The role of acetone solvent in the reaction of 3-chloropropionyl- and 2-chloropropionyl-isothiocyanate with hydrazine

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Abstract. The reaction of 3-chloropropionylisothiocyanate with hydrazine did not give the expected bis(3-chloropropionylthioureido)hydrazine but instead 3-Chloro-N-[5,5-dimethyl-4-(4-oxo-5,6-dihydro-4H-[1,3]thiazin-2-yl)-4,5-dihydro-[1,3,4]thiadiazol-2-yl]-propionamide was obtained with 23.56% yield. On the other hand 2-thiocyanatopropionylisothiocyanate undergoes crisscross cycloaddition leading to the formation of 3,3,7,7-Tetramethyl-tetrahydro-[1,2,4][triazolo[1,2-a][1,2,4]triazole-1,5-dithione with 96.1% yield. The mechanisms involving the acetone solvent for both reactions are discussed. Both compounds were evaluated to their antioxidant activity using DPPH radical scavenging method. The results shows that compound 2 with EC50 > 1000 μM is less active than 1 (EC50 of 76 μM) may be due to the S-alkylated on the structure which eliminated its activity. Compound 1 showed higher activity than ascorbic acid (EC50 of 561.36 μM) so that it is potential as antioxidant agent.

1. Introduction
The participation of solvent in particular acetone in various reactions is well known. However, the driving force or factor that brings about the participation in certain reaction but not in the others is not well understood. Organo-isothiocyanate is a well-known source for the formation of thiourea moiety. The ease of the reaction has produced numerous carbonoylthioureas of the type RC(O)NHC(S)NHR’ such as N-benzoyl-N’phenyl thiourea[1] and carbonothioyl-benzamide[2]. The ambidentate nature of the anion has displayed diversity of role in many organic and inorganic reactions. It is now understood that the halogenocarbonyl undergoes nucleophilic substitution at the ipso carbon leading to the formation of carbonylisothiocyanate, an important intermediate for the thiourea formation. On the contrary, the 2-halogenocarbonyl where the halogen is at β-position to the carbonyl the sulfur atom will replace the halogen to give thiocyanatocarbonyl. The later will always give cyclized products when reacted with amine compounds such as in the formation of 3,5-diphenyl-1H-1,2,4-triazole[3]. In addition to the ambidentate nature of the anion it also allow the participation of solvent in particular acetone which normally end up as cyclized product containing dimethyl group in the ring system as shown in Safin et al[4]. The established nucleophilic substitution of carbonylisothiocyanate has produced some bis(thiourea) such as 1,2-Bis(N’-benzoylthioureido)-4- chlorobenzene[5] and 1,2-bis(N’-benzoylthioureido)cyclohexane[6].
N,N’-Bis(benzamidothiocarbonyl)hydrazine[7] is an example of bis-thiourea in which the thiourea moieties are linked by the shortest linker, hydrazine. Ethylenediamine is another linker that has displayed bis-thiourea[8] and cyclized product[9]. In this paper, the participation of acetone in the reaction of 3-chloropropionylisothiocyanate and 2-thiocyanatopropionylisothiocyanate containing both thiocyanate and isothiocyanate unit with hydrazine with the initial aim to produce bis-thiourea but end-up with cyclized products is discussed. We also screened the antioxidant activity of both products by using in-vitro DPPH radical scavenging method.

2. Experimental Details

2.1. Materials

All chemicals used in this research were purchased from Sigma-Aldrich and Merck. Acetone was dried with molecular sieves before used. Elemental Analysis data were collected using ThermoFinnigan Instrument. Infrared spectra were recorded with FTIR Perkin Elmer 100 Spectrophotometer in the region of 400-4000 cm⁻¹ by using KBr pellet method. ¹H and ¹³C NMR spectrum were collected using Bruker AVANCE III 600 MHz with deuterated DMSO as solvent.

Single crystal data was collected using Bruker SMART APEX CCD Diffractometer with graphite monochromatic Mo Ka radiation source. Crystal structure was solved by the SHELXS-2013 program and refined by SHELXL-2013 program[10].

