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Synthesis and Spectroscopic Studies of Alkyl Substituted Thiourea Derivatives Towards the Selected Antibacterial Activities

Wan M. Khairul[1*], Siti Aishah Suskri[1], Rafizah Rahamathullah[1, 2], Noraznawati Ismail[3]
[1]School of Fundamental Science, Universiti Malaysia Terengganu,
21030 Kuala Terengganu, Malaysia
[2]Faculty of Engineering Technology, Universiti Malaysia Perlis, Sungai Chuchuh,
02100 Padang Besar, Perlis, Malaysia
[3]Institute of Marine Biotechnology, Universiti Malaysia Terengganu,
21030 Kuala Terengganu, Terengganu, Malaysia
[*]e-mail: wmkhairul@umt.edu.my

Abstract

In this study, new members of alkyl substituted thiourea derivatives of (R-C(O)NHC(S)NHCH$_2$CH(CH$_3$)NHC(S)NHC(O)-R) in which R represents alkyl group namely isobutyryl and pivaloyl were successfully synthesized for their antibacterial activities. The compounds were spectroscopically and analytically characterized via infrared (IR) spectroscopy, ultraviolet–visible (UV–vis) spectroscopy, CHNS elemental analysis as well melting point analysis. In turn, they were screened for antibacterial activity using selected bacteria namely, *Micrococcus* sp., *Salmonella typhi*, *Aeromonas* sp., *Klebsiella pneumoniae*, *Bacillus cereus* and also *Escherichia coli*. From the antibacterial test, all synthesized compounds were found to exhibit great potential results as antibacterial agents, except for **1** which did not show inhibition activity towards *Klebsiella pneumoniae.* Thus, these proposed molecular structures have given ideal indication to act as antibacterial agents which have utmost potential in pharmaceutical and medical industries in the near future.

Keywords spectroscopic, isobutyryl, pivaloyl, alkyl-thioureas, antibacterial agent

INTRODUCTION

The demand for developing effective antibacteria agents to prevent pathogenic microorganisms has increased significantly due to rising rates of diseases and infections induced by various types of bacteria. Recently, the coordination chemistry of substituted thiourea have received much attention due to their potential molecular framework to be applied in biologically activities [11, 20, 12, 1] such as anti-inflammatory, antiulcer, anticancer, antimutagenic, antidiabetic, analgesic and antimicrobial. The uniqueness of thiourea derivatives are due to the existence of both carbonyl (C=O) and thiocarbonyl (C=S) groups in the structure [5] which gives efficient effects towards bactericidal action. In addition, they are widely known to exhibit important biological activities due to its π-electron clouds and lone pair of nitrogen, sulphur and oxygen atoms in their structure which able to coordinate to metal centres either in form of neutral ligands, monoanions or dianions [14,13].

In this respect, lots of previous works reported on the exploration of varying substituent group on aromatic and heterocyclic rings of the thiourea moiety to determine their different properties such as physical and chemical, crystal structure, thermal behavior and applied widely in numerous applications [2, 18] including in biological and medicinal application. Regarding to this matter, a series of alkyl substituted thiourea derivatives with different alkyl substituents namely isobutyryl and pivaloyl were successfully synthesised as potential antibacterial candidates. These alkyl groups were selected in this study due to their effect of electron donating group which expected to generate ideal hydrophobic characters in which the compounds can penetrate and disrupt the cell membrane easily. Therefore, this work involved the combination of multiple approaches namely synthesis, characterization and evaluation of antibacterial activity towards standard Gram-negative and Gram-positive bacteria.

