Synthesis, structurale elucidation and antioxidant study of Ortho-substituted N,N’-bis(benzamidothiocarbonyl)hydrazine derivatives

Syadza Firdausiah¹, S A Hasbullah² and B M Yamin²

¹ Chemistry Department, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Jl. Perintis Kemerdekaan 10 Tamalanrea Makassar, Sulawesi Selatan, 90245, Indonesia
² School of Chemical Science and Food Technology, Faculty Science and Technology, National University of Malaysia, Bangi, Selangor, 43600, Malaysia

E-mail: syadza.f@gmail.com

Abstract. Some bis(thiourea) compounds have been reported to possess excellent performance in pharmaceutical and environmental fields because of their ability to form chelating complexes with various anions and metal ions. Structurally for carbonyl thiourea derivatives, to become a chelating agent, it must adopt cis-configuration. In the present study, four new bis(thiourea) derivatives namely N,N’-bis(o-fluorobenzamidothiocarbonyl)hydrazine (1), N,N’-bis(o-chlorobenzamidothiocarbonyl)hydrazine (2), N,N’-bis(o-nitrobenzamidothiocarbonyl)-hydrazine (3), and N,N’-bis(o-methylbenzamidothiocarbonyl)hydrazine (4) were successfully synthesized and characterized by CHNS microelemental analysis, FTIR, UV-Vis, and ¹H and ¹³C NMR spectroscopy. However chemical crystallography study showed that both thiourea moieties in compound (2) and (3) adopt trans geometry. Therefore they are potential monodentate ligand with two active moieties. DPPH radical scavenging experiment showed that compound (1), (2), and (4) exhibited higher antioxidant activity than ascorbic acid (Vitamin C).

1. Introduction
Thiourea derivatives have diverse potential applications in many fields. In pharmaceutical field, some thiourea derivatives showed the potential application as inhibitor of urease [1], antimicrobial agents [2–5] and in human cancer treatment [6]. Thiourea derivatives also applicable as potentiometric sensor due to the presence of C=S and amine groups which are easy to form complexes with metal ions [7]. Studies on the structure-activity relationship revealed that thiourea moiety played important role in their antioxidant study [8].

The modifications on the N side of thiourea have been reported by many researchers. Some N,N’-disubstituted thiourea compounds showed good performance as HIV binding inhibitors [9]. Thiourea compound bearing chitosan moiety was also reported as good antibacterial and antifungal agent [3]. Other thiourea derivatives bearing allyl and triazole moieties were also active as antiviral and antituberculosis agent [10]. Carbonyl thioureas also showed potential in industrial [11] and agrochemical fields [12].
The metal complexes of some thiourea derivatives have been found as new classes of antifungal agent [13, 14] and showed moderate toxicity against human cancer cell lines [15]. The other thiourea-metal complexes have also been used for metal ion removal from water environment and as component of chemosensors [16, 17].

Bis(thiourea) compounds with hydrazine spacer have been widely used as building blocks in synthesis of many heterocyclic compounds. For example, bis(thiourea)hydrazines which substituents are alkyl groups such as methyl, ethyl, propyl and butyl have been used for synthesis of triazolidine compounds [18]. Those bis(thiourea)hydrazine compounds have also been reported as building blocks for synthesis of novel thiazolone azines which are ideal candidates for electrochemical absorption and redox indicators due to their capability to form stable radical cations [19]. The synthesis of biologically active compounds, bis-oxathiaaza[3.3.3]propellanes, have been carried out using bis(thiourea)hydrazines containing aromatic groups such as phenyl, benzyl and allyl [20].

Other aromatic bis(thiourea)hydrazine compounds were also potential in pharmaceutical field as anticonvulsant agent and selective agent for grand mal epilepsy [24].

There are less structure of bis(thiourea) have been reported so far compared to monothiourea compounds. To the best of our knowledge, there are only five crystal structures of bis(thiourea) compounds utilizing hydrazine linker have been reported in CCDC (The Charnbridge Crystallographyc Data Centre). They are dithiobiurea [25], bis(N-phenylthiourea) [26], 1-(2,3,4,6-tetra-O-β-D-glucopyranosyl)-3-thioureidothiourea monohydrate [27], N,N'-bis(benzamidothiocarbonyl)hydrazine [28] and 1,6-bis-phosphorylated-2,5-dithiobiurea [29].

In this research, bis(carbonylthiourea) with the shortest linker N-N (N-C₉-N; n=0), namely bis(arylthiourea)hydrazine (Figure 1), is designed and synthesized, which X is o-fluoro, o-chloro, o-nitro and o-methyl. Figure 1 shows predicted structure of synthesized compounds. If all C=S and C=O bonds are in cis-configuration about the C-N and N-N bonds, the compound can act as tetradeinate ligand. However, if both C=S bonds are in trans-configuration about the N-N bond but C=S and C=O bonds are in cis-configuration about their C-N bonds, the compound can provide two bidentate active sites metal chelations. The characterizaion and molecular structure of the compounds including their antioxidant properties are presented.

