

Synthesis, Characterization and Antibacterial Screening of 3-Hydroxy-2-[3-(2/3/4-Methoxybenzoyl)Thioureido]Butyric Acid

M. S. M. Yusof, R. Ramli, S. K. C. Soh, N. Ismail, N. Ngah

Abstract—This study presents the synthesis of a series of methoxybenzoylthiourea amino acid derivatives. The compounds were obtained from the reactions between 2/3/4-methoxybenzoyl isothiocyanate with threonine. All of the compounds were characterized via mass spectrometry, ^1H and ^{13}C NMR spectrometry, UV-Vis spectrophotometer and FT-IR spectroscopy. Mass spectra for all of the compounds showed the presence of molecular ion $[\text{M}]^+$ peaks at m/z 312, which are in agreement to the calculated molecular weight. For ^1H NMR spectra, the presence of OCH_3 , $\text{C}=\text{S}-\text{NH}$ and $\text{C}=\text{O}-\text{NH}$ protons were observed within range of δ_{H} 3.8-4.0 ppm, 11.1-11.5 ppm and 10.0-11.5 ppm, respectively. ^{13}C NMR spectra in all compounds displayed the presence of OCH_3 , $\text{C}=\text{O}-\text{NH}$, $\text{C}=\text{O}-\text{OH}$ and $\text{C}=\text{S}$ carbon resonances within range of δ_{C} 55.0-57.0 ppm, 165.0-168.0 ppm, 170.0-171.0 ppm and 180.0-182.0 ppm, respectively. In UV spectra, two absorption bands have been observed and both were assigned to the $n-\pi^*$ and $\pi-\pi^*$ transitions. Six vibrational modes of $\nu(\text{N}-\text{H})$, $\nu(\text{O}-\text{H})$, $\nu(\text{C}=\text{O}-\text{OH})$, $\nu(\text{C}=\text{O}-\text{NH})$, $\nu(\text{C}=\text{C})$ aromatic and $\nu(\text{C}=\text{S})$ appeared in the FT-IR spectra within the range of 3241-3467 cm^{-1} , 2976-3302 cm^{-1} , 1720-1768 cm^{-1} , 1655-1672 cm^{-1} , 1519-1525 cm^{-1} and 754-763 cm^{-1} , respectively. The antibacterial activity for all of the compounds was screened against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhimurium* and *Escherichia coli*. However, no activity was observed.

Keywords—Methoxybenzoyl isothiocyanate, amino acid, threonine, antibacterial.

I. INTRODUCTION

THIOUREA is a compound that contains carbon, hydrogen, sulphur and nitrogen atoms in its molecular structure [1]. Since the introduction of first thiourea derivatives by Nencki in 1873 [2], different types of thiourea derivatives have been synthesized such as arylthiourea [3], [4], alkylthiourea [5], acylthiourea [6], [7], aroylthiourea [8], disubstituted and trisubstituted thiourea [9], symmetrical thiourea [10], unsymmetrical thiourea [11], [12] and many others. Among all, benzoylthiourea derivatives which fall under aroylthiourea group have received great attention from researchers worldwide. This is due to the presence of delocalized phenyl and carbonyl groups as bioactive components instead of only thione and amino group in thiourea compound. Benzoylthiourea derivatives were very well known for their diverse biological activity such as antibacterial [13],

antituberculosis and antifungal [14], urease inhibitor [15]. This compound can coordinate to metal atom to form stable coordination complexes [16]-[18].

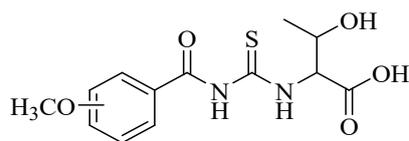
Amino acids consist of amino group, carboxy group and also side chain. Amino acids are classified according to their capacity of interacting with water. Polar amino acids easily interact with water as they contain hydroxyl group which is capable of making hydrogen bonding. For nonpolar amino acids, their poor interaction with water made them capable of maintaining the 3-dimensional structure of protein. Meanwhile, acidic amino acids can be identified by the presence of negatively charged carboxylate group. Basic amino acids bear a positive charge which can accept proton from water to form conjugate acid [19]. The properties of amino acids are different depending on group/orientation of the side groups attached. However, in this paper, only substituted-methoxybenzoyl isothiocyanate in reaction with threonine are reported.

