Asian Journal of Chemistry; Vol. 28, No. 3 (2016), 596-600

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.19430

Synthesis, Spectroscopic Studies and Antibacterial Activity of New Lauroyl Thiourea Amino Acid Derivatives

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Received: 20 June 2015; *Accepted*: 6 August 2015; *Published online*: 5 December 2015; AJC-17658

Four new thiourea compounds have been successfully synthesized and characterized from combination of lauroyl chloride, ammonium thiocyanate and simple amino acids in acetone. The structure of the respective compounds, namely 3-(3-dodecanoyl-thioureido)propionic acid **(R1)**, 2-(3-dodecanoyl-thioureido)-3-methyl butyric acid **(R2)**, (3-dodecanoyl-thioureido)acetic acid **(R3)** and 2-(3-dodecanoyl-thioureido)-3-phenyl propionic acid **(R4)** are confirmed by combination of spectroscopic techniques such as infrared, ultraviolet and nuclear magnetic resonance. The antibacterial activity of these compounds are investigated towards selected bacteria and the results show that compound **R4** displays better antibacterial activity compared to **R1**-**R3** and as well as to few reported compounds. Compound **R4** shows good antibacterial activity towards two Gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium*, with inhibition zone approximately 8 mm wide and minimum inhibitory concentration (MIC) 50 µg/mL, respectively. The good results given by **R4** might be attributed by the presence of alkyl and phenyl group that increases the lipophilicity and stability of the compounds.

Keywords: Dodecanoyl, Lauroyl thiourea, Antibacterial activity, Amino acids.

INTRODUCTION

The synthesis of new thiourea derivatives has increased rapidly due to their great interest in biological and pharmaceutical research [1-4]. The thioamide moiety in the thiourea backbone is responsible for the pharmacological activities by the presence of delocalized electrons that stabilize the molecules [5-11]. Thiourea with benzoyl derivatives are the most common compounds investigated for biological activities with respect to their high stability and delocalized electrons at benzoyl and thione moieties [12-17]. In contrast, the research involving alkylthiourea is more limited compared to benzoylthiourea due to the challenge in the synthesis. However, Kang *et al.* [18] have recently reported the facile synthesis of alkylthiourea derivatives. The study also reveals that the molecules with long alkyl chain are potent against hepatitis C virus (HCV) in the cell-based subgenomic hepatitis C virus replicon assay. On the other hand, the study proved that the use of aliphatic chain can contribute to the improvement in bacteriostatic effects [19]. In line with our interest in the design of thiourea derivatives suitable for biological applications, we have designed thiourea from combination of three bioactive compounds such as lauryl chloride, thiourea and amino acids. In this study, we report the facile synthesis of four new lauroyl thiourea amino acid derivatives, characterization by spectroscopic studies and evaluation on the antibacterial activity. To the best of our knowledge, only few thiourea-lauryl compound has been reported while combination of the three units, lauryl chloride, thiourea and amino acid is new [20].

EXPERIMENTAL

All chemicals or reagents used were purchased from standard supplier (Merck and Sigma Aldrich) and used as received without further purification. Melting points were measured using BÜCHI melting point B-545. The infrared spectra were recorded on a Fourier transform-infrared spectrometer, Perkin Elmer spectrum 100 in the range of 4000-400 cm^{-1} using potassium bromide pellets. For UV-visible analysis, all compounds were recorded by using Spectrophotometer Shimadzu UV-1601PC in 1 cm^3 cuvette in methanolic solution for absorbance analysis. ^1H NMR and ^{13}C NMR spectra were recorded using Bruker Avance III 400 spectrometer with deuterated chloroform ($CDCl_3$) and deuterated dimethyl

sulfoxide (DMSO) as the solvents and chemical shift values were given in parts per million (ppm) relative to solvent resonances as internal standard.

General methods

Syntheses of lauroyl thiourea derivatives: Lauroyl chloride (3.5 g, 0.015 mol) and ammonium thiocyanate (1.142 g, 0.015 mol) in acetone (25 mL) was stirred for 1 h to give white precipitate which indicates the formation of lauroyl isothiocyanate. β-Alanine (1.337 g, 0.015 mol) was added dropwise to the mixture and further heated at refluxed for another 5 h. After the reaction was completed, the mixture was filtered into a beaker of ice, collected by filtration, washed with acetone and dried under vacuum to give **R1**. β-Alanine was then replaced with valine, glycine and phenylalanine and reacted with lauroyl isothiocyanate to produce compounds **R2**, **R3** and **R4** in the similar manner describes for **R1**.