![Figure 1](image-url)

**Figure 1.** The predicted structure of synthesized compounds. (a) Tetradeinate ligand; (b) bidentate ligand.
2. Experimental

2.1. Material and methods

All reagents were used without further purification. The solvent was treated with molecular sieve before used. C, H, N and S analyses were performed using a ThermoFinnigan Instrument. FTIR spectra were recorded using KBr pellet on Perkin Elmer 100 Spectrophotometer in the region of 400 – 4000 cm⁻¹. ¹H and ¹³C NMR spectra were obtained using Bruker Avance III 400 MHz. Electronic spectra were recorded on UV-1800 240V Shimadzu Spectrophotometer. Crystal data was recorded on Bruker SMART APEX CCD Diffractometer. The structure was solved using SHELXS97 [30] and was refined by full matrix least squares on F² [30].

2.2. Synthesis

A solution of aroyl chloride (4 mmol) was added to 0.304 g of ammonium thiocyanate (4 mmol) in acetone. The mixture was stirred at room temperature for 15 minutes and the precipitate was filtered out. Hydrazine hydrate as many as 0.1 g (2 mmol) was added to the filtrate and the mixture was refluxed for 3 hours. Then, the residue was filtered out. The precipitate was washed with dichloromethane and ethanol/H₂O 1:1.

N,N'-bis(o-fluorobenzamidothiocarbonyl)hydrazine (I): Yield 57%; colourless solid, m.p 271.2-272.0 °C. IR (KBr pellet, cm⁻¹): v(N-H)3424; v(C=O)1671; δ(N-H)1612; v(C-N)1229; v(C=S)1079; (C=S)F)1150. ¹H-NMR (DMSO-d₆, 400 MHz): δ 7.35-7.74 (m, 4H); 12.35 (s, 1H). ¹³C-NMR (DMSO-d₆, 100 MHz): δ 116.8; 125.3; 131.1; 134.9; 158.5; 161.1; 166.1; 171.6. Anal. Calc. For C₉H₇N₂O₂S₂F₂: C,45.22; H,2.98; N,13.83; S,15.68. Found: C,45.97; H,2.88; N,14.0; S,16.8. λmax 335 nm, ε 15,578.75 L.mol⁻¹.cm⁻¹.

N,N'-bis(o-chlorobenzamidothiocarbonyl)hydrazine (2): Yield 88%; colourless solid, m.p ºC. IR (KBr pellet, cm⁻¹): v(N-H)3237, v(C=O)1686, δ(N-H)1591, v(C-N)1225, v(C=S)1100, (C=Ar-Cl)1040. ¹H-NMR (DMSO-d₆, 400 MHz): δ 7.45-7.68 (m, 4H); 12.57 (s, 1H); 13.94 (s, 1H). ¹³C-NMR (DMSO-d₆, 100 MHz): δ 127.4; 130.0; 130.2; 130.6; 132.9; 134.3; 168.3; 171.6. Anal. Calc. For C₁₀H₈N₂O₂S₂Cl₂: C,44.97; H,2.83; N,13.11; S,15.01. Found: C,45.22; H,2.98; N,13.83; S,15.68. λmax 334 nm, ε 14,580.25 L.mol⁻¹.cm⁻¹.

N,N'-bis(o-nitrobenzamidothiocarbonyl)hydrazine (3): Yield 52%; yellowish solid, m.p ºC. IR (KBr pellet, cm⁻¹): v(N-H)3271, v(C=O)1686, δ(N-H)1591, v(C-N)1250, v(C=S)1067, (C=Ar-NO₂) Asym. 1518, sym. 1349. ¹H-NMR (DMSO-d₆, 400 MHz): δ 7.81-8.27 (m, 4H); 12.67 (s, 1H); 13.86 (s, 1H). ¹³C-NMR (DMSO-d₆, 100 MHz): δ 124.8; 130.1; 130.4; 132.5; 135.2; 145.9; 168.0; 171.29. Anal. Calc. For C₁₀H₈N₂O₄S₂: C,42.85; H,2.70; N,18.74; S,14.30. Found: C,43.23; H,3.24; N,19.40; S,14.69. λmax 331.5 nm, ε 6,501.46 L.mol⁻¹.cm⁻¹.

N,N'-bis(o-methylbenzamidothiocarbonyl)hydrazine (4): Yield 50%; colourless solid, m.p ºC. IR (KBr pellet, cm⁻¹): v(N-H)3246, v(C=O)1677, δ(N-H)1600, v(C-N)1226, v(C=S)1061. ¹H-NMR (DMSO-d₆, 400 MHz): δ 2.41 (3H, s); 7.29-7.54 (m, 4H); 12.26 (s, 1H). ¹³C-NMR (DMSO-d₆, 100 MHz): δ 19.9; 126.1; 128.8; 131.2; 131.7; 133.8; 136.6; 171.0; 171.8. Anal. Calc. For C₁₀H₁₃N₀₂S₂: C,55.94; H,4.69; N,14.50; S,16.59. Found: C,56.26; H,5.44; N,15.12; S,17.27. λmax 323.5 nm, ε 14,392.52 L.mol⁻¹.cm⁻¹.

