Synthesis, Spectroscopic Studies, and Biological Activities of Acylthiourea Derivatives as Potential Anti-Bacteria Agents

Wan M. Khairul[1,*], Aziah Ahmad Ariffin[1], Noraznawati Ismail[2], Adibah Izzati Daud[1, 3]
[1]School of Fundamental Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
[2]Institute of Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
[3]Faculty of Engineering Technology, Universiti Malaysia Perlis (UniMAP), Aras 1, Blok S2, Kampus UniCITI Alam, Sungai Chuchuh, Padang Besar, 02100, Perlis, Malaysia
[*]e-mail: wmkhairul@umt.edu.my

Abstract

Thiourea is known to be a unique class of organic compounds especially acylthiourea since they consist excellent H-bonds donor and acceptor where it has strong ability to form metal complexation with various metal salts. Therefore, six members of acylthiourea derivatives (TH1-TH6) have been successfully synthesized and characterised using selected spectroscopic and analytical methods namely Fourier-Transform Infrared (FT-IR) spectroscopy, UV-visible (UV-vis) spectrophotometer, and CHNS elemental analysis. In turn, all the synthesised TH1-TH6 were used to investigate the anti-bacterial activity towards selected bacteria of both Gram-negative and positive namely *Salmonella typhi* and *Bacillus cereus* respectively. TH1-TH6 revealed to have good activity towards *S. typhi* and *B. cereus* with specific and broad-range spectrum activity.

Keywords thiourea; spectroscopic; antibacterial; synthesis; characterisation

INTRODUCTION

The occurrence of resistance towards antibacterial mediators is widely known to be severe health problem in our daily life today. Currently, the devotion has been engrossed on the multi-drug resistance resulting from the extensive use and abuse of classical antimicrobial drugs. Nowadays, bacterial resistance against antibiotics is an ongoing main problem in the therapy of bacterial infections [16]. The development of effective ocular drugs of antimicrobial agents is one of the crucial aspects and a big challenge in medicinal chemistry, whereas to date, there is still lack of concern regarding ocular healing mediations. Nowadays, various types of active compounds featuring new derivatives have been synthesised from organic compounds [18, 8]. Moreover, thiourea moiety is classified as one of the organic compounds containing carbon, nitrogen, hydrogen, and sulfur elements that have currently gained a lot of attention from many researchers. New derivatives of thiourea is synthesised by organic chemist which in turn are investigated towards any biological cells or any system of interest. Thiourea derivatives show a wide range of biological activity including anti-bacterial [1], anti-fungal [14], anticancer [11] and also as a plant growth regulator properties [13]. The implication of previous work lies in the prospect that the future generation thiourea derivatives might be more efficient as antimicrobial and anticancer agents owing to the presence of both carbonyl (C=O) and thiocarbonyl (C=S) groups [15]. However, a depth investigation involving the structure and the activity of the thiourea derivatives and their stability under biological conditions are essential. These comprehensive studies could be helpful in designing more effective antimicrobial and anticancer agents for the therapeutic use. This study consists of combination of multiple approaches such as, synthesis, structural elucidation, and the evaluation of thiourea derivatives towards *S. typhi* and *B. cereus* by investigating their inhibition zone (mm). The active materials of antibacterial agents introducing thiourea derivatives were synthesised, and studied by typical spectroscopic analysis namely Fourier Transform Infrared (FT-IR) spectroscopy, UV-visible spectrophotometer, and CHNS elemental analysis. TH1-TH6 (molecular structures as shown in Figure 1) however, have been published elsewhere [4], however, some alterations in the synthetic work, advance characterisation and biological studies have been carried out and discussed in this contribution in details.