Structural study is one of the most attractive methods of molecular modeling and studying. This study usually focuses on substituents effect and positional isomerism. In general, substituents are divided into electron donating group (EDG) and electron withdrawing group (EWG). EDG stabilizes the π system by electron donation while EWG causes π system to destabilize after withdrawal of electron density. Due to the interesting role of substituents, many studies related to substituents effect with different attachment to the *ortho*-, *meta*- and *para*- positions of aromatic ring have been carried out [20]. However, most of the reported studies focused on the effect of substituents towards the biological activity [13], [14] and other applications [16]-[18]. In this study, the aim is to focus on the effect of substituents and positional isomerism toward the FT-IR, UV-Vis and NMR data obtain.

Owing to the versatility of benzoylthiourea derivatives, this study has focused on the synthesis of a series of methoxybenzoylthiourea amino acid derivatives. The research design which involves combination of substituted-methoxybenzoylthiourea and amino acid (Fig. 1) was chosen; because, these compounds are interesting to explore due to the difficulties of synthesizing.

M. S. M. Yusof*, R. Ramli, S. K. C. Soh, N. Ismail are with the Universiti Malaysia Terengganu, Terengganu, Malaysia (*e-mail: mohdsukeri@umt.edu.my).

N. Ngah is with the International Islamic University Malaysia, Pahang, Malaysia.



Where OCH₃ at:

Ortho : 2-MeOBTT

Meta : 3-MeOBTT

Para : 4-MeOBTT

Fig. 1 Molecular structure of *o/m/p*-MeOBTT

II. EXPERIMENTAL

Physical Measurements

Chemicals in this study were used without further purification. Chemicals were purchased from Merck (*o/m/p*-methoxybenzoyl chloride), Acros Organics (Threonine) and R & M Chemicals (Ammonium thiocyanate). Mass spectra were recorded using Gas Chromatograph Mass Spectrometer GCMS-QP2010 Ultra. The NMR samples were characterized at Institute Marine Biotechnology, UMT and have been recorded by Bruker Avance III 400 MHz Spectrometer. UV samples were run in methanol and recorded by UV-1800 Shimadzu UV spectrophotometer. The IR spectra were recorded using FT-IR Perkin Elmer 100 Spectrometer.

Preparation of 3-Hydroxy-2-[3-(2-Methoxybenzoyl)Thioureido]Butyric Acid (2-MeOBTT)

The procedure applied was proposed earlier by [20]. A solution of ammonium thiocyanate (0.23 g, 3.0 mmol) in acetone (30 mL) was added dropwise into the two neck round bottomed-flask containing 2-methoxybenzoyl chloride (0.51 g, 3.0 mmol). The mixture was stirred for 10 minutes. A solution of 30 mL of threonine (0.36 g, 3.0 mmol) in 30 mL of acetone was added into the reaction mixture. The mixture was stirred and refluxed for *ca.* 6 hours. Reaction progress was monitored using Thin Layer Chromatography (TLC). Solution was filtered into a beaker containing ice cubes to afford the titled compound. Experimental data: Yellow solid; 0.29 g, yield 30.61%; EI-MS: [M]⁺ *m/z* = 312 (1%); ¹H NMR (DMSO-*d*₆) ppm: 1.136, 3.998, 4.312, 4.809, 7.159, 7.270, 7.655, 7.893, 11.112, 11.132; ¹³C NMR (DMSO-*d*₆) ppm: 21.38, 57.12, 63.97, 66.47, 113.27-157.94, 165.67, 171.22, 180.96; UV-Vis (MeOH): λ_{max}, nm (ε, Lmol⁻¹cm⁻¹) 250.2 (39000), 290.0 (26400); IR (KBr pellets): ν(N-H) 3447 cm⁻¹ (m), ν(O-H) 3302 cm⁻¹ (m), ν(C=O-OH) 1769 cm⁻¹ (s), ν(C=O-NH) 1659 cm⁻¹ (s), ν(C=C) 1526 cm⁻¹ (s), ν(C=S) 757 cm⁻¹ (m).