2.3. Single crystal X-Ray determination

Single crystal data were collected using Bruker SMART APEX CCD Diffractometer with graphite monochromated Mo Kα radiation source. A suitable crystal with size less than 0.5 mm was selected under microscope and mounted on the end of the glass fibre. The fibre was then mounted on a goniometer head before the X-ray experiment started. Raw data was collected by using SMART system (Siemens, 1997) and data reduction was done by using SAINT software (Siemens, 1997). Structure was solved directly by using SHELXTL [30].
2.4. Antioxidant Screening

The antioxidant activity of the thiourea was screened by using DPPH radical scavenging method. 300 μM each solution of DPPH (1.5 mL) in 96% ethanol was added to sample solutions (each 1.5 mL) of different concentrations (100, 200, 300, 400, 500, and 600 μM) in DMSO. The mixtures were allowed to react in the dark place at room temperature. After 30 minutes, the absorbance values were measured at 517 nm. DMSO was used as negative control. The scavenging activity of each sample was calculated as the percentage reduction of DPPH absorbance (Q) using the following formula:

$$Q = 100(A_c - A_s)/A_c$$

(1)

Where $A_c$ is the absorbance of the sample and $A_s$ is the absorbance of negative control. The results were compared to ascorbic acid activity as positive control. The EC$_{50}$ values of the sample and ascorbic acid were determined by using Four Parameter Logistics in SigmaPlot 12.0.

3. Results and discussion

3.1. Synthesis

The procedure and reaction schemes are outlined in Figure 2. The synthesis of bis(arylthiourea)hydrazine (1-4) was carried out by two steps of reaction. The first step was the nucleophilic substitution of chlorine atom to form the isothiocyanate compound. The next step was the nucleophilic addition of hydrazine nitrogen on the isothiocyanate carbon atom to produce the thiourea compounds.

![Figure 2](image-url)

Figure 2. Synthesis of bis(arylthiourea)hydrazine consist of (a) Reaction of aryl chloride with ammonium thiocyanate at room temperature for 15 minutes, (b) Reaction of aryl isothiocyanate with hydrazine at reflux condition for 3 hours

3.2. Chemistry

All compounds show their UV-vis spectra with only one maximum absorption peak between 331,5-335 nm due to n→π* transition of C=O bonds. The expected two maximum absorption of n→π* and π→π* transitions of C=S bond were not observed due to the cut point of DMSO solvent occured at 268 nm and the π→π* transition normally occurs at shorter wavelength (<250 nm).

The compound which has amide group contains both π systems and unshared electron pairs and will show two absorption peaks due to n→π* transition at longer wavelength (>300 nm) and a π→π* transition at shorter wavelength (<250 nm) [31]. Refat & El-Metwaly reported that the thiosemicarbazide compound, (E)-N-(5-bromo-2-hydroxy-benzylidene)-1,4-dioxo-3,4-dihydrophthalazine-2(IH-carbo-thioamide, showed two absorption peak at 350 and 270 nm for n→π* and π→π* transitions, respectively[32]. In 2013, Huang et al. revealed that the benzoyl thiourea compound in acetonitrile has two absorption peaks at 211 and 288 nm which assigned to E$_2$ and B bands, respectively [33]. Osman reported that each thiourea compounds showed an absorption of n→π* transition of C=S bond at 272-309 nm as shoulder peak [34].
The infrared spectra of the products are almost similar. The thione C=S stretching bands appeared in the range of 1100-1061 cm\(^{-1}\) indicating the presence of thioketo functional groups. The sharp and strong bands about 1680 cm\(^{-1}\) are the carbonyl C=O amide stretching frequencies. The C-N stretching frequencies are between 1250 and 1225 cm\(^{-1}\). Compound 3 has higher C=O stretching frequencies than the other compounds due to the electron withdrawing characteristic of nitro group. The strong bands between 3424 and 3237 cm\(^{-1}\) are due to N-H stretching frequencies. The bending N-H frequencies can be observed around 1616-1572 cm\(^{-1}\).

Due to the low solubility of the products in many organic solvents, less than 5 mg of each compound were able to dissolve in 560 µl of DMSO-d\(_6\), they are insoluble in other deuterated solvents. Thus, in some compounds, the signals of all NH protons were not observable. It is due to exchange of proton with deuterium of the solvent. The exchangeable protons like –OH and –NH give broad signals in their NMR spectra, and it completely disappear if the concentration of the sample is low [31]. \(^1\)H NMR spectrum of all compounds exhibited broad singlet signals at 12.26 – 12.67 ppm, which were assigned to the NH(1) and NH(1’) protons.

The thioamide proton NH(2) and NH(2’) appear at lower field (13.94-13.86 ppm) than amide proton NH(1) and NH(1’). The protons are very deshielded due to the interaction with d\(_6\)-DMSO and intramolecular hydrogen bondings [35]. The integration value of thioamide and amide protons are one, indicating the symmetry properties of the molecule at N-N bond. The multiplet signals between 7.29 and 8.27 ppm are assigned to protons of aromatic ring. But due to the low concentration of the samples, the multiplicity and the coupling constant in their NMR spectrum were not observable. Their \(^{13}\)C NMR spectra showed signals at 166.1-171.0 and 171.29-171.8 ppm for C=O (amide) and C=S (thioamide), respectively. The signal of aromatic carbons was observed at 116.80-161.1 ppm.

3.3. X-ray Crystallography
In the present study, crystal of compound 2 and 3 were obtained from DMSO. The crystals are not stable in the air. They became white powder after about 3 hours upon exposure to air. Therefore, the crystals were coated with special oil for the X-ray crystallography analysis.

![Figure 3](image_url)

**Figure 3.** The molecular structure of 2 drawn at 50% probability displacement ellipsoid. The dashes lines indicated intramolecular hydrogen bonds.