2.2. Synthesis Method

2.2.1. Synthesis of 3,3,7,7-Tetramethyl-tetrahydro-[1,2,4]-triazolo[1,2-a]-[1,2,4]triazole-1,5-dithione 1.

A solution of 2-chloropropionylchloride (4 mmol) in dry acetone was treated with ammonium thiocyanate (8 mmol) for 15 minutes at room temperature. The mixture was filtered off and the filtrate was added with hydrazine hydrate (2 mmol). The mixture was heated under reflux for 3 hours and was evaporated at room temperature. The precipitate was washed with cool acetone and EtOH/H₂O (1:1) to yield the pure product. The crystalized product was analyzed by X-ray Single Crystal Diffractometer. White crystalline solid was obtained in 96.17% yield. mp 179.8 °C. IR (KBr, ν, cm⁻¹): 3084.76 (N-H secondary amine), 2979.86 (C-H methyl), 1217.99 (C-N), 1077.98 (C=S). ¹H-NMR (DMSO-d₆) δ(ppm): 1.66 (s, 12H, CH₃-); 9.53 (s, 2H, NH). ¹³C-NMR (DMSO-d₆) δ(ppm): 25.7 (4C); 79.6 (2C); 169.4 (2C).

2.2.2. Synthesis of 3-chloro-N-[5,5-dimethyl-4-(4-oxo-5,6-dihydro-[1,3,4]thiadiazol-2-yl)-4,5-dihydro-[1,3,4]thiadiazol-2-yl]-propionamide 2.

A solution of 3-chloropropionylchloride (4 mmol) in dry acetone was treated with ammonium thiocyanate (4 mmol) for 15 minutes at room temperature. The mixture was filtered off and the filtrate was added with hydrazine hydrate (2 mmol). The mixture was heated under reflux for 3 hours and was evaporated at room temperature. The precipitate was washed with cool acetone and EtOH/H₂O (1:1) to yield the pure product. The crystalized product was analyzed by X-ray Single Crystal Diffractometer. White solid was obtained in 23.54% yield. mp 202.2-203.2 °C. IR (KBr, ν, cm⁻¹): 3104.99 (N-H secondary amine), 2908.39 (C-H methyl), 1697.21 (C=O), 1187.32 (C-N), 710.86 (C=S). ¹H-NMR (DMSO-d₆) δ(ppm): 1.994 (s, 6H, (CH₃)₂-); 2.435 (t, 2H, CH₂-); 2.893 (t, 2H, CH₂-); 3.146 (t, 2H, CH₂-); 3.815 (t, 2H, CH₂-); 12.017 (s, 1H, NH-). ¹³C-NMR (DMSO-d₆) δ(ppm): 175.48; 172.72; 169.72; 151.46; 38.60; 33.35; 30.38; 28.74; 26.92.
2.3. Antioxidant Study

The antioxidant activity of both products was screened by using DPPH radical scavenging method[11]. When the deep violet DPPH free radical reacts with a compound that can donate hydrogen atom, DPPH-H will be formed and that violet colour will turn to the pale yellow (Scheme 1).

\[
\text{Diphenylpicrylhydrazyl (free radical)} \rightarrow \text{Diphenylpicrylhydrazine (nonradical)}
\]

Scheme 1. Reaction of DPPH free radical

300 μM solution of DPPH (1.5 mL) in 96% ethanol was added to sample solutions (each 1.5 mL) of different concentrations (20 – 1000 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:

\[
Q = 100(\frac{A_c - A_s}{A_c})\%
\]

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

3. Results and Discussion

3.1. Chemistry

Syntheses of \(1\) and \(2\) have been done with two steps of reactions (Scheme 2 and 3). The reactions were started with substitution of each 2-chloropropionyl chloride and 3-chloropropionyl chloride with ammonium thiocyanate using dry acetone as the solvent. The next step was hydrazinolysis with half-fold equimolar of hydrazine hydrate. Both compounds were determined by their melting points and characterized by their IR, \(^1\)H NMR, \(^{13}\)C NMR, and crystallography data.