Antibacterial assay: The antibacterial activities of compounds **R1**-**R4** were screened against test strains of Gram-positive (*Bacillus subtilis* ATCC 11774, *Staphylococcus epidermidis* ATCC 13518 and *Staphylococcus aureus* ATCC 25923) and Gram-negative (*Escherichia coli* ATCC 11775 and *Salmonella typhimurium* ATCC 14128) strains using common well diffusion method. Mueller-Hinton media were seeded with bacterial inoculum using cotton swab. Wells of 6 mm diameter were bored into the media using sterile cork borer and 90 µL of the diluted compounds at a dose range of 10-0.01 mg/mL were added in each well. Streptomycin (Abtek Biologicals Ltd) was used as the positive control while methanol served as negative control. All plates were incubated overnight at 37 °C. The antibacterial activities were evaluated by measuring the zones of inhibition (mm) and minimum inhibitory concentrations (MIC).

Spectral data

3-(3-Dodecanoyl-thioureido)propionic acid (R1): Compound **R1** was obtained as white solid. Yield: 4.25 g (85.84 %); m.p. 83-84 ºC; FT-IR (KBr pellets, ν_{max}, cm^{-1}): 3292 (NH), 3200 (OH), 1694 (C=O carboxylic), 1634 (C=O amide), 1443 (C-N), 720 (C=S). ^{1}H NMR ($CDCl_3$, ppm): 0.89 (3H, t, *J* = 6.8 Hz, CH_3), 1.27 (16H, s, 8×CH_2), 1.61-1.68 (2H, m, *J* = 7.3 Hz, CH_2), 2.17-2.52 (2H, m, *J* = 8.1 Hz, CH_2), 2.36 (2H, t, *J* = 7.6 Hz, CH_2), 2.61 (2H, t, *J* = 5.8 Hz, CH_2), 3.55 (1H, t, 6.0 Hz, NH), 6.25 (1H, s, NH), 10.85 (1H, s, OH); ^{13}C NMR ($CDCl_3$, ppm): 14.12 (CH_3), 22.69-31.91 (9×CH_2), 33.98 (CH_2-CO), 34.04 (CH_2-COOH), 36.75 (CH_2-NH), 177.31 (C=O-NH, C=O-OH), 179.47 (C=S).

2-(3-Dodecanoyl-thioureido)-3-methyl-butyric acid (R2): Compound **R2** was obtained as white solid. Yield: 3.08 g (57.44 %); m.p. 87-88 ºC; FT-IR (KBr pellets, ν_{max}, cm^{-1}): 3332 (NH), 2922-3332 (OH, broad), 1712 (C=O carboxylic), 1650 (C=O amide), 1415 (C-N), 723 (C=S). ^{1}H NMR ($CDCl_3$, ppm): 0.89 (3H, t, *J* = 7.0 Hz, CH_3), 0.97 (3H, d, *J* = 7.0 Hz, CH_3), 1.00 (3H, d, *J* = 7.0 Hz, CH_3), 1.06-1.09 (6H, m, *J* = 5.8 Hz, CH_2), 1.64-1.69 (2H, m, *J* = 7.9 Hz, CH_2), 2.24-2.30 (8H, m, *J* = 5.2 Hz, CH_2), 2.36-2.38 (4H, m, *J* = 3.4 Hz, CH_2), 4.60-4.62 (1H, m, *J* = 4.4 Hz, CH), 4.99 (1H, d, *J* = 4.2 Hz, CH), 6.13 (1H, d, *J* = 8.4 Hz, NH), 9.16 (1H, s, OH), 11.03 (1H, s, NH); ^{13}C NMR ($CDCl_3$, ppm): 14.17 ((2 × CH_3)-CH), 17.70 (CH_3), 19.02-36.64 (10 × CH_2), 25.77 (CH-(CH_3)$_2$), 57.21 (CH-NH), 174.19 (C=O-NH), 176.44 (C=O-OH), 179.94 (C=S).