\(N,N’\)-bis(2-chlorobenzamidothiocarbonyl) hydrazine (2) crystallized in monoclinic system with space group of C2/c. The crystal system and refinement data are given in Table 1. The crystal is centrosymmetric about the mid-point of the N2-N2a bond (Figure 3). The sulfur S2 atom is disordered and was treated by parting the atom into two different occupancies.
The benzene (C1-C6 and C1A-C6A) and the bis(thiourea) fragment, O1/N1/N2/C6/C7/C8/O1A/N1A/N2A/C6A/C7A/C8A, are planar with maximum deviation of 0.030(3)Å for C8 and N2 atoms from the least square planes. The dihedral angle between these two planes is 67.73(11)°. The thiourea moieties have trans geometry. This configuration is contributed by intramolecular N2-H2···S1A and N2-H2···O1 hydrogen bonds.

There are four intramolecular hydrogen bonds of 2 (Table 2). Both N2-H2···O1 and N2-H2···S1A of the centro-symmetric molecule form two pseudo six-membered rings {(C7/N1/C8/N2/H2···O1) and (C7A/N1A/C8A/N2A/H2A···O1A)} and two five-membered rings {(C8/N2/N2A/H2A···S1) and (C8/N2/N2A/H2A···S1A)}.

Table 1. Crystal data and structure refinement of 2.

| Parameter                          | Data                                      |
|------------------------------------|-------------------------------------------|
| Empirical formula                  | C_{16}H_{12}N_{4}O_{2}S_{2}Cl_{2}, 2(C_{2}H_{6}OS) |
| Formula weight                     | 583.57                                    |
| Temperature                        | 301(2) K                                  |
| Wavelength                         | 0.71073 Å                                 |
| Crystal system, space group        | Monoclinic, C 2/c                         |
| Unit cell dimensions               | a = 29.7268(11) Å, α= 90°                |
|                                    | b = 5.1077(2)                             |
|                                    | c = 18.3219(7), γ= 90°                   |
| Volume, Z                          | 2710.56(18) Å, 4                         |
| Density (calculated)               | 1.430 Mg/m³                               |
| Absorption coefficient             | 0.581 mm⁻¹                                |
| F(000)                             | 1208                                      |
| Crystal size                       | 0.500 x 0.220 x 0.110 mm³                 |
| Theta range for data collection    | 2.938 to 28.359°                          |
| Index ranges                       | -38<=h<=39, -6<=k<=6, -                    |
| Reflections collected              | 24<=l<=24                                 |
| Independent reflections            | 32985                                     |
| Completeness to theta = 25.242°    | 3372 [R(int) = 0.0448]                    |
| Refinement method                  | Full-matrix least-squares on F^2          |
| Data / restraints / parameters     | 99.8 %                                    |
| Goodness-of-fit on F^2             |                                           |
| Final R indices [R>2sigma(I)]      |                                           |
| R indices (all data)               | 3372 / 1 / 166                            |
| Largest diff. peak and hole        | 1.109                                     |
| R1 = 0.0596, wR2 = 0.1572          |                                           |
| R1 = 0.0887, wR2 = 0.1760          |                                           |
| 0.777 and -0.508 e.Å⁻³             |                                           |
Table 2. Hydrogen bonds geometry of 2 (Å and °)

| D-H···A         | d(D-H) | d(H···A) | d(D···A) | <(DHA) |
|-----------------|--------|----------|----------|--------|
| N1-H1···O2#2    | 0.86   | 1.99     | 2.845(3) | 178    |
| N2-H2A···O1     | 0.86   | 1.91     | 2.580(4) | 134    |
| N2-H2A···S1     | 0.86   | 2.55     | 2.932(3) | 108    |
| C10-H2A2···O1#4 | 0.96   | 2.54     | 3.437(6) | 156    |
| C4-H4···O2#5    | 0.93   | 2.54     | 3.230(4) | 132    |

Symmetry transformations used to generate equivalent atoms:

#1 -x,-y+1,-z   #2 x,y,z   #3 x+1/2,y+3/2,z   #4 -x+1/2,y+5/2,-z+1/2   #5 x,y+1,z

The molecular packing of 2 showed that the molecules are linked by (N1-H1···O2), (C10-H2A2···O1) and (C4-H4···O2) intermolecular hydrogen bonds, where the oxygen atom of the DMSO is connected to two thiourea molecules to form 2-dimensional network (Figure 4).

The selected bond lengths and angles of 2 is shown in Table 3. All bond lengths and angles are in normal ranges [36]. The bond length of N2-C8 is shorter than N1-C7. This double bond character is due to the resonance effect with C=S thiocarbonyl groups.