MATERIALS AND METHODS

Materials and instrumentation

All chemicals and solvents used in this study were purchased from various standard commercial suppliers (Sigma-Aldrich, Merck, Fisher Scientific and R & M Chemical) and used as received without any further purification. The infrared (IR) spectrum was recorded on Fourier Transform Infrared (FT-IR) Perkin Elmer 100 Spectrophotometer by using potassium bromide (KBr) pellets in the spectral range of 4000-400 cm^{-1}. For UV-Visible Absorption Spectroscopy (UV-Vis), the spectrum was recorded in the range of 200-400 nm via Spectrophotometer Shimadzu UV-1601PC in methanolic solution with concentration $1x10^{-5}$ M. Meanwhile, the CHNS Analyzer Flash Elemental Analyzer 1112 series was used to determine the percentage weight of the microelements of C, H, N and S. The determination of melting point of the synthesized compounds were carried out using the Stuart Scientific Melting Point Apparatus SMP3.

General Methodology

The synthetic work-up have been reported in previous works by [4, 6, 7], however some alterations in synthetic work and further characterization on the spectroscopic and analytical tasks were carried out for the interest of this report are discussed further. The mixture of alkyl chloride in acetone was added with equimolar amount of ammonium thiocyanate and designated amines in acetone and was put on reflux with constant stirring for *ca*. 30 minutes. When adjudged completion via thin layer chromatography (TLC) using the solvent system (hexane:ethyl acetate) (3:2), the reaction mixture was filtered and the filtrate was allowed to cool at room temperature. The yellowish filtrate was added with several ice cubes and then filtered to obtain yellow precipitate. The synthetic pathway for the preparation of alkyl-thiourea is summarized in Figure 1.

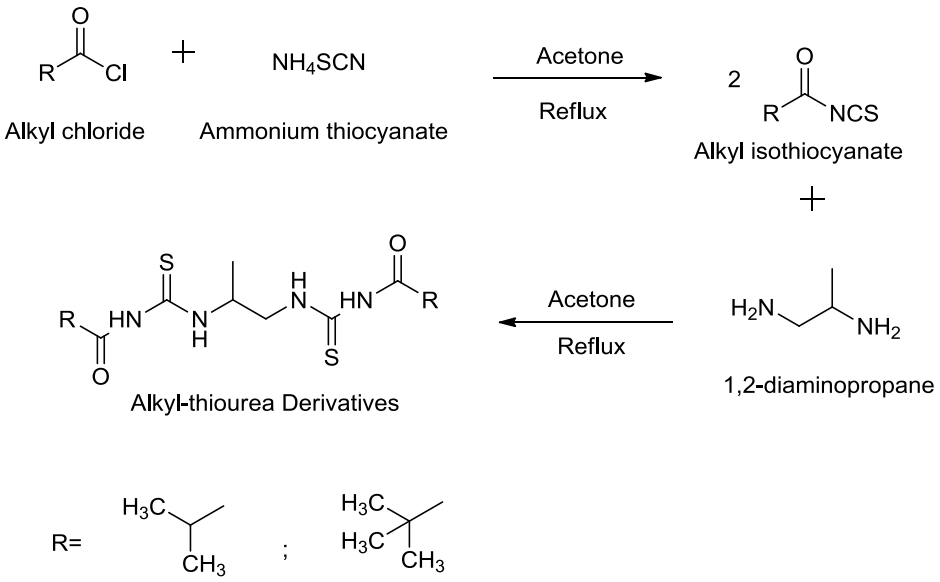

Figure 1 Synthetic pathway for the preparation of alkyl-thioureas

Synthesis of N^1,N^3-bis(isobutyrylcarbamothioyl) propane (1)

The experimental details with regard to the synthesis of 1 was altered from the methods carried out by Douglass & Dains 1934 involving suspension of isobutyryl chloride (1.07 g, 10 mmol) in 50 mL acetone was added dropwise to a solution of ammonium thiocyanate (0.76 g, 10 mmol) in 50 mL acetone. Afterwards, the reaction mixture was then heated at reflux condition with constant stirring for *ca*. 30 minutes before the addition of 1, 2-diamminopropane (0.37 g, 5 mmol). After stirring at reflux continuously for 4 hours, the colour of the mixture solution turned from white to pale yellow solution. After adjudged completion by using TLC (hexane: ethyl acetate; 3:2) the reaction mixture was cooled to room temperature.