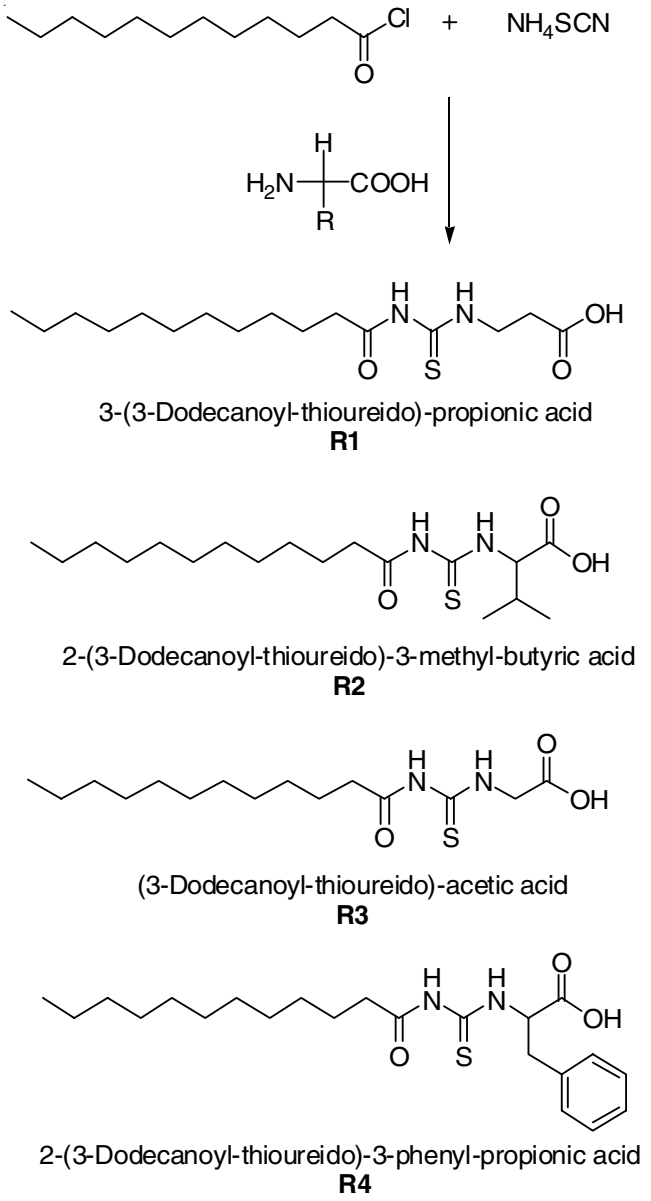
(3-Dodecanoyl-thioureido)acetic acid (R3): R3 was obtained as pale yellow solid. Yield: 2.61 g (55.07 %); m.p. 81-83 ºC; FT-IR (KBr pellets, ν_{max}, cm^{-1}): 3320 (NH), 3197 (OH), 1705 (C=O carboxylic), 1643 (C=O amide), 1406 (C-N), 720 (C=S). ^{1}H NMR (DMSO-d_6, ppm): 0.85 (3H, t, *J* = 6.8 Hz, CH_3), 1.24 (18H, s, 9×CH_2), 1.47 (2H, s, CH_2), 2.17 (2H, t, *J* = 7.4 Hz, CH_2), 3.71 (1H, s, NH), 8.08 (1H, t, *J* = 5.8 Hz, NH), 11.25 (1H, s, OH); ^{13}C NMR (DMSO-d_6, ppm): 14.40 (CH_3), 22.57-34.13 (10×CH_2), 41.05 (CH_2-NH), 171.96 (C=O-NH), 172.98 (C=O-OH), 174.79 (C=S).