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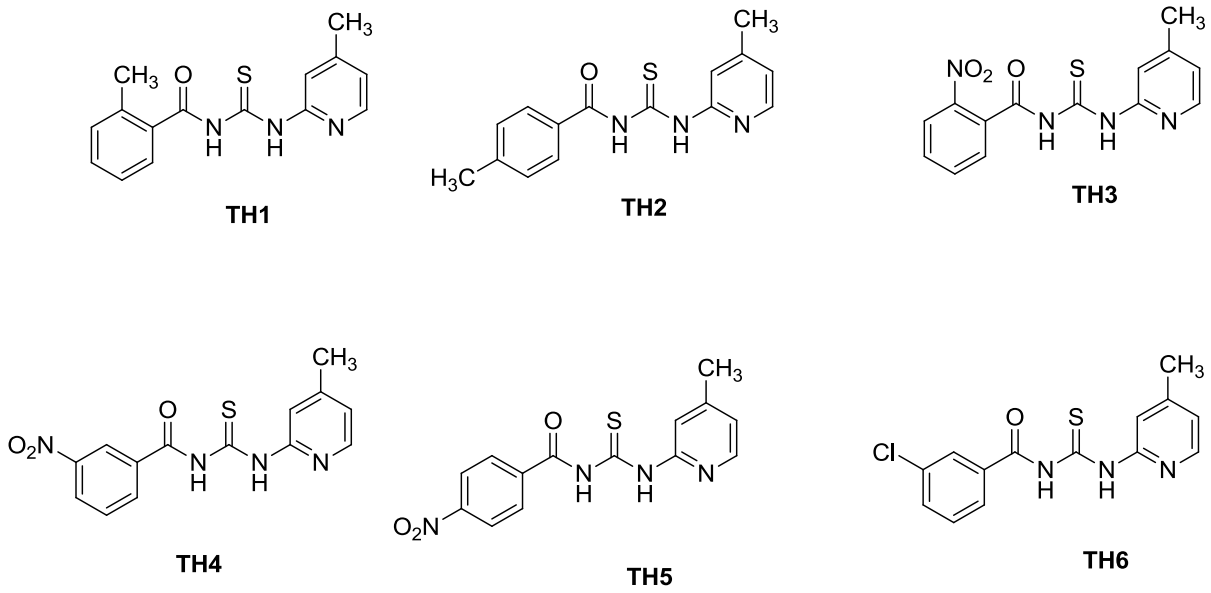

Figure 1 Molecular structures of acylthiourea derivatives (TH1 – TH6)

RESEARCH METHODOLOGY

Materials and general synthetic work-up

All synthetic work-up have been done under ambient atmosphere without inert environment to eliminate moisture during experimental work-up. Chemicals used were acquired from numerous standard suppliers (Merck, Fisher Scientific, R&M Chemicals, and Sigma-Aldrich) and used as received without extra purification. Infrared (IR) spectra of all synthesised compounds were recorded from KBr pellets via Fourier Transform Infrared (FT-IR) Perkin Elmer 100 spectrophotometer in the spectral range 4000 – 450 cm^{-1}. Besides, CHNS elemental analysis was carried out by CHNS Flashea 1112 series. Meanwhile, UV-visible analysis spectra of all synthesised compounds (TH1-TH6) were recorded by Shidmadzu UV-vis 1601 series in methanolic solution of concentration 1 x 10^{-5}M. In addition, the melting point was obtained in the range 20°C - 360°C by Stuart Scientific model SMP3.

Preparation of N-(4-methylpyridine)-N'-(2-methylbenzoylthiourea) (TH1)

The experimental procedure of thiourea derivatives (TH1 – TH6) followed the general method in synthesizing typical thiourea derivatives which have been described before in previous literature [3]. However, several modifications in synthetic work-up have been made, improved, and deliberated in this occasion. Figure 2 illustrates general synthetic pathway to synthesis acylthiourea derivatives. A solution of 2-methylbenzoyl chloride (2.01 g, 13 mmol) in 20 ml acetone was transferred dropwise into a solution of ammonium thiocyanate (0.98 g, 13 mmol) in 20 ml acetone. The reaction mixture was stirred for ca. 10 minutes in a two necked 250 ml round-bottomed flask. A solution of 2-amino-4-methylpyridine (1.41 g, 13 mmol) in 50 ml acetone was added dropwise to the mixture. The reaction mixture was continued at reflux for ca. 1 hour. The evolvement of the reaction was examined using Thin Layer Chromatography (TLC). When the reaction completed, the mixture was cooled to room temperature filtered to remove white precipitate. The filtrate attained was added to several blocks of ice cubes, in turn, filtered to obtain yellowish precipitate. The precipitate next, recrystallised from acetone to yield title compound TH1 (53%) as yellowish solid. IR (KBr): ν(N-H) 3325 cm^{-1}, ν(C=O) 1683 cm^{-1}, ν(C-N) 1329 cm^{-1} ν(C=S) 729 cm^{-1}. Require: C, 63.1; H, 5.3; N, 14.7; S, 11.2 %. Found: C, 63.5; H, 5.1; N, 16.5; S, 12.7 %. Melting Point; 158.1-159.8°C.