Scheme 2. Synthesis reaction of compound 1

The infrared spectra of compound 1 shows four important bands at 3085, 2980, 1218 and 1102 cm\(^{-1}\) for stretching N-H, methyl, C-N and C=S groups, respectively. The singlet signal in the \(^1\)H NMR spectrum at \(\delta\) 9.53 ppm corresponds to the proton of NH groups. The 12 integration signal at \(\delta\) 1.66 ppm represents the protons on the four symmetry methyl groups. While in the \(^{13}\)C NMR spectrum, the signals at \(\delta_c\) 25.7, 79.6 and 169.4 ppm are assigned for methyl, quartenary and thione carbons, respectively.
The molecular structure of 1 and 2 are given in figure 1. Compound 1 crystallized in monoclinic system with $P_{2\text{I}}\overline{1}$ space group, while compound 2 crystallized in orthorhombic system with $P_{bca}$ space group. The crystallographic and refinement data of both crystals are shown in Table 1. The similar molecular structure and data of compound 1 has been reported by Safin et al. The bond length and angle (Table 2) are similar in the previously report.
Table 2. The Bond Length and Angles of Crystal 1 and 2

| Bond Length (Å) | 1       | 2       |
|-----------------|---------|---------|
| S(1)-C(3)       | 1.672(2)| 1.528(2) |
| N(1)-C(3)       | 1.330(2)| 1.290(2) |
| N(1)-N(1)#1     | 1.394(3)| 1.340(3) |
| N(1)-C(2)       | 1.466(2)| 1.409(2) |
| N(2)-C(3)#1     | 1.328(2)| 1.290(2) |
| N(2)-C(2)       | 1.469(2)| 1.409(2) |
| C(2)-C(5)       | 1.506(3)| 1.480(3) |
| Cl(1A)-C(1)     | 1.907(8)| 1.907(8) |
| Cl(1B)-C(1)     | 1.693(7)| 1.693(7) |
| S(1)-C(4)       | 1.740(3)| 1.740(3) |
| S(1)-C(5)       | 1.847(3)| 1.847(3) |
| S(2)-C(8)       | 1.780(4)| 1.780(4) |
| S(2)-C(11)      | 1.780(4)| 1.780(4) |
| O(1)-C(3)       | 1.209(4)| 1.209(4) |
| O(2)-C(9)       | 1.231(4)| 1.231(4) |
| N(1)-C(4)       | 1.359(4)| 1.359(4) |
| N(1)-C(5)       | 1.376(4)| 1.376(4) |
| N(2)-C(4)       | 1.277(4)| 1.277(4) |
| N(3)-C(8)       | 1.347(4)| 1.347(4) |
| N(3)-C(9)       | 1.352(4)| 1.352(4) |
| C(1)-C(2)       | 1.469(7)| 1.469(7) |
| C(2)-C(3)       | 1.517(5)| 1.517(5) |
| C(5)-C(7)       | 1.511(5)| 1.511(5) |
| C(5)-C(6)       | 1.520(5)| 1.520(5) |
| C(9)-C(10)      | 1.504(5)| 1.504(5) |

| Bond Angle (°)  | 1       | 2       |
|-----------------|---------|---------|
| C(3)-N(1)-N(1)#1| 110.73(17)| 110.73(17) |
| C(3)-N(1)-C(2)  | 138.80(14)| 138.80(14) |
| N(1)#1-N(1)-C(2)| 109.44(17)| 109.44(17) |
| C(3)#1-N(2)-C(2)| 115.48(14)| 115.48(14) |
| N(2)-C(2)-N(1)  | 97.06(12)| 97.06(12) |
| N(2)-C(2)-C(5)  | 112.2(4) | 112.2(4) |
| N(1)-C(2)-C(5)  | 112.2(4) | 112.2(4) |
| N(2)-C(2)-C(4)  | 112.2(4) | 112.2(4) |
| N(1)-C(2)-C(4)  | 112.2(4) | 112.2(4) |
| C(5)-C(2)-C(4)  | 112.2(4) | 112.2(4) |
| N(2)#1-C(3)-N(1)| 112.50(16)| 112.50(16) |
| N(2)#1-C(3)-S(1)| 127.68(13)| 127.68(13) |
| N(1)-C(3)-S(1)  | 125.99(13)| 125.99(13) |
| C(4)-S(1)-C(5)  | 90.18(14)| 90.18(14) |
| C(8)-S(2)-C(11)| 98.16(17)| 98.16(17) |