Figure 4. Molecular packing of compound 2 viewed down a axis.
The dashed lines indicate intermolecular hydrogen bonds.
Table 3. Selected bond lengths and angles of 2

| Bond Length | Bond Angle |
|-------------|------------|
| S1 – C8     | 1.655(3)   |
| N2 – N2a    | 1.372(3)   |
| N2 – C8     | 1.331(3)   |
| N1 – C8     | 1.391(4)   |
| O1 – C7     | 1.220(3)   |
| C – Cl      | 1.733(3)   |
| O1 – C7     | 121.7(3)   |
| C6 – C7 – N1| 114.7(2)   |
| O1 – C7 – N1| 123.6(3)   |
| N1 – C8 – S1| 115.0(2)   |
| N2 – C8 – S1| 119.4(2)   |
| O1 – C7     | 119.4(2)   |

*N,N'-bis(2-nitrobenzamidothiocarbonyl)hydrazine* (3) crystallized in triclinic crystal system with space group of P1. The unit cells of the crystal are a=8.6373(12) Å, b=8.7962(12) Å, c=13.5691(18) Å, α=94.440(4)°, β=93.877(4)° and γ=118.458(4)°, V=897.2(2) Å³ and Z=1. Crystal system and refinement parameters are shown in Table 4.

The molecule has the centre of inversion at N3-N3A bond (Figure 5). Both thiourea moieties have trans geometry. The nitrobenzoyl group is trans to the thiono group about the C-N bond. The bis(thiourea) fragment (S1/O3/N2/N3/C7/C8/S1A/O3A/N2A/N3A/C7A/C8A) and benzene {((C1-C6) and (C1A-C6A)} groups are planar with maximum deviation 0.046(4) Å for N2 atom. The bis(thiourea) fragment is perpendicular to the benzene fragment by dihedral angle of 87.9(3)°. The large dihedral angle is due to the bulky nature of the nitro group that cause the steric hindrance between the benzene and the thiourea fragment.

**Figure 5.** The molecular structure of 3 drawn at 50% probability displacement ellipsoid. The dashes lines indicated intramolecular hydrogen bonds.
Table 4. Crystal data and structure refinement of 3.

| Parameter                                      | Data                                      |
|------------------------------------------------|-------------------------------------------|
| Empirical formula                              | C_{16}H_{12}N_{6}O_{6}S_{2}, 4(C_{2}H_{6}O) |
| Formula weight                                 | 761.01                                    |
| Temperature                                    | 301(2) K                                   |
| Wavelength                                     | 0.71073 Å                                  |
| Crystal system, space group                    | Triclinic, P1                              |
| Unit cell dimensions                           | a = 8.6373(12) Å, α = 94.440(4)°          |
|                                              | b = 8.7962(12) Å, β = 93.877(4)°          |
|                                              | c = 13.5691(18) Å, γ = 118.458(4)°        |
| Volume, Z                                      | 897.2(2) Å³, 1                            |
| Density (calculated)                           | 1.464 Mg/m³                                |
| Absorption coefficient                         | 0.551 mm⁻¹                                 |
| F(000)                                         | 412                                        |
| Crystal size                                   | 0.490 x 0.440 x 0.310 mm³                  |
| Theta range for data collection                | 2.888 to 26.497°                           |
| Index ranges                                   | -10 ≤ h ≤ 10, -11 ≤ k ≤ 11, -12 ≤ l ≤ 12  |
| Reflections collected                          | 45496                                      |
| Independent reflections                        | 3674 [R(int) = 0.0588]                     |
| Completeness to theta = 25.242°                | 99.5 %                                     |
| Refinement method                              | Full-matrix least-squares on F²            |
| Data / restraints / parameters                 | 3674 / 2 / 230                             |
| Goodness-of-fit on F²                          | 1.157                                      |
| Final R indices [I > 2σ(I)]                    | R1 = 0.0684, wR2 = 0.1928                  |
| R indices (all data)                           | R1 = 0.0856, wR2 = 0.2093                  |
| Largest diff. peak and hole                    | 0.627 and -0.488 e.Å⁻³                    |

There are two pseudo six-membered rings, (C7/N2/C8/N3/H3A···O3) and (C7A/N2A/C8A/N3A/H3AA···O3A) and two five-membered rings, (C8/N3/N3A/H3AA···S1) and (C8A/N3A/N3/H3A···S1A) in the molecule due to N3-H3···O3A and N3-H3A···S1 intramolecular hydrogen bonds (table 5). In the crystal structure, the molecule is linked to two DMSO solvent by N2-H2A···O4 intermolecular hydrogen bonds. In addition, the linked DMSO molecule is also connected to another DMSO molecule by C10-H10B···O5 intermolecular hydrogen bonds which form short polymeric chain consist of five molecules and arrange along ab face (Figure 6).
Table 5. Hydrogen bonds geometry of 3 (Å and °)

| D-H···A     | d(D-H)  | d(H···A)  | d(D···A)  | <(DHA) |
|------------|---------|-----------|-----------|--------|
| N2-H2A···O4#2 | 0.86(4) | 1.92(4)   | 2.759(5)  | 165(4) |
| N3-H3A···O3   | 0.85(4) | 2.00(4)   | 2.609(4)  | 127(4) |
| N3-H3A···S1   | 0.85(4) | 2.47(4)   | 2.922(5)  | 114(3) |
| C10-H10B···O5#4 | 0.96    | 2.45      | 3.336(8)  | 153    |

Symmetry transformations used to generate equivalent atoms:
#1 -x+1,-y,-z+1  #2 x+1,y,z  #3 x,y,z  #4 -x+1,-y+1,-z+1

Figure 6. Molecular packing of compound 3 viewed down c axis. The dashed lines indicate intermolecular hydrogen bonds.