2-(3-Dodecanoyl-thioureido)-3-phenyl-propionic acid (R4): R4 was obtained as yellow solid. Yield: 3.14 g (51.66 %); m.p. 134-136 ºC; FT-IR (KBr pellets, ν_{max}, cm^{-1}): 3316 (NH, OH), 1722 (C=O carboxylic), 1712 (C=O amide), 1463 (C-N), 720 (C=S). ^{1}H NMR (DMSO-d_6, ppm): 0.84-0.87 (7H, m, *J* = 4.2 Hz, CH_3 + 2×CH_2), 1.07-1.11 (2H, m, *J* = 7.0 Hz, CH_2), 1.35-1.39 (2H, m, *J* = 6.3 Hz, CH_2), 1.47-1.51 (2H, m, *J* = 7.0 Hz, CH_2), 2.00-2.03 (2H, m, *J* = 6.0 Hz, CH_2), 2.32-2.36 (2H, m, *J* = 6.6 Hz, CH_2), 2.81-2.84 (2H, m, *J* = 7.9 Hz, CH_2), 3.03-3.06 (2H, m, *J* = 6.3 Hz, CH_2), 3.10 (2H, t, *J* = 7.0 Hz, CH_2), 4.42 (2H, d, *J* = 3.5 Hz, CH_2), 5.05 (1H, t, *J* = 6.3 Hz, CH), 7.14-7.28 (5H, m, *J* = 7.7 Hz, Ar-H), 8.09 (1H, s, NH), 10.97 (1H, d, *J* = 7.7 Hz, NH), 11.28 (1H, s, OH); ^{13}C NMR (DMSO-d_6, ppm): 14.37 (CH_3), 22.60-34.12 (10×CH_2), 37.22 (CH_2-Ph), 53.69 (CH-NH), 126.69-138.25 (C_6H_5), 172.58 (C=O-NH), 173.70 (C=O-OH), 174.89 (C=S).

RESULTS AND DISCUSSION

The synthesis of four new thiourea compounds was described. Compounds 3-(3-dodecanoyl-thioureido)propionic acid **(R1)**, 2-(3-dodecanoyl-thioureido)-3-methyl butyric acid **(R2)**, (3-dodecanoyl-thioureido)acetic acid **(R3)** and 2-(3-dodecanoyl-thioureido)-3-phenyl propionic acid **(R4)** were prepared by combination of equimolar amounts of lauroyl chloride, ammonium thiocyanate and amino acid in acetone. The first stage of the synthesis requires preparation of lauroyl isothiocyanate from reaction between lauroyl chloride and ammonium thiocyanate. The obtaining lauroyl isothiocyanate was then combined with respective amino acids to give the products as clear solution. This solution was poured into a beaker containing ice cubes for rapid precipitation where white solid was immediately formed in the water solution. Compounds **R1**-**R4** were collected and further characterized by elemental analysis, FTIR, UV-visible and NMR spectroscopy techniques. The general schematic diagram on the synthesis of the compounds is outlined in **Scheme-I**.

Thioamide band (NH–C=S) are commonly indicated at 1500, 1300, 1100 and 750 cm^{-1} in the IR spectra. The characteristic band in 1600-1500 cm^{-1} region is originated from the deformation of NH and the stretching mode of C=C. The C-N stretching is commonly indicated in region 1300-1400 cm^{-1} while the C=S stretching coupled with S-C-N bending normally appeared at range 800-700 cm^{-1}. In the IR spectra of these four compounds, several distinctive peaks that assigned to ν(N-H), ν(O-H), ν(C=O carboxylic acid), ν(C=O amide), ν(C-N) and



Scheme-I: Synthesis of lauroyl thiourea amino acid derivatives

ν(C=S) were indicated in 3316-3292, 3322-2922, 1722-1634, 1463-1406 and 723-720 cm^{-1} region, respectively. The absence of isothiocyanate (N=C=S) stretching which previously appeared at 2125 cm^{-1} indicates the coupling of lauroyl isothiocyanate with amino acid to form thioamide (HNC=S) [21]. In compound **R4**, the NH band is overlapped with OH band and gives a broad peak around that area. Another important peak for C-H stretching which attributed by aliphatic chain is observed at 2900 cm^{-1} in all compounds. A distinctive peak for C=O carboxylic acid and C=O amide were detected at 1722-1694 cm^{-1} and 1712-1634 cm^{-1}, respectively. The absorption for C=O carboxylic acid appeared at higher frequency compared to C=O amide due to the higher electronegativity of oxygen compared to nitrogen. The C-N stretching was indicated at 1463-1406 cm^{-1} while the important thione band, C=S stretching was found at 723-720 cm^{-1}. The lower frequency of the C=S indicates that combination with amino acids does not give significant effect to the double bond character of the thione [22]. The IR spectra for compounds **R1-R4** is listed in Table-1.