Preparation of N-(4-methylpyridine)-N'-(4-methylbenzoylthiourea) (TH2)

Yielding 45% yellowish precipitate, TH2 was synthesised from 4-methylbenzoyl chloride (1.96 g, 13 mmol), ammonium thiocyanate (0.96 g, 13 mmol), and 2-amino-4-methylpyridine (1.37 g, 13 mmol) in a same manner as for TH1. IR (KBr): ν(N-H) 3286 cm^{-1}, ν(C=O) 1681 cm^{-1}, ν(C-N) 1335 cm^{-1} ν(C=S) 733 cm^{-1}. Require: C, 63.1; H, 5.3; N, 14.7; S, 11.2 %. Found: C, 62.4; H, 5.1; N, 15.0; S, 14.6 %. Melting Point; 183.1-185.9°C.

Preparation of N-(4-methylpyridine)-N'-(2-nitrobenzoylthiourea) (TH3)

Yielding 58% yellowish precipitate, TH3 was synthesised from 2-nitrobenzoyl chloride (2.01 g, 10.8 mmol), ammonium thiocyanate (0.82 g, 10.8 mmol), and 2-amino-4-methylpyridine (1.17 g, 10.8 mmol) in a same manner as for TH1. IR (KBr): ν(N-H) 3131 cm^{-1}, ν(C=O) 1676 cm^{-1}, ν(C-N) 1347 cm^{-1}, ν(C-S) 785 cm^{-1}. Require: C, 53.1; H, 3.8; N, 17.71; S, 10.1 %. Found: C, 53.2; H, 3.8; N, 18.6; S, 11.8 %. Melting Point; 158.9-161.5°C.

Preparation of N-(4-methylpyridine)-N'-(3-nitrobenzoylthiourea) (TH4)

Yielding 62% white-off precipitate, TH4 was synthesised from 3-nitrobenzoyl chloride (2.01 g, 10.8 mmol), ammonium thiocyanate (0.82 g, 10.8 mmol), and 2-amino-4-methylpyridine (1.17 g, 10.8 mmol) in a same manner as for TH1. IR (KBr): ν(N-H) 3414 cm^{-1}, ν(C=O) 1674 cm^{-1}, ν(C-N) 1349 cm^{-1} ν(C-S) 716 cm^{-1}. Require: C, 53.1; H, 3.8; N, 17.7; S, 10.1 %. Found: C, 52.5; H, 4.4; N, 18.3; S, 11.6 %. Melting Point; 194.5-202.5°C.

Preparation of N-(4-methylpyridine)-N'-(4-nitrobenzoylthiourea) (TH5)

Yielding 62% yellow precipitate, TH5 was synthesised from 4-nitrobenzoyl chloride (2.01 g, 10.8 mmol), ammonium thiocyanate (0.82 g, 10.8 mmol), and 2-amino-4-methylpyridine (1.17 g, 10.8 mmol) in a same manner as for TH1. IR (KBr): ν(N-H) 3340 cm^{-1}, ν(C=O) 1664 cm^{-1}, ν(C-N) 1345 cm^{-1}, ν(C-S) 711 cm^{-1}. Require: C 53.1; H, 3.8; N, 17.7; S, 10.1 %. Found: C, 55.6; H, 3.3; N, 16.8; S, 11.0 %. Melting Point; 192.5-193.8°C.