The important bond lengths and angles is presented in Table 6. The bond lengths and angles are in normal ranges (Table 6). All bond lengths and angles are in normal ranges [36].
Table 6. Selected bond lengths and angles of 3.

| Bond Length (Å) | Bond Angle (°) |
|-----------------|----------------|
| S1 – C8         | 1.663(4)       |
| N3 – N3a        | 1.371(6)       |
| N3 – C8         | 1.322(5)       |
| N2 – C8         | 1.387(5)       |
| N2 – C7         | 1.365(5)       |
| O3 – C7         | 1.210(5)       |
| C5 – N1         | 1.458(7)       |
| O3 – C7 – C6    | 121.6(4)       |
| C6 – C7 – N2    | 113.4(4)       |
| O3 – C7 – N2    | 124.7(4)       |
| N2 – C8 – N3    | 115.8(3)       |
| N3 – C8 – S1    | 123.7(3)       |
| C8 – N3 – N3a   | 119.4(4)       |

3.4. Antioxidant Screening

DPPH is a stable free radical molecule that has maximum absorption at 515-518 nm, thus it has purple colour. When DPPH accepts an electron or H radical from other molecule, it is converted into a non-radical molecule. The purple colour generally disappears and it can be measured from the changes in absorbance. Based on this property, DPPH was extensively used to evaluate the antioxidant activity of many compounds.

Table 7. The EC$_{50}$ values of the products and ascorbic acid

| Product | EC$_{50}$ (μM) |
|---------|----------------|
| 1       | 123.09         |
| 2       | 374.89         |
| 3       | 611.89         |
| 4       | 215.60         |
| Ascorbic Acid | 561.36     |

The antioxidant activity of the synthesized compounds on the DPPH radical was evaluated according to the methods of Molyneux with some modifications [37]. Various research groups have used widely different experimental procedures which differed in the concentration of DPPH, incubation time and reaction solvent. It caused the EC$_{50}$ values for even the standard ascorbic acid significantly different. For examples, by using the DPPH concentration of 50 μM in buffered methanol and 30 minutes of incubations, the EC$_{50}$ of ascorbic acid is 11.5 μM [38]. But when using the DPPH concentration of 100 μM in DMSO and 60 minutes of incubation, the EC$_{50}$ value of ascorbic acid is 68 μM [8]. Therefore, it is not possible to compare the results of the similar compounds and ascorbic acid of different experimental protocols [37].

The antioxidant activity of all compounds and ascorbic acid is presented in table 7. It can be seen that the EC$_{50}$ values of all compounds by DPPH free radical method are higher than the antioxidant activity of ascorbic acid, except for compound 3.

The proposed reaction mechanism of DPPH scavenging by synthesized compounds is shown in figure 6. The thiourea compounds stabilized the DPPH radical by two reaction mechanism involving hydrogen donor and electron transfer [8,39]. The thione C=S group transferred an electron to form a new bond with the DPPH radical. To stabilize the radical formed, the H atom from N-H thioamide.
group was donated to the other DPPH radical. The presence of two thiourea moieties, a bis(thiourea) molecule should be able to stabilize 4 DPPH radicals much effectively.

![Diagram](image.png)

**Figure 7.** Proposed reaction mechanism of DPPH scavenging by \( N,N' \)-bis(benzamidocarbonyl)hydrazine derivatives

Generally, the presence of electron withdrawing groups can enhance the antioxidant activity because they could stabilize the radical formed \[40\]. The low antioxidant activity of compound 3 may be due to the steric hindrance to the DPPH radicals to attack the \((NH)1\) groups. Further study about the structure activity relationship is needed to reveal the influence of the structures of the synthesized compounds to their antioxidant activity.

4. Conclusion
Four new \( N,N' \)-bis(benzamidothiocarbonyl)hydrazine (X-PhC(O)NHC(S)NH), where X = \( o \)-fluoro, \( o \)-chloro, \( o \)-nitro and \( o \)-methyl groups have been successfully synthesized and characterized by spectroscopic methods. Chemical crystallographic study of \( N,N' \)-bis(\( o \)-fluorobenzamidothiocarbonyl)hydrazine and \( N,N' \)-bis(\( o \)-chlorobenzamidothiocarbonyl)hydrazine showed that both thiourea moieties adopt trans geometry. Thus, monodentate coordination with metal is most likely to occur in a complexation reaction.

All the compound showed higher antioxidant activity against DPPH free radical than ascorbic acid (EC\(_{50}\) of 561.36 \( \mu \)M), except compound 3 (EC\(_{50}\) of 611.89 \( \mu \)M). Compound 1 has the highest antioxidant activity with EC\(_{50}\) of 123.09 \( \mu \)M, due to the presence of fluoro atom in \( o \)-position. The computational docking study is also needed to identify the potential important interaction between the products and the radical molecule for further antioxidant study.