TABLE-1
INFRARED DATA OF **R1-R4**

Compd.	ν(NH) (cm^{-1})	ν(OH) (cm^{-1})	ν(C=O) (cm^{-1})	ν(C-N) (cm^{-1})	ν(C=S) (cm^{-1})
R1	3292	3200	1694, 1634	1443	720
R2	3332	2922-3322	1712, 1650	1415	723
R3	3320	3197	1705, 1643	1406	720
R4	NH overlap with OH at 3316		1722, 1712	1463	720

The electronic spectra of all the four compounds, **R1-R4** are recorded in methanol in UV-visible region at range 190 and 400 nm. In the UV spectra, the absorbance peaks for C=S and C=O are indicated at range 269-275 nm, respectively. In this study, the absorption band for compounds **R1**-**R4** was observed at 270 nm which assigned to the n-π* transitions that commonly reported for C=S and C=O. In comparison of all spectra, it was observed that **R4** exhibited broader band in the area of 275 nm which corresponded to the mixture of π-π* and n-π* transition. This might be due to the contribution of conjugated π bond in phenyl ring which interfere with the absorption of C=S at 270 nm, thus resulting to the broadening of the peak (Fig. 1).

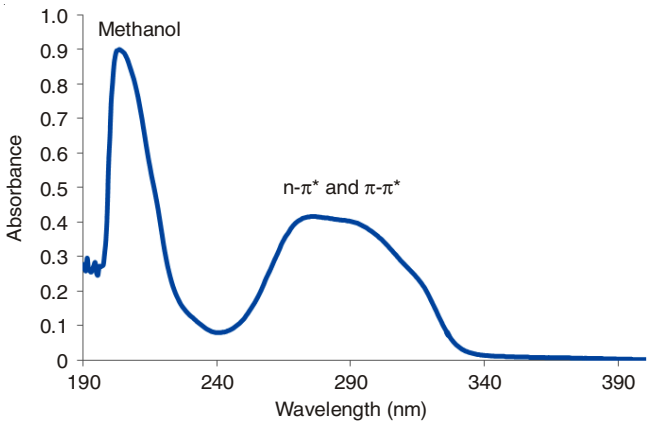


Fig. 1. UV-visible spectra of **R4**

The ^{1}H NMR spectra for **R1-R4** showed signals for alkyl absorption in the range of δ_H 0.84-3.10 ppm. Two signals representing proton for N-H were observed around δ_H 3.55-10.97 ppm. The NH amide next to the C=O group is more deshielded compared to the NH group neighbouring to the C=S. In the ^{1}H NMR spectra of **R4** (Fig. 2(a)), the aromatic protons were displayed as multiplets at δ_H 7.14-7.28 ppm in around similar range to several reports [23,24]. Meanwhile the OH proton was observed at δ_H 9.16-11.28 ppm which also common. In the ^{13}C NMR spectra [Fig. 2(b)], the carbonyl and thione (C=S) carbon were clearly observed in the range of 171.96-177.31 and 174.79-179.94 ppm respectively [25]. The C=S resonance was identified as the most deshielded carbon in the spectra attributed to the presence of two nitrogen atoms in thiourea moiety [26]. The signals for aromatic (Ar) carbon resonances of **R4** was indicated at range 126.6-138.2 ppm and this is closed to the value reported by Liu and co-workers [27].

Antibacterial activity: The inhibition zones were observed for the test strains bacteria, indicating that the lauroyl thiourea