Preparation of N-(4-methylpyridine)-N'-(3-chlorobenzoylthiourea) (TH6)

Yielding 67% orange precipitate, TH6 was synthesised from 3-chlorobenzoyl chloride (2.01, 11.5 mmol), ammonium thiocyanate (0.87 g, 11.5 mmol), and 2-amino-4-methylpyridine (1.24 g, 11.5 mmol) in a same manner as for TH1. IR (KBr): ν(NH) 3340 cm^{-1}, ν(C=O) 1664 cm^{-1}, ν(C-N) 1345 cm^{-1} ν(C-S) 711 cm^{-1}. Require: C, 54.9; H, 3.9; N, 13.7; S, 10.4%. Found: C, 54.8; H, 3.6; N, 13.5; S, 11.0 %. Melting Point; 135.7-136.4°C.

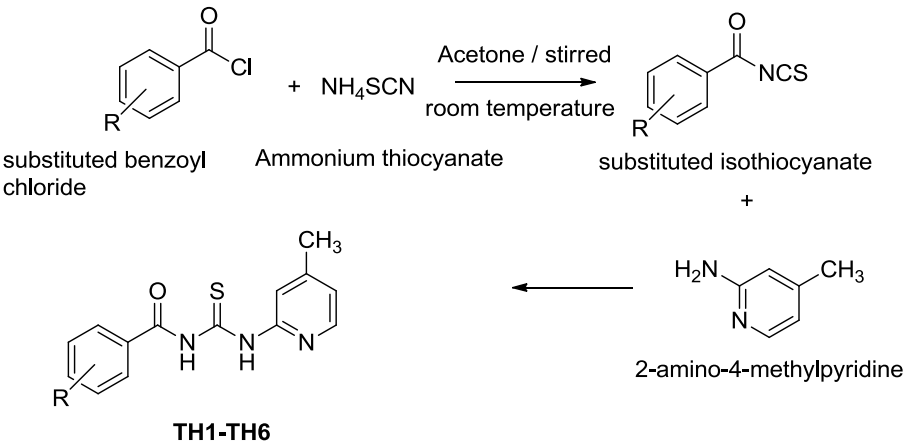

Figure 2 General synthetic work-up for TH1-TH6

Antibacterial test for synthesised compounds

Bacteria strains used in this study are Gram-positive and negative bacteria namely *Salmonella typhi* and *Bacillus cereus* were obtained from Microbiological Laboratory, School of Fundamental Science, Universiti Malaysia Terengganu. TH1 – TH6 were dissolved in DMSO with concentration 1 mg/mL. Paper disc with diameter 6 mm have been autoclaved at 121°C before used. Later, the paper discs were dipped into TH1 – TH6 solution, and the discs were put onto the surface of agar containing growth bacteria. The plates were then inverted and overnight at 27°C before the zones of inhibition were observed after 24 hours.

RESULTS AND DISCUSSION

Spectroscopic studies

Infrared (IR) spectra of TH1 – TH6 indicated four distinctive important bands which are, ν(N-H), ν(C=O), ν(C-N), and ν(C=S) in which the intensity ranging from weak, modest and strong intensities. The absorption peaks for carbonyl moieties ν(C=O) can be observed at 1673 –1685cm^{-1} which deceptively decreasing in frequencies in comparison to the ordinary carbonyl absorption found at 1710 cm^{-1} due to its conjugated behaviour arising from resonance of phenyl ring and the formation of intra-molecular hydrogen bonding with secondary amine (N-H) [17]. In fact, the presence of strong electron donating group of methyl, weaken the bonding of carbonyl group, therefore absorption bands of C=O shifted to the lower frequency. Besides, the ν(N-H) stretching vibration of secondary amine can be observed at 3475 cm^{-1} – 3150 cm^{-1} which is in same argumentas reported by [2]. Moreover, the absorption bands for ν(C-N) occurred at higher frequency in aromatic due to the resonance effect increased in the double bond characteristics between the rings with the devoted electronegative nitrogen atom as stated by [12]. Indeed, the presence of ν(C=S) in TH1 – TH6 were proven to be thiourea derivatives, were seen in the region between 711 cm^{-1} - 784 cm^{-1} with strong intensity which was consistent to the previous work carried out by [5]. Table 1 shows the summarised data of Infra-red analysis for TH1-TH6.

