoculums was inoculated with 10 mL of culture medium at increasing concentration of the compounds (50, 80, and 100 ppm) dissolved in DMSO. The mixtures were shaken at 150 rpm at 37°C. The inoculum with only DMSO mixture was used as control. The aliquots of each replicate were taken at 1 h interval for 6 h. UV/Visible spectrometer Optima SP-300 was used to analyse the amount of transmittance (T). The antibacterial activities of the compounds were determined from the graph as ln N_t versus time. N_t is related to the number of colony forming units/mL. The ln N_t values were calculated based on the formula of ln N_t = 27.1–8.56T (*E. coli*) and ln N_t = 27.4–10.3T (*S. aureus*) [35].

3.3.2. Disc Diffusion Method. Disc diffusion method was used due to the insolubility of compounds in medium. *E. coli* ATCC 25922 and *S. aureus* S48/81 were used as inoculum where they were cultured in Mueller-Hinton broth and incubated at 37°C with permanent shaking at 150 rpm overnight. The bacterial suspension was then inoculated onto the entire surface of a Mueller-Hinton agar plate with a sterile cotton-tipped swab to form an even lawn. Sterile filter paper disc impregnated with 10 μL of the compound in DMSO was placed on the surface of agar plate using a sterile pair of forceps. The plates were then incubated at 37°C for 24 h. The zones of inhibition were measured in millimetre (mm) to estimate the potency of the test compounds [29].

4. Conclusion

A series of aspirin derivatives **1**–**12** with various types of carbon substituents were successfully synthesised and demonstrated against *E. coli* and *S. aureus*. The compounds showed moderate to excellent antibacterial activities compared to aspirin alone. The substituents at *meta* position gave better activity compared to *ortho* and *para* position. Aspirin derivative with shorter chain length showed moderate activity, compared to the longer alkyl chain. The properties of the substituents have contributed to the activities and potential usage in pharmaceutical industries.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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