The yellowish filtrate was added with ice cubes and then filtered to obtain white precipitate which was then recrystallized from hot methanol to afford crystalline solid of the title compound (60 % yield). IR (KBR): ν(N-H) 3369 cm^{-1}, ν(C=O) 1659 cm^{-1}, ν(C-N) 1264 cm^{-1}, ν(C-S) 758 cm^{-1}. Anal. Calcd for $C_{13}H_{24}N_4O_2S_2$: C, 46.96 H, 7.28 N, 16.85, S, 19.29. Found C, 46.41 H, 7.65 N, 16.23, S, 19.52.

Synthesis of N^1,N^3-bis(pivaloylcarbamothioyl) propane (2)

The white crystalline solid of **2** was prepared from the reaction between pivaloyl chloride (1.21 g, 10 mmol), ammonium thiocyanate (0.76 g, 10mmol) and 1,2-diamminopropane (0.37 g, 5 mmol) in the same manner as described above (65%). m.p (197°C). IR (KBR): ν(N-H) 3220 cm^{-1}, ν(C=O) 1685 cm^{-1}, ν(C-N) 1272 cm^{-1}, ν(C-S) 715 cm^{-1}. Anal. Calcd for $C_{15}H_{28}N_4O_2S_2$: C, 49.97 H, 7.83 N, 15.54, S, 17.79. Found C, 49.64 H, 7.32 N, 15.05, S, 17.32.

Antibacterial activity

The synthesized compounds were screened for their antibacterial activity against two bacteria species of Gram-positive (*Micrococcus* sp. and *Bacillus cereus*) and Gram-negative (*Salmonella typhi*, *Aeromonas* sp., *Klebsiella pneumoniae*, *Escherichia coli*) via Agar well diffusion assay. Ampicilin was used as a standard antibiotic, 1 mg of compounds were dissolved in 1 ml of DMSO. To undergo antibacterial assay, nutrient agar petri plates were prepared with sterile cotton swabs, respective bacterial colony lawns were prepared with 5 ml pepton water for 1 minute followed by culturing the bacteria on the agar gel. After 24 h of incubation, the inhibition zone was measured and the results were determined as the diameter of inhibition zone around the discs in mm.

RESULTS AND DISCUSSION

Physical Properties of alkyl-thioureas (1-2)

Melting Point Analysis

Compounds 1 and 2 started to melt at around 187-197°C and achieved small range of melting point temperature (1-2°C) which represent higher purity of the compounds. The experimental value obtained were supported with the previous study where the range of melting point for thiourea derivatives are between 170 °C to 220 °C [8]. In addition, the features of 1 and 2 that having different alkyl group which present the differences in terms of molecular weight. Therefore, 2 which has higher molecular weight required more energy to break down the intermolecular attraction between atoms and thus the melting point was higher than the lower molecular weight of 1.

CHNS Elemental Analysis

The weight percentages of carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) elements for **1** and **2** were compared with the theoretical values in order to determine the percentage of exact elements presence in the compounds. Based on the result, the target compounds of alkyl-thiourea revealed good agreement with slightly small difference value compared to theoretical value. Therefore, it can be concluded that they have been successfully synthesized and indicated fairly pure compounds analytically by showing good agreement between calculated and experimental value data for C, H, N and S elemental analysis.

IR Spectroscopy Analysis

IR spectra of alkyl-thioureas revealed all the expected band of interest namely ν(N-H), ν(C=O), ν(C-N), ν(C-O) and ν(C=S). Two bands of ν(N-H) can be identified in the range of 3369-3199cm^{-1} which corresponded to the asymmetric and symmetric stretching vibrations of ν(N-H) in the secondary thioamide moiety. The assignment of NH at above 3000 cm^{-1} had been examined to be the existance of C=O…H-N intramolecular hydrogen bond characteristic of -C(O)NHS(O)NH- moiety [9, 16, 15]. The C-H alkane