Preparation of 3-Hydroxy-2-[3-(3-Methoxybenzoyl)Thioureido]Butyric Acid (3-MeOBTT)

An amount of (0.51 g, 3.0 mmol) 3-methoxybenzoyl chloride, (0.23 g, 3.0 mmol) ammonium thiocyanate and (0.36 g, 3.0 mmol) threonine have been used for the preparation of 3-MeOBTT. The title compound 3-MeOBTT was synthesized in the same manner as described earlier. Experimental data: Yellow solid; 0.312 g, yield 33.33%; EI-MS: [M]⁺ *m/z* = 312

(1%); ¹H NMR (DMSO-*d*₆) ppm: 1.145, 3.846, 4.324, 4.868, 7.194-7.560, 11.330, 11.506; ¹³C NMR (DMSO-*d*₆) ppm: 20.83, 55.34, 63.33, 66.00, 113.03-158.99, 167.97, 170.81, 181.44; UV-Vis (MeOH): λ_{max}, nm (ε, Lmol⁻¹cm⁻¹) 250.0 (2940), 285.5 (2230); IR (KBr pellets): ν(N-H) 3242 cm⁻¹ (m), ν(O-H) 2976 cm⁻¹ (m), ν(C=O-OH) 1727 cm⁻¹ (s), ν(C=O-NH) 1676 cm⁻¹ (s), ν(C=C) 1519 cm⁻¹ (s), ν(C=S) 754 cm⁻¹ (m).

Preparation of 3-Hydroxy-2-[3-(4-Methoxybenzoyl)Thioureido]Butyric Acid (4-MeOBTT)

An amount of (1.36 g, 8.0 mmol) 4-methoxybenzoyl chloride, (0.61 g, 8.0 mmol) ammonium thiocyanate and (0.95 g, 8.0 mmol) threonine have been used for the preparation of 4-MeOBTT. The title compound 4-MeOBTT was synthesized in the same manner as described earlier. Experimental data: White solid; 0.37 g, yield 14.69%; EI-MS: [M]⁺ *m/z* = 312 (2%); ¹H NMR (Acetone-*d*₆) ppm: 1.310, 3.915, 4.581, 5.160, 7.086, 8.063, 10.065, 11.472; ¹³C NMR (Acetone-*d*₆) ppm: 20.15, 55.18, 63.59, 67.03, 113.99-163.84, 166.80, 170.32, 182.10; UV-Vis (MeOH): λ_{max}, nm (ε, Lmol⁻¹cm⁻¹) 216.0 (51610), 278.2 (80060); IR (KBr pellets): ν(N-H) 3467 cm⁻¹ (m), ν(O-H) 3243 cm⁻¹ (m), ν(C=O-OH) 1720 cm⁻¹ (s), ν(C=O-NH) 1656 cm⁻¹ (s), ν(C=C) 1520 cm⁻¹ (s), ν(C=S) 763 cm⁻¹ (m).

Antibacterial Assay

The antibacterial screening was carried out using disc-diffusion method based on procedure proposed by [20], and the laboratory work was done at Belangkas Laboratory. Mueller-Hinton Agar was prepared and poured into petri dish. Four bacterial strains; *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhimurium* and *Escherichia coli* were grown in nutrient broth and incubated for 24 hours. The samples were then dissolved in DMSO with the concentration of 1 mg/mL. An amount of 70 μL mixture of samples with DMSO was impregnated on the AA discs and left to dry overnight. Each of the bacteria was uniformly spread using sterile cotton swab on a sterile Petri dish MH agar. Streptomycin which is the positive control was also placed on the agar. The agar was incubated overnight. The inhibition zones for all positive and negative controls and compounds were measured the next day.

III. RESULTS AND DISCUSSION

A series of methoxybenzoylthiourea amino acid derivatives has been synthesized and characterized via MS, NMR, UV and IR. All three compounds were obtained from the reactions between *o/m/p*-methoxybenzoyl isothiocyanate with threonine.