The intermolecular hydrogen bonds were formed between H(N2) and S1. Thus, there are four hydrogen bonds in each molecule (figure 1b). This hydrogen bond stabilized the crystal structure and formed polymeric chain.

![Figure 1. Molecular Structure of (a) Compound 1; (b) Compound 2](image-url)

The molecular structures of compound 1 and 2 show that there were cyclization processes in their formation. Both of the compounds have the geminal dimethyl groups due to the involving of acetone in the reaction. The proposed reaction mechanism of 1 has been explained in Safin et al.[4].
Scheme 4. Proposed Reaction Mechanism of 1 by criss-cross cycloaddition

The reaction is involving two-fold equimolar of acetone to formed aldazine as intermediate, then continued to the crisscross cycloaddition to formed 1, as shown in scheme 4. The proposed reaction mechanism of 2 is given in Scheme 5. We assume that bis(3-chloropropionylthioureido)hydrazine was formed from reaction between 3-chloropropionylisothiocyanate and hydrazine. The presence of chloro substituent at β position led to the cyclization reaction. The second cyclization took place due to the involving of one equimolar of acetone in the reaction and formed compound 2.

Scheme 5. Proposed reaction mechanism of 2 (i) Formation of bis-thiourea; (ii) 1\textsuperscript{st} cyclization reaction; (iii) reaction with acetone; (iv) 2\textsuperscript{nd} cyclization reaction
3.2. Antioxidant Study

An antioxidant compound is the compound that can easily provide hydrogen atoms or donate electrons to the radical molecules. The study of scavenging activity of compound 1 and 2 by DPPH radical scavenging method, as shown in Table 3, revealed that compound 1 exhibited higher antioxidant activity with EC50 of 76 μM than ascorbic acid (EC50 of 561.36 μM).

| Compound | EC50 (μM) |
|----------|-----------|
| 1        | 76.00     |
| 2        | >1000     |
| Ascorbic Acid | 561.36   |

Table 3. Antioxidant activity of synthesized compounds by DPPH method

Many studies reported that the presence of thiourea moiety in the molecules enhance their antioxidant activity[12]–[14]. The C=S bonds in compound 1 can easily stabilized the odd electron of DPPH radicals and led to the hydrogen abstraction of the N-H bonds[15], as shown in Scheme 6.

Scheme 6. Proposed scavenging of DPPH radicals by compound 1

Compound 2 showed very low activity with EC50 more than 1000 μM (did not reach Q of 50%). This is may be due to the S-alkylation of two thiourea moiety on the molecule. This S-alkylated structure (figure 2) eliminated its antioxidant activity[15].

Figure 2. S-alkylated in compound 2
4. Conclusion

Two unexpected compounds, 3,3,7,7-Tetramethyl-tetrahydro-[1,2,4]triazolo [1,2-a][1,2,4]triazole-1,5-dithione 1 and 3-Chloro-N-[5,5-dimethyl-4-(4-oxo-5,6-dihydro-4H-[1,3]thiazin-2-yl)-4,5-dihydro-[1,3,4]thiadiazol-2-yl]- propionamide 2 have been synthesized through cyclization reaction, and characterized. The crystals of compound 1 and 2 revealed monoclinic and orthorhombic crystal systems, respectively. The antioxidant study showed that compound 2 has very low antioxidant activity (EC50 > 1000 μM) due to the S-alkylated molecular structure, while compound 1 has good antioxidant activity with EC50 of 76 μM, thus it is potential as an antioxidant agent.

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