stretching, strong absorption bands of C-N and C-O for these compounds were observed in range of 3012–2703 cm^{-1}, 1383–1252 cm^{-1}, and 1172–1160 cm^{-1} respectively. In addition, the existence of new absorption bands for C=O and C=S can be observed in the range of 1685-1659cm^{-1} and 758-715cm^{-1} respectively, in which it highly indicated the compound was indeed thiourea derivatives. The strong absorption of ν(C=O) stretching band that appeared at around 1685-1659cm^{-1}, apparently decreased in frequency compared to the ordinary carbonyl compounds which a signature for the presence of intramolecular hydrogen bond towards N-H moiety [19]. Additionally, ν(C=S) stretching vibration can be clearly noticed at a low frequency of 758-715cm^{-1} due to less double bond character and lower nucleophilic character of sulfur atom in C=S moiety that were in close agreement with other several reported series of thiourea derivatives [10, 17].

UV-Visible Spectroscopy Analysis

Electronic transition spectra of these molecules were recorded in methanol solution in 1 cm^3 cuvette with concentration of 1 x 10-5 M , and they exhibited two principal bands that are believed to arise from the carbonyl (C=O) and thiocarbonyl (C=S). The absorption band of C=O chromophore can be observed in the range of λ_{max} 220nm to 223nm due to the effect of π-conjugation of carbonyl and thus the transition of π→π* shifted to the longer wavelength. Strong absorption band was identified in the region around λ_{max} 269-271nm can be attributed to π→π* and n→π* transitions was due to the presence of overlapping C=O and C=S moietis.

Antibacterial Studies

The synthesized compounds were screened for their antibacterial activities against pathogenic bacteria such as Gram-positive (*Micrococcus* sp. and *Bacillus cereus*) and Gram-negative (*Salmonella typhi*, *Aeromonas* sp., *Klebsiella pneumoniae, Escherichia coli*) by using disk diffusion method (Kirby-Bauer method). The ampicillin act as positive control was used to give a ideal comparison between antibacterial activity of positive control and alkyl-thiourea. Meanwhile, dimethyl sulfoxide (DMSO) was applied as negative control since all compounds were soluble in this solvent and DMSO gave no influence towards inhibition of bacterial strains. The tests were repeated three times and the results are summarized in Table 1.

Table 1 Antibacterial bioassay screening of alkyl-thioureas (1-2) against tested strains

Compound	Bacteria activity*					
	Gram-positive		Gram-negative			
	Micrococcus sp.	*Bacillus cereus*	*Salmonella typhi*	*Aeromonas* sp.	*Klebsiella pneumoniae*	*Escherichia coli*
1	1	2	1	3	-	2
2	1	4	1	1	3	2
positive control (*Ampicilin*)	27	1	7	1	2	15
negative control (DMSO)	-	1	1	2	1	-

*Zone of inhibition (radius, mm).

Compounds 1 and 2 showed to be active against all tested selected bacterial strains excluding 1 which did not show inhibition activity towards *Klebsiella pneumoniae*. However, most of investigated compounds with alkly group inhibited the growth of bacteria with moderate activity which may arise because of their

low lipophilicity where they do not penetrate into the microorganisms as easily as the acyl thiourea derivatives as reported previously [3]. Thus, from this preliminary study, alkyl-thiourea have an ability to change the membrane and inhibit the growth of selected bacteria.

CONCLUSION

New derivatives of alkyl thiourea compounds featuring isobutyryl and pivaloyl substituents have been successfully designed and characterized to act as potential materials in the application as an antibacterial agents. The compounds were spectroscopically and analytically characterized via Infrared (IR) spectroscopy, Ultraviolet–visible (UV–vis) spectroscopy, CHNS elemental analysis as well melting point analysis. Based on their bacterial activity, all compounds exhibited moderate activity towards selected studied bacteria. Compound 2 which consists of pivaloyl substituent exhibited to give higher activity compared to 1 due to strong donating effect and hydrophobic characters. In conclusion, throughout this study, these new class of thiourea derivatives have an ability to inhibit the growth of bacteria, therefore, further investigation on various molecular system of alkyl-thiourea with different types of substituents should be under spotlight for further development in the field of pharmaceutical interest.

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