Mass Spectra Analysis

All of the compounds were successfully characterized by electron impact mass spectrometry (EI-MS). Based on the mass spectra of all compounds, molecular ion peak was observed at *m/z* 312. Each compound: 2-MeOBTT, 3-MeOBTT and 4-MeOBTT recorded very weak relative intensities of 1%, 1% and 2%, respectively. The ions generated for mass spectrometry using electron impact (EI)

has often led to the appearance of very weak intensity of molecular ion peak, or it might not be seen at all [22]. The presence of base peak which corresponded to the methoxybenzoyl fragment was observed at m/z 135 with relative intensity of 100%. The results achieved were in agreement to the molecular weight of the synthesized compound, which indicated that the compounds were indeed methoxybenzoylthiourea amino acid derivatives.

NMR Spectra Analysis

The ^1H NMR spectra reveal the presence of methyl, methine and aromatic proton resonances in the range of δ_{H} 1.1-1.3 ppm, 4.3-5.2 ppm and 7.0-8.1 ppm, respectively. To note, the presence of singlet resonance at δ_{H} 3.922 ppm, 3.847 ppm and 3.852 ppm for 2-MeOBTT, 3-MeOBTT and 4-MeOBTT, respectively, proved the presence of methoxy protons [23]-[24]. Two amide protons, C=S-NH and C=O-NH, observed at the low field region in the range of δ_{H} 11.1-11.5 ppm and 10.0-11.5 ppm, might be due to electronegativity effect of nitrogen atom. The absence of proton resonances for OH and C=O-OH since they are easily dissociate and exchange with H_2O [23]. Meanwhile, ^{13}C NMR spectra displayed the appearance of all of the carbon resonances of methyl, methoxy, methine, aromatic, C=O-NH, C=O-OH and C=S at approximately δ_{C} 20.1-21.4 ppm, 55.0-57.0 ppm, 63.3-67.1 ppm, 113.0-163.8 ppm, 165.0-168.0 ppm, 170.0-171.0 ppm and 180.0-182.0 ppm, respectively. Three main carbon signals, which are C=O-NH, C=O-OH and C=S, experienced deshielding effects probably due to the formation of intramolecular hydrogen bonding and also the electronegativity of the oxygen and sulphur atoms [25]. All of the NMR spectral data are listed in Table I.

TABLE I
NMR DATA FOR METHOXYBENZOYLTHIOUREA AMINO ACID DERIVATIVES

Compound	2-MeOBTT, δ	3-MeOBTT, δ	4-MeOBTT, δ
Proton NMR			
CH_3	1.136	1.145	1.310
OCH_3	3.998	3.846	3.915
CH	4.312, 4.809	4.324, 4.868	4.581, 5.160
CH aromatic	7.159-7.893	7.194-7.560	7.086, 8.063
C=O-NH	11.112	11.506	10.065
C=S-NH	11.132	11.330	11.472
Carbon NMR			
CH_3	21.38	20.83	20.15
OCH_3	57.12	55.34	55.18
CH	63.97, 66.47	63.33, 66.00	63.59, 67.03
CH aromatic	113.27-157.94	113.03-158.99	113.99-163.84
C=O-NH	165.67	167.97	166.80
C=O-OH	171.22	170.81	170.32
C=S	180.96	181.44	182.10

UV Spectra Analysis

UV spectra showed the existence of two absorption bands corresponded to the $n-\pi^*$ and $\pi-\pi^*$ transitions. The first absorption band was observed in the range of λ_{max} 216.0-252.0 nm and was assigned to the C=O group. The second absorption band was assigned to C=S group and appeared at

λ_{max} 278.2-290.0 nm. The results are presented in Table II and showed good agreement with previous study [26]. However, 4-MeOBTT has recorded a slightly lower wavelength for the primary absorption band which appeared at λ_{max} 216.0 nm. In contrast, 2-MeOBTT and 3-MeOBTT have their primary absorption bands observed at *ca.* λ_{max} 250.0 nm. This might be due to the increasing electron drift from electron releasing para-methoxy group to the electron withdrawing carbonyl group (C=O-NH) through the π bond of phenyl ring, which has led to the significant increase in absorptivity of the compounds [27].