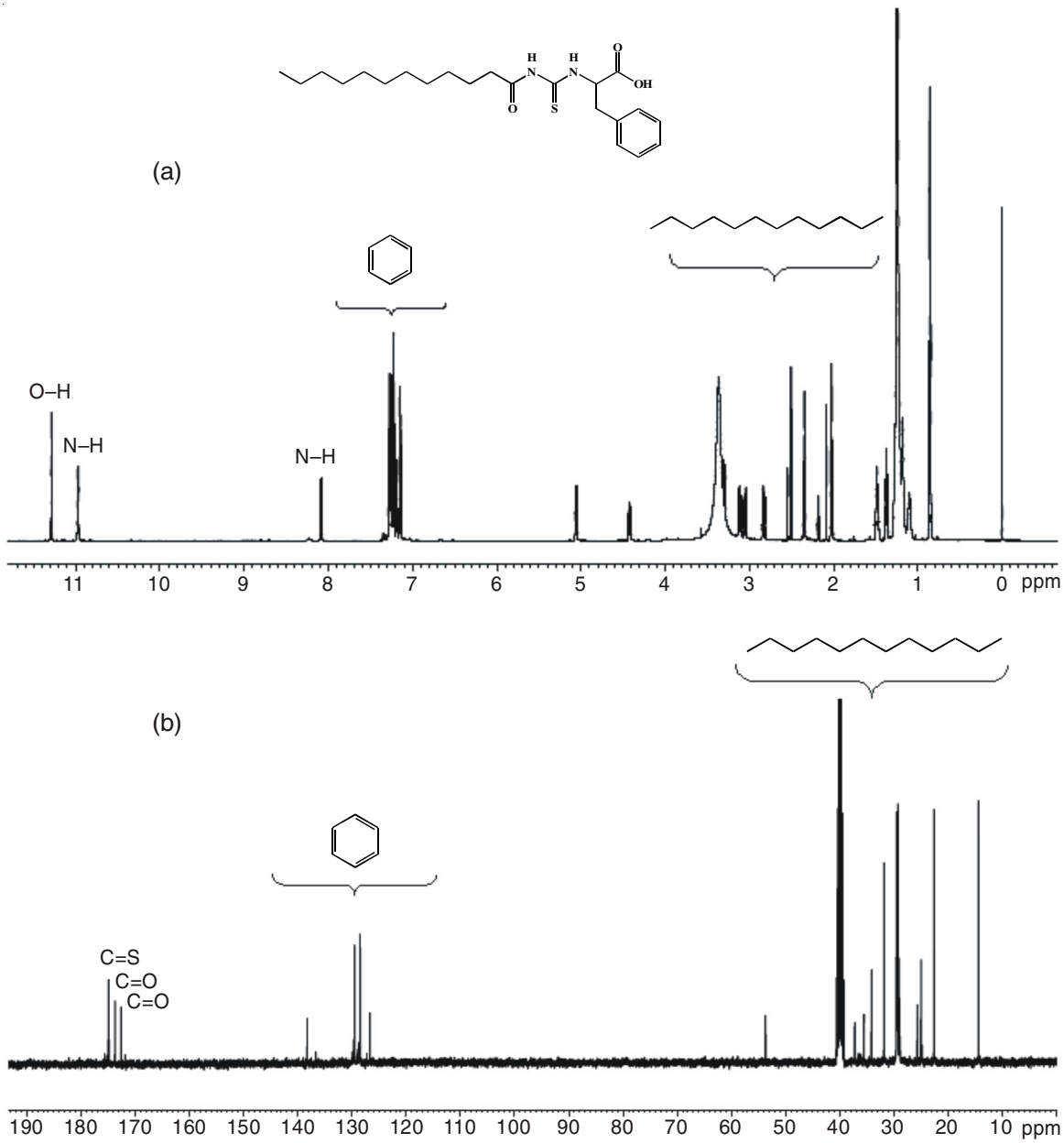


Fig. 2. (a) ^{1}H NMR of **R4** and (b) ^{13}C NMR of **R4**

amino acid derivatives were able to inhibit the growth of bacterial strains tested. The experimental results showed that **R4** has better antibacterial activity against the Gram-negative bacteria *S. typhimurium* and *E. coli*, with inhibition zone of 17 mm indicating strong activity (Fig. 3). This is slightly similar or higher than those antibacterial activity of thiourea derivatives reported in the literature [28]. The second active compound is **R2**, with inhibition zone of 16 mm. As **R2** has bulky structure compared to **R3** and **R1**, the antibacterial activity of **R2** is expected to be better than those two. The very weak activity against all bacteria is displayed by compound **R3**, having acetic acid on one of the pendant arms (derived from glycine). However, the inhibition data performed by **R3** is better compared to some amino acid derivatives reported by many researchers [29-31]. This result suggests that combination of three bioactive units is effective in the design of thiourea molecules as antibacterial agents.