Acknowledgement
We thank all staff of School of Chemical Science and Food Technology, FST UKM and The Centre for Research and Instrumentation (CRIM) UKM for providing all the facilities. We also acknowledged LPDP (Indonesia Endowment Fund for Education) of Indonesian Ministry of Finance for the scholarship.
References

[1] Khan K M, Naz F, Taha M, Khan A, Perveen S, Choudhary M I and Voelter W 2014 Synthesis and in vitro urease inhibitory activity of N,N’-disubstituted thioureas. Eur. J. Med. Chem. 74 314–23

[2] Karipcin F, Atis M, Sariboga B, Celik H and Tas M 2013 Structural , spectral , optical and antimicrobial properties of synthesized J. Mol. Struct. 1048 69–77

[3] Mohamed N a and Abd El-Ghany N a 2012 Preparation and antimicrobial activity of some carboxymethyl chitosan acyl thiourea derivatives. Int. J. Biol. Macromol. 50 1280–5

[4] Saeed S, Rashid N, Jones P G, Ali M and Hussain R 2010 Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. Eur. J. Med. Chem. 45 1323–31

[5] Vega-Pérez J M, Periñañi I, Argandoña M, Vega-Holm M, Palo-Nieto C, Burgos-Morón E, López-Lázaro M, Vargas C, Nieto J J and Iglesias-Guerra F 2012 Isoprenyl-thiourea and urea derivatives as new farnesyl diphosphate analogues: synthesis and in vitro antimicrobial and cytotoxic activities. Eur. J. Med. Chem. 58 591–612

[6] Koca İ, Özgür A, Coşkun K A and Tutar Y 2013 Synthesis and anticancer activity of acyl thioureas bearing pyrazole moiety. Bioorg. Med. Chem. 21 3859–65

[7] Jumal J, Yamin B M, Ahmad M and Heng L Y 2012 Mercury Ion-Selective Electrode With Self–plasticizing Poly(n–buthylacrylate) Membrane Based On 1,2-Bis(N’ benzoylthioureido)cyclohexane As Ionophore APCBEE Procedia 3 116–23

[8] Arif A, Abdul N, Yehye W A, Alhadi A A and Kadir F A 2014 European Journal of Medicinal Chemistry PASS-assisted design , synthesis and antioxidant evaluation of new butylated hydroxytoluene derivatives 87 564–77

[9] Sivan S K, Vangala R and Manga V 2013 Molecular docking guided structure based design of symmetrical N,N’-disubstituted urea/thiourea as HIV-1 gp120-CD4 binding inhibitors. Bioorg. Med. Chem. 21 4591–9

[10] Tatar E, Rollas S and Clercq E De 2008 Synthesis of some novel thiourea derivatives obtained from 5-[(4- thiones and evaluation as antiviral / anti-HIV and anti-tuberculosis agents 43

[11] Alkan C, Tek Y and Kahraman D 2011 Preparation and characterization of a series of thiourea derivatives as phase change materials for thermal energy Turk J Chem 35 769–77

[12] Xu X, Qian X, Li Z, Huang Q and Chen G 2003 Synthesis and insecticidal activity of new substituted N-aryl-N’-benzoylthiourea compounds J. Fluor. Chem. 121 51–54

[13] Criado J J, Gheorghe R, Gonz F J, Hermosa M R, Sanz F, Manzano J L, Monte E and Rodr E 2004 N-benzoyl- N 0-alkylacylated and their complexes with Ni ( II ) , Co ( III ) and Pt ( II ) – crystal structure of 3-benzoyl-1-butil-1-methyl-thiourea : activity against fungi and yeast 98 1307–14

[14] Rodríguez-Fernández E, Manzano J L, Benito J J, Hermosa R, Monte E and Criado J J 2005 Thiourea, triazole and thiadiazine compounds and their metal complexes as antifungal agents. J. Inorg. Biochem. 99 1558–72

[15] Alkan C, Tek Y and Kahraman D 2011 Preparation and characterization of a series of thiourea derivatives as phase change materials for thermal energy Turk J Chem 35 769–77

[16] Xu X, Qian X, Li Z, Huang Q and Chen G 2003 Synthesis and insecticidal activity of new substituted N-aryl-N’-benzoylthiourea compounds J. Fluor. Chem. 121 51–54

[17] Criado J J, Gheorghe R, Gonz F J, Hermosa M R, Sanz F, Manzano J L, Monte E and Rodr E 2004 N-benzoyl- N 0-alkylacylated and their complexes with Ni ( II ) , Co ( III ) and Pt ( II ) – crystal structure of 3-benzoyl-1-butil-1-methyl-thiourea : activity against fungi and yeast 98 1307–14

[18] Rodríguez-Fernández E, Manzano J L, Benito J J, Hermosa R, Monte E and Criado J J 2005 Thiourea, triazole and thiadiazine compounds and their metal complexes as antifungal agents. J. Inorg. Biochem. 99 1558–72

[19] Rauf M K, Badshah A, Gielen M, Ebihara M, Vos D De and Ahmed S 2009 Synthesis , structural characterization and in vitro cytotoxicity and anti-bacterial activity of some copper ( I ) complexes with N , N 0 -disubstituted thioureas J. Inorg. Biochem. 103 1135–44

[20] Vinithra G, Suganya S and Velmathi S 2013 Naked eye sensing of anions using thiourea based chemosensors with real time application Tetrahedron Lett. 54 5612–5

[21] Fan L, Luo C, Lv Z, Lu F and Qiu H 2011 Removal of Ag+ from water environment using a novel magnetic thiourea-chitosan imprinted Ag+. J. Hazard. Mater. 194 193–201

[22] Taylor P, Jing X, Xu F, Zhu Q, Ren X, Yan C and Wang J 2005 Novel and Efficient Cyclization Procedure for the Synthesis of 2,5-Disubstituted-1,3,4-thiadiazoles Without Using Any Ring-Closing Reagents Synth. Commun. 42 3251–60