TABLE II
UV DATA FOR METHOXYBENZOYLTHIOUREA AMINO ACID DERIVATIVES

Compound	Wavelength (λ_{max} , nm)/ Extinction coefficient (ϵ , L/mol/cm)	Possible assignment
2-MeOBTT	250.2 (39000)	$n-\pi^*$ and $\pi-\pi^*$
	290.0 (26400)	$n-\pi^*$ and $\pi-\pi^*$
3-MeOBTT	250.0 (2940-shoulder)	$n-\pi^*$ and $\pi-\pi^*$
	285.5 (2230)	$n-\pi^*$ and $\pi-\pi^*$
4-MeOBTT	216.0 (51610)	$n-\pi^*$ and $\pi-\pi^*$
	278.2 (80060)	$n-\pi^*$ and $\pi-\pi^*$

IR Spectra Analysis

There are six main vibrational modes observed in the IR spectra of all of the compounds which are $\nu(\text{N-H})$, $\nu(\text{O-H})$, $\nu(\text{C=O-OH})$, $\nu(\text{C=O-NH})$, $\nu(\text{C=C})$ aromatic and $\nu(\text{C=S})$. All peaks were found in the range of 3241-3467 cm^{-1} , 2976-3302 cm^{-1} , 1720-1768 cm^{-1} , 1655-1672 cm^{-1} , 1519-1525 cm^{-1} and 754-763 cm^{-1} , respectively (Table III).

TABLE III
FT-IR DATA FOR METHOXYBENZOYLTHIOUREA AMINO ACID DERIVATIVES

Compound	$\nu(\text{N-H})$, cm^{-1}	$\nu(\text{O-H})$, cm^{-1}	$\nu(\text{C=O-OH})$, cm^{-1}	$\nu(\text{C=O-NH})$, cm^{-1}	$\nu(\text{C=C})$, cm^{-1}	$\nu(\text{C=S})$, cm^{-1}
2-MeOBTT	3447	3302	1769	1659	1526	757
3-MeOBTT	3242	2976	1727	1676	1519	754
4-MeOBTT	3467	3243	172	165	1520	763

Based on the IR spectral data, the $\nu(\text{N-H})$ and $\nu(\text{O-H})$ appeared as broad peak and were observed in the normal stretching values recorded for thiourea compound [28]. It was found that the $\nu(\text{C=O-OH})$ showed decreasing wavenumber following the ortho-, meta- and para-positions of methoxy group. The electron donating effect and the steric effect in these compounds influenced the wavenumber recorded.

Antibacterial Activity Analysis

All of the compounds were screened against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhimurium* and *Escherichia coli* to study their antibacterial properties. The result showed that no compounds were able to inhibit the growth of all of the tested bacterial strains. The reactivity of compounds played a major part in any reaction. The reactivity of benzoyl isothiocyanate, benzoylthiourea amino acid and also amino acids have been studied by using Gaussian 09 software [29]. Based on the analysed data on frontier orbital of Highly Occupied Molecular Orbital (HOMO) and Lowest

Unoccupied Molecular Orbital (LUMO) of benzoylthiourea amino acid, the result demonstrated that the carboxylic acid group was unreactive. It can be concluded that reactivity of compounds may also be the reason for the negative result of the bacterial inhibition.

IV. CONCLUSION

In short, three methoxybenzoylthiourea amino acid derivatives were successfully obtained from the condensation reactions between *o/m/p*-methoxybenzoyl isothiocyanate with threonine. The molecular structure of all of the compounds was confirmed using the combination of MS, NMR, UV-Vis and FT-IR. The antibacterial properties of the synthesized compounds were screened against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhimurium* and *Escherichia coli*. However, negative result was obtained for the bacterial activity evaluation which might be related to the reactivity of the compounds.

ACKNOWLEDGMENTS

The authors would like to dedicate appreciation to the Ministry of Education Malaysia for RAGS Research Grant No: 12-040-0040 and FRGS 59390, Universiti Malaysia Terengganu and IIUM Kuantan Campus for their contribution towards this research project.