Table 1 The IR data of TH1-TH6

Compounds	ν(N-H)	ν(C=O)	ν(C-N)	ν(C=S)
TH1	3237 (m)	1683 (s)	1329 (s)	720 (s)
TH2	3282 (s)	1681 (s)	1334 (s)	751 (m)
TH3	3310 (m)	1677 (w)	1348 (s)	715 (s)
TH4	3134 (w)	1674 (m)	1347 (s)	702 (s)
TH5	3245 (m)	1677 (m)	1351 (s)	717 (s)
TH6	3308 (w)	1673 (s)	1336 (s)	726 (m)

The electronic absorption spectra of TH1 - TH6 were recorded in methanolic solution obtained in a 1 cm path length quartz cell with concentration of 1 x 10^{-5} M which resulted two major bands of interest, attributed from C=O, C=S, and aromatic moieties. Absorption band for C=O chromophore in TH1 – TH6 were observed at λ_{max}in the range 229.6- 267.4 nm with strong and broad bands. For the thiol (C=S) chromophore, the absorption of weak and broad bands were observed in the range λ_{max}, 272.6 - 308.6 nm. The presence of broad absorption bands is due to multiple vibrational mode presence in each electronic transition level. Table 2 shows the λ_{max} and molar absorptivity of TH1 – TH6.

Table 2 The summarised data of λ_{max} and molar absorptivity of TH1 – TH6

Compound	λ_{max} (ε / $M^{-1}cm^{-1}$)	
	C=O	C=S
TH1	237.6 (46740)	272.7 (37880)
TH2	267.4 (71610)	307.2 (83130)
TH3	253.6 (74570)	302.6 (45370)
TH4	229.6 (47940)	256.0 (29250)
TH5	262.8 (48050)	262.8 (48050)
TH6	253.4 (15860)	275.2 (84290)

TH1 – TH6 exhibited bathochromic shift (red shift), due to the presence of auxochromes on the C=O chromophores. The auxochromes featuring donating and withdrawing groups contain unshared (non-bonding) electrons pairs; shifted the λ_{max} to higher wavelength. The presence of bathochromic shift, proven the existence of the mixed $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions in these synthesised compounds of unsaturated bonds.

Antibacterial Screening

Antibacterial studies of all synthesised compounds TH1-TH6 were assayed in order to determine the potential and possibilities of TH1 – TH6 to act as antibacterial agents, involving two types of bacteria, Gram-negative and positive. The differences between these two types of bacteria were differing in their chemical composition and structural arrangement of the entire bacteria cell walls [9]. The bacteria inhibition zone was observed and determined. In this work, ampicillin which is an antibiotic was used as a positive control while dimethylsulfoxide (DMSO) was used as negative control. Figure 3 presents the comparison graph of the inhibitory activity of bacterial strains under treatment of TH1-TH6.

