
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, 'H and 13C NMR spectrometry, UV-Vis spectrophotometer and FT-IR spectroscopy. Mass spectra for all of the compounds showed the presence of molecular ion [M] + peaks at m/z 312, which are in agreement to the calculated molecular weight. For 'H NMR spectra, the presence of OCH3, C=S-NH and C=O-NH protons were observed within range of δH 3.8-4.0 ppm, 11.1-11.5 ppm and 10.0-11.5 ppm, respectively. 13C NMR spectra in all compounds displayed the presence of OCH3, C=O-NH, C=O-OH and C=S carbon resonances within range of δC 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-π* and π-π* transitions. Six vibrational modes of ν(N-H), ν(O-H), ν(C=O-OH), ν(C=O-NH), ν(C=C) aromatic and ν(C=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, 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 arylthiourea 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.
**Preparation of 3-Hydroxy-2-[3-(3-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-d6) ppm: 1.310, 3.915, 4.581, 5.160, 7.086, 8.063, 10.065, 11.472; 13C NMR (Acetone-d6) ppm: 20.15, 55.18, 63.59, 67.03, 113.99-163.84, 166.80, 170.32, 182.10; UV-Vis (MeOH): λmax, nm (ε, Lmol⁻¹cm⁻¹) 216.0 (51610), 278.2 (80060); IR (KBr pellets): ν(N-H) 3467 cm⁻¹ (m), ν(C=O-NH) 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 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*, *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus epidermidis* 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 concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An amount of 70 µL mixture of samples with concentration 1 mg/mL. An am
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 \\text{n}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 methoxybenzothiourea amino acid derivatives.

**NMR Spectra Analysis**

The $^1$H NMR spectra reveal the presence of methyl, methine and aromatic proton resonances in the range of $\delta_1$ 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 $\delta_1$ 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 $\delta_1$ 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$_2$O [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 $\delta_1$: 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.

**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 $\lambda_{max}$ 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 $\lambda_{max}$ 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 $\lambda_{max}$ 216.0 nm. In contrast, 2-MeOBTT and 3-MeOBTT have their primary absorption bands observed at ca. $\lambda_{max}$ 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 $\pi$ bond of phenyl ring, which has led to the significant increase in absorbitivity of the compounds [27].

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**TABLE I**

| 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=O-SH | 11.112 | 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 |

**TABLE II**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wavelength ($\lambda_{max}$ nm)</th>
<th>Extinction coefficient ($\varepsilon$, L/mol/cm)</th>
<th>Possible assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-MeOBTT</td>
<td>250.2 (39000)</td>
<td>n-\pi* and \pi-\pi*</td>
<td></td>
</tr>
<tr>
<td>3-MeOBTT</td>
<td>250.0 (2940~shoulder)</td>
<td>n-\pi* and \pi-\pi*</td>
<td></td>
</tr>
<tr>
<td>4-MeOBTT</td>
<td>278.2 (80060)</td>
<td>n-\pi* and \pi-\pi*</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE III**

<table>
<thead>
<tr>
<th>Compound</th>
<th>n(H$_2$O), cm$^{-1}$</th>
<th>n(O-H), cm$^{-1}$</th>
<th>n(C=O), cm$^{-1}$</th>
<th>n(C=O-NH), cm$^{-1}$</th>
<th>n(C=O-SH), cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOBTT</td>
<td>2-</td>
<td>3447</td>
<td>3302</td>
<td>1769</td>
<td>1659</td>
</tr>
<tr>
<td></td>
<td>4-</td>
<td>3242</td>
<td>2976</td>
<td>1727</td>
<td>1676</td>
</tr>
<tr>
<td>MeOBTT</td>
<td>2-</td>
<td>3467</td>
<td>3243</td>
<td>172</td>
<td>165</td>
</tr>
</tbody>
</table>

Based on the IR spectral data, the \nu(N-H) and \nu(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(C=O-NH) 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-methoxybenzyl 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.

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REFERENCES