REFERENCES

- [1] W. S. H. W. Zulkiplee, A. N. A. Halim, Z. Ngaini, M. A. M. Ariff, H. Hussain, "Bis-thiourea bearing aryl and amino acids side chains and their antibacterial activities," *Phosphorus Sulfur Silicon Relat. Elem.*, vol. 189, no. 6, pp. 832-838, Jun. 2014.
- [2] G. Binzet, F. M. Emen, U. Flörke, T. Yesilkaynak, N. Külcü, H. Arslan, "4-Chloro-*N*-(*N*-(6-methyl-2-pyridyl)-carbamothioyl)benzamide," *Acta Crystallogr. Sect. E. Struct. Rep. Online*, vol. 65, no. 1, pp. 81-82, Jan. 2009.
- [3] S. Jadhav, L. Khillare, M. Rai, A. Durrani, "Synthesis, characterization and antimicrobial activity of substituted arylthiourea," *Rasayan J. Chem.*, vol. 3, no. 1, pp. 27-31, Jan. 2010.
- [4] T. Kimura, T. Eto, D. Takahashi, K. Toshima, "Stereocontrolled photoinduced glycosylation using an aryl thiourea as an organo photoacid," *Org. Lett.*, vol. 18, no. 13, pp. 3190-3193, Jun. 2016.
- [5] H. Marquez, E. R. Perez, A. M. Plutin, M. Morales, A. Loupy, "Synthesis of 1-benzoyl-3-alkylthioureas by transamidation under microwave in dry media," *Tetrahedron Lett.*, vol. 41, pp. 1753-1756, Mar. 2000.
- [6] N. Gunasekaran, S. W. Ng, E. R. T. Tiekink, R. Karvembu, "Hypodentate coordination of *N,N*-di(alkyl/aryl)-*N'*-acylthiourea derivatives in Cu(I) complexes," *Polyhedron*, vol. 34, pp. 41-45, Feb. 2012.
- [7] S. S. Elkholy, H. A. Salem, M. Eweis, M. Z. Elsabee, "Synthesis and characterization of some acyl thiourea derivatives of chitosan and their biocidal activities," *Int. J. Bio. Macromol.*, vol. 70, pp. 199-207, Sep. 2014.
- [8] Y. Zhao, C. W. Ge, Z. H. Wu, C. N. Wang, J. H. Fang, L. Zhu, "Synthesis and evaluation of aroylthiourea derivatives of 4-β-amino-4'-O-demethyl-4-desoxy podophyllotoxin as novel topoisomerase II inhibitors," *Eur. J. Med. Chem.*, vol. 46, pp. 901-906, Mar. 2011.
- [9] H. Y. Yuen, W. Henderson, A. G. Oliver, "Nickel(II) complexes of di- and tri-substituted thiourea mono- and di-anions," *Inorg. Chim. Acta*, vol. 368, pp. 1-5, Mar. 2011.
- [10] P. P. Kumavat, A. D. Jangale, D. R. Patil, K. S. Dalal, J. S. Meshram, D. S. Dalal, "Green synthesis of symmetrical *N,N'*-disubstituted thiourea derivatives in water using solar energy," *Environ. Chem. Lett.*, vol. 11, pp. 177-182, Jun. 2013.
- [11] P. K. Mohanta, S. Dhar, S. K. Samal, H. Ila, H. Junjappa, "1-(methylthiocarbonyl)imidazole a useful thiocarbonyl transfer reagent for synthesis of substituted thioureas," *Tetrahedron*, vol. 56, pp. 629-639, Jan. 2000.
- [12] A. Yahyazadeh, Z. Ghasemi, "Synthesis of unsymmetrical thiourea derivatives," *European Chemical Bulletin*, vol. 2, no. 8, pp. 573-575, Apr. 2013.
- [13] M. Atis, F. Karipcin, B. Sariboga, M. Tas, H. Celik, "Structural, antimicrobial and computational characterization of 1-benzoyl-3-(5-chloro-2-hydroxyphenyl)thiourea," *Spectrochim. Acta Mol. Biomol. Spectrosc.*, vol. 98, pp. 290-301, Dec. 2012.
- [14] M. M. Zhao, X. Y. Dong, G. Li, Y. H. Yang, Y. J. Zhang, X. Q. Yang, "Antituberculosis and antifungal activities of synthesized benzoylthiourea derivatives," *Asian J. Chem.*, vol. 25, no. 13, pp. 7548-7550, Sep. 2013.