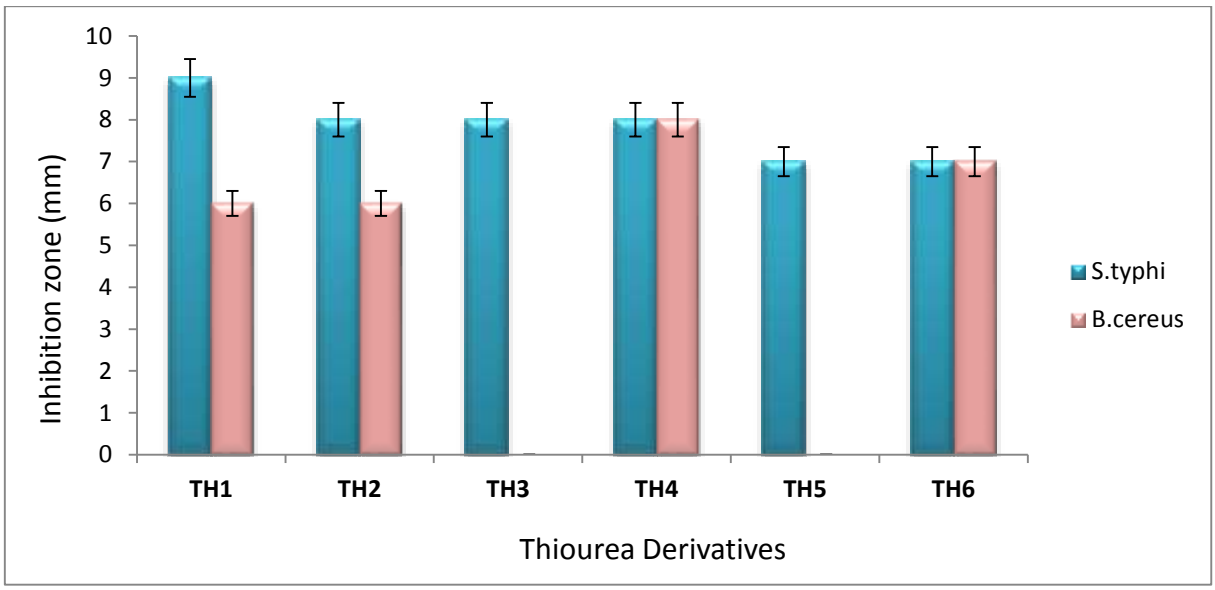

Figure 3 Comparison graph of the inhibitory activity of bacterial strains under treatment of TH1-TH6

Result obtained shown that most of the synthesised compounds indicated their inhibition zone towards *S.typhi* and *B.cereus* at the minimum concentration of 1 mg/mL. TH3 and TH5 revealed to have specific activity due to the ability of both compounds to inhibit only the growth of Gram-negative bacteria, while TH1, TH2, TH4, and TH6 gave broad-range spectrum activities in which can inhibit both Gram-positive and negative bacteria strains. All synthesised compounds (TH1-TH6) have inhibition zone towards *S.typhi* bacteria strain in range 7 – 9 mm which indicated moderate antibacterial activity. However, for *B.cereus*

bacteria strain, TH1, TH2, TH4, and TH6 revealed positive results in range 6 – 8 mm. In fact, the structure of Gram-positive coated by murein makes any drugs easily to penetrate into bacteria cell walls and inhibit the growth of bacteria at any suitable concentration. Whilst, Gram-negative bacteria cell walls are somehow thicker than Gram-positive bacteria, contains secondary lipid bilayer (outer membrane) outside coated murein of cell wall. However, in this study, thiourea derivatives of TH1-TH6 gave positive inhibition result around 6 – 9 mm of weak activity towards both Gram-negative and positive bacteria, in same arguments with previous reported publications [7]. The inhibition results of treated bacteria strains by TH1-TH6 of thiourea derivatives were expected to surround the cell wall surfaces and avoid the escape of intracellular components [6]. In addition, the presence of potential active functional groupsof C=O, C=S, and N-H in all thiourea derivatives protonated in DMSO solution were further reacted with carboxyl and phosphate groups of bacterial surfaces in which consequently gave antibacterial activity against both types of bacteria [10].

CONCLUSION

Six derivatives of thiourea compounds (TH1-TH6) have been synthesised, characterised and assayed biologically to act as antibacterial agents. For structural elucidation of TH1-TH6, all synthesised compounds were spectroscopically characterised via FT-IR spectroscopy, UV-visible spectrophotometer, and CHNS elemental analysis. From biological investigation, TH1, TH2, TH4, and TH6 exhibited broad-range spectrum activities towards *B.cereus* and *S.typhi* bacteria strains. In contrast, only TH3 and TH5 revealed specific activity towards *S.typhi* bacteria strain. To conclude, throughout this study, the synthesised thiourea derivatives have shown an ability to inhibit the growth of bacteria strains.

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