Further study on the MIC properties was carried out on the four compounds following the standard procedures. From

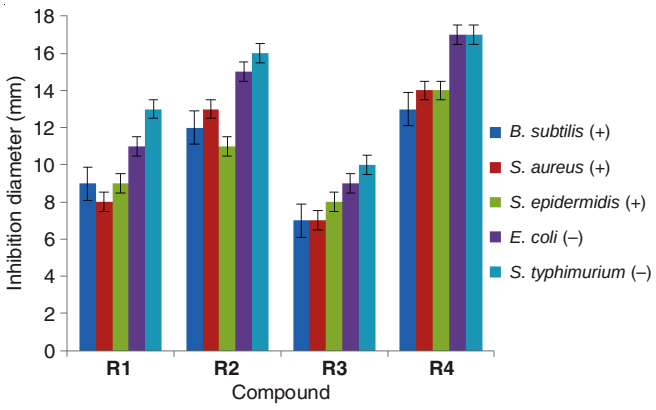


Fig. 3. Antibacterial screening of LTAA derivatives

this study, it was confirmed that **R4** adopts the lowest MIC (50 µg/mL) with inhibition zone of 8 mm against *S. typhimurium* and *E. coli*. The strongest antibacterial activity of **R4** was

attributed by lipophilicity of phenyl ring in **R4**. It is important to take into account that compound with phenyl moiety adopts lipophilic character that allowed better penetration into the microorganisms which inhibit their growth [32]. In addition to that, the presence of delocalized electron at thioamide moeities and also at the conjugated phenyl groups may also attributed to the improvement of antibacterial properties of compound **R4** [32-34]. In this experiment, compounds **R3** and **R1** display very weak antibacterial activities towards the test bacterias with MIC, 1000 µg/mL (Fig. 4).

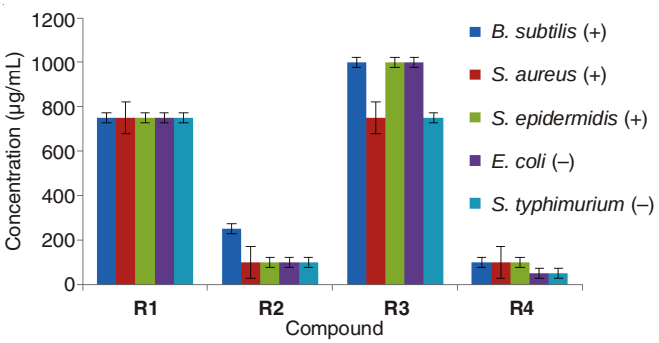


Fig. 4. Zone of inhibition and minimum inhibitory concentration of **R1**-**R4**

This study also showed that **R4** has greater MIC results compared to two similar compounds reported in the literature namely lauryl-poly-L-lysine (LAA) [35] and 2-[*N'*-(2-acetoxy-benzoyl)thioureido]-3-phenylpropanoic acid (AAT) [21]. Fig. 5 shows comparison of the MIC value of most similar reported compounds (LAA and AAT) investigated by other researchers compared with **R4**, all against *E. coli*. The lowest MIC value given by **R4** has showed that combination of amino acid and lauryl units can improve antibacterial performance of thiourea derivatives.

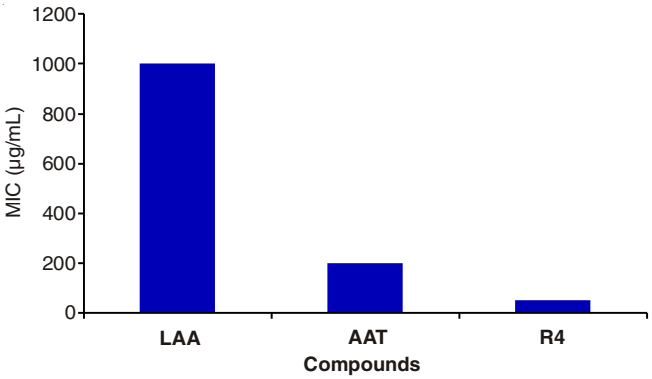


Fig. 5. Antibacterial activity of reported compounds and **R4** against *E.coli*

Conclusion

In conclusion, four new thiourea compounds with lauryl chloride and amino acid containing pendant arms have been successfully designed, synthesized and characterized *via* common spectroscopic techniques. Among of all compounds, compound **R4** has shown the strongest antibacterial activity towards two Gram-negatives bacteria, S. *typhimurium* and *E. coli* due to its better lipophilicity behaviour. The high stability of **R4** is also affected by the presence of high density conjugated electrons from phenyl and thione moieties.

ACKNOWLEDGEMENTS

The authors greatly acknowledge the scientific support from University Malaysia Terengganu. Malaysia Genome Institute (MGI) is also acknowledged for NMR service. Special thanks go to Ministry of Higher Education Malaysia for financial support through Research Acculturation Grants Scheme (RAGS Vot 57077).

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