[23] Hammami A, C. Kosheiry H and Armand M 2004 A New Family of 2(3H)-Thiazolone Azines as Precursors to Air-Stable Radical Cations Bull. Chem. Soc. Jpn. 77 165–7
[20] Hassan A A, Mohamed N K, Makhlouf M M, Brase S and Nieger M 2015 Synthesis of Oxaza- and Bis-oxathiaaza[3.3.3]propellanes from Dicyanomethylene-1,3-indanedione and 2,5-Dithiobiureas Synthesis (Stuttg.). 47 3036–42
[21] Simonov Y A, Fonari M S, Zaworotko M J, Abourahma H, Lipkowski J, Ganin V and Yavolovskii A A 2003 From 1D strands to extended molecular assemblies in the binary compounds of dithiooxamide and dithiobiurea with crown ethers Org. Biomol. Chem 1 2922–9
[22] Donia A M, Atia A A and Heniesh A M 2008 Efficient removal of Hg (II) using magnetic chelating resin derived from copolymerization of bisthiourea / thiourea / glutaraldehyde Sep. Purif. Technol. 60 46–53
[23] El S, Ghazy S, Mohamed G, El A, Ahmed O, Gammal A and Yousef T 2010 Flotation-separation of toxic metal ions from aqueous solutions using thiosemicarbazide derivatives as chelating agents and oleic acid as a surfactant Eur. J. Chem. 1 76–83
[24] Nevagi R J, Dhake A S, Narkhede H I and Kaur P 2012 Synthesis and Structural Study Biomol. Chem. 10 1342–50
[25] Pignedoli A, Peyronel G and Antolini L 1975 The Crystal and Molecular Structure of Dithiobiurea, N,N′-bis(thiocarbamoyl)hydrazine (NH2.CS.NH2) Acta Crystallogr. Sect. B Struct. Crystallogr. Cryst. Chem. B31 1903–6
[26] Akinchan N T, Drozdzewski P M and Battaglia L P 2002 Crystal structure and spectroscopic characterization of bis (N-phenylthiourea) J. Chem. Crystallogr. 32 91
[27] Weidong S, Jin Y, Lifei B and Wang X 2009 1-(2,3,4,6-tetra-O-β-D-glucopyranosyl)-3-thioureidothiourea monohydrate Acta Crystallogr. Sect. E Struct. Reports Online E65 o242
[28] Yusof M S M, Yamin B M and Shamsuddin M 2003 N-(N-benzoylhydrazinocarbothioyl)benzamide Acta Crystallogr. Sect. E Struct. Reports Online 59 o810–1
[29] Safin D a., Babashkina M G, Bolte M and Klein A 2009 The influence of the spacer Z on N-phosphorylated bis-thioureas and 2,5-dithiobiurea Z[C(S)NHP(O)(OiPr)2]2 (Z=NHCH2CH2NH, NHCH2NH, NHCH2NH2, NNNH) crystal design Polyhedron 28 1403–8
[30] Sheldrick G M 2008 A short history of SHELEX. Acta Crystallogr. A. 64 112–22
[31] Pavia D L, Lampman G M and Kriz G S 2001 Introduction to Spectroscopy (Thomson Learning, Inc.)
[32] Refat M S and El-Metwaly N M 2012 Spectral, thermal and biological studies of Mn(II) and Cu(II) complexes with two thiosemicarbazide derivatives. Spectrochim. Acta. A. Mol. Biomol. Spectrosc. 92 336–46
[33] Huang Y, Dong X, Zhang L, Chai W and Chang J 2013 Structure – property correlation of benzoyl thiourea derivatives as organogelators J. Mol. Struct. 1031 43–8
[34] Osman U M and Yamin B M 2012 Synthesis and Structural Study of Bis-Thiourea Moieties with Aromatic Linkers 1535–8
[35] Wilson D, Arada M D L A, Alegret S and del Valle M 2010 Lead(II) ion selective electrodes with PVC membranes based on two bis-thioureas as ionophores: 1,3-bis(N′-benzoylthioureido)benzene and 1,3-bis(N′-furoylthioureido)benzene. J. Hazard. Mater. 181 140–6
[36] Allen F H, Kennard O, Watson D G, Brammer L and Orpen A G 2002 Tables of Bond Lengths determined by X-Ray and Neutron Diffraction. Part 1–19
[37] Molyneux P 2004 The use of the stable free radical diphenylpicryl- hydrazyl ( DPPH ) for estimating antioxidant activity Songklanakarin J. Sci. Technol. 26 211–9
[38] Sharma O P and Bhat T K 2009 DPPH antioxidant assay revisited Food Chem. 113 1202–5
[39] Nguyen D T, Le T H and Bui T T T 2013 Antioxidant activities of thiosemicarbazones from substituted benzaldehydes and N-(tetra-O-acetyl-β-D-galactopyranosyl)thiosemicarbazide. Eur. J. Med. Chem. 60 199–207
[40] Khan I, Ali S, Hameed S, Rama N H, Hussain M T, Wadood A, Uddin R, Ul-Haq Z, Khan A, Ali S and Choudhary M I 2010 Synthesis, antioxidant activities and urease inhibition of some new 1,2,4-triazole and 1,3,4-thiadiazole derivatives Eur. J. Med. Chem. 45 5200–7