- [15] A. Saeed, M. S. Khan, H. Rafique, M. Shahid, J. Iqbal, "Design, synthesis, molecular docking studies and *in vivo* screening of ethyl 4-(3-benzoylthioureido) benzoates as urease inhibitors," *Bioorg. Chem.*, vol. 52, pp. 1-7, Feb. 2014.
- [16] D. M. H. Al-Mudhaffar, D. S. Al-Edani, S. M. Dawood, "Synthesis, characterization and biological activity of some complexes of some new amino acid derivatives *N*-(benzoylamino)-thioxomethyl-amino acid (HL)," *J. Korean Chem. Soc.*, vol. 54, no. 5, pp. 506-514, Jun. 2010.
- [17] M. S. Rathod, S. Z. Jadhao, "Synthesis and structural investigation of nickel metal-ligand (thiourea derivative) complexes," *J. Chem. Pharm. Res.*, vol. 4, no. 3, pp. 1629-1631, 2012.
- [18] C. Li, W. Yang, H. Liu, M. Li, W. Zhou, J. Xie, "Crystal structures and antifungal activities of fluorine-containing thioureido complexes with nickel(II)," *Molecules*, vol. 18, pp. 15737-15749, Dec. 2013.
- [19] G. Wu, "Amino acids, biochemistry and nutrition," CRC Press, 2010.
- [20] N. Ngah, Darman, B. M. Yamin, S. W. Ng, "2-(3-(4-Methoxybenzoyl)thioureido)-3-methylbutyric acid," *Acta Crystallogr. Sect. E. Struct. Rep. Online*, 62(5):o1903-o1904, May. 2006.
- [21] N. I. M. Halim, K. Kassim, A. H. Fadzil, B. M. Yamin, "Synthesis, characterisation and antibacterial studies of 1,2-bis(*N'*-2,3 and 4-methoxybenzoylthioureido)-4-nitrobenzene," *APCBEE Procedia*, vol. 3, pp. 129-133, Dec. 2012.
- [22] R. M. Silverstein, F. X. Webster, D. J. Kiemle, "Spectrometric Identification of Organic Compounds," 7th Edition. New York: John Wiley & Sons, Inc. 2005.
- [23] N. Ameram, B. M. Yamin, "Synthesis and characterization *o*-, *m*- and *para*-toluyl thiourea substituted *para*-pyridine and ethyl pyridine as a chromoionophore," *IOSR Journal of Applied Chemistry*, vol. 4, no. 6, pp. 59-67, Jun. 2013.
- [24] J. Han, H. Dong, Z. Xu, J. Wang, M. Wang, M. "Synthesis and activity of novel acylthiourea with hydantoin," *Int. J. Mol. Sci.*, vol. 14, pp. 19526-19539, Sep. 2013.
- [25] M. S. M. Yusof, R. H. Jusoh, W. M. Khairul, B. M. Yamin, "Synthesis and characterisation a series of *N*-(3,4-dichlorophenyl)-*N'*-(2,3 and 4-methylbenzoyl)thiourea derivatives," *J. Mol. Struct.*, vol. 975, pp. 280-284, Jun. 2010.
- [26] S. A. Zakaria, S. A. Muharam, M. S. M. Yusof, W. M. Khairul, M. A. Kadir, B. M. Yamin, "Spectroscopic and structural study of a series of pivaloylthiourea derivatives," *The Malaysian J. Analytical Sciences*, vol. 15, no. 1, pp. 37-45, 2011.
- [27] S. Kumar, "Spectroscopy of organic compounds," 2006.
- [28] H. M. Abosadiya, S. E. Ashoor, B. M. Yamin, "Synthesis and x-ray structure study of *cis-trans* 3-(3-biphenyl carbonylthioureido)propanoic acid(I) and *N*-(4-biphenyl carbonyl)-*N'*-(3-hydroxyphenyl)thiourea(II)," *Rasayan J. Chem.*, vol. 2, no. 3, pp. 572-576, Jul. 2009.
- [29] F. Odame, E. C. Hosten, R. Betz, K. Lobb, Z. R. Tshentu, "Characterization of some amino acid derivatives of benzoyl isothiocyanate: Crystal structures and theoretical prediction of their reactivity," *J. Mol. Struct.*, vol. 1099, pp. 38-48, Nov. 2015.