Malaysian Journal of Chemistry, 2017, Vol. 19(2), 118 – 123

One-pot Synthesis of 3,3,7,7-Tetramethyl-tetrahydro-[1,2,4]triazolo[1,2-a][1,2,4]triazole-1,5-dithione and its Biological Activities

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3,3,7,7-Tetramethyl-tetrahydro-[1,2,4]triazolo[1,2-a][1,2,4]triazole-1,5-dithione 1 has been synthesized from 2-chloropropionyl chloride ammonium thiocyanate, and hydrazine in 96% yield. Criss-cross cycloaddition involving aldazine produced from the acetone-hydrazine reaction was proposed for the mechanism. The structure was confirmed by micro-elemental analysis, FTIR, ^{1}H and ^{13}C NMR spectroscopy and ESI mass spectrometry. Compound **1** crystallized in monoclinic crystal system with P21/c space group. Antibacterial screening against *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, and Gram-negative bacteria: Enterobacter aerogenes, Klebsiella pneumoniae, Proteus vulgaris, Escherichia coli,* and *Pseudomonas aeruginosa* were conducted. The highest activity was against *S. epidermidis* (MIC = 1.563 mg/ml and MBC = 1.563 mg/ml) and *Escherichia coli* (MIC = 1.563 mg/ml and MBC = 1.563 mg/ml) thus exhibiting a moderate antibacterial activity.

Key words: Antibacterial activity; Criss-cross cycloaddition; X-ray crystal structure; triazole compound

Received: January 2016; Accepted: April 2017

The reaction between isothiocyanate and diamine compounds usually lead to the formation of bis-thiourea. The diamine acts as a linker of two thiourea groups at N,N' positions. For example, N,N'-Bis (benzamidothiocarbonyl) hydrazine has hydrazine group as the linker of the two benzamidothiocarbonyl groups [1]. Some bis-thioureas with phenylene diamines linkers have also been synthesized [2–5]. Bis- phosporilated thiourea is the other example where ethylenediamine is the linker [6].

In the present study, we investigated the reaction between 2-chloropropionychloride **2**, ammonium thiocyanate and hydrazine in acetone as the solvent with an objective to synthesize bis-chloronatedthiourea linked by hydrazine. Unexpectedly, a triazole compound, tetrahydro-3,3,7,7-tetramethyl- [1,2,4] triazolo [1,2-a][1,2,4] triazole-1,5-dithione **1**, was obtained instead of the expected bis-thiourea.

It is known that triazole compounds have been widely used especially in pharmaceutical field [8]. Some triazole compounds possess high cytotoxicity against human cancer cell lines [7,9,10] and good antimicrobial activities against some bacteria. Kùçùkgùzel *et al.* in 2008 revealed the moderate activities of some triazole compounds as antivirus and antituberculosis agents [11].

In fact the synthesis of tetrahydro-3,3,7,7-tetramethyl-[1,2,4]triazolo [1,2-a][1,2,4]triazole-1,5-dithione has been reported by Safin *et al.* involving several steps [12]. But in this paper, we report the synthesis, characterisation and the X-ray structure of the product. The antibacterial activity against nine bacteria, *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Enterobacter aerogenes, Klebsiella pneumonia,*

Proteus vulgaris, Escherichia coli, and *Pseudomonas aeruginosa* were also investigated.

EXPERIMENTAL DETAILS

Materials

All chemicals used in this research were purchased from Sigma-Aldrich and Merck. Acetone was dried with molecular sieves before use. Elemental analysis data were collected using Thermo Finnigan Instrument. Infrared spectra were recorded with FTIR Perkin Elmer 100 Spectrophotometer in the region of 400–4000 cm^{-1} by using KBr pellet method. ^{1}H and ^{13}C NMR spectrum were collected using Bruker AVANCE III 600 MHz with deuterated DMSO as the solvent. Mass spectral ESI measurement was carried out on Dionex Bruker MicroToF Q Instrument.

Single crystal data was collected using Bruker SMART APEX CCD diffractometer with graphite monochromatic Mo Kα radiation source. The crystal structure was solved by the SHELXS-97 program and refined by SHELXL-97 program [13].

Synthesis of 1

A solution of 2-chloropropionylchloride **2** (4 mmol) in dry acetone was treated with ammonium thiocyanate (8 mmol) for 15 min 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 h and was evaporated at room temperature. The precipitate was washed with EtOH/H_2O (1:1) and dried under vacuum. The white solid was recrystallized from CH_2Cl_2/EtOH solvents (1:1).

The white crystalline solid was obtained in 96% yield. mp 178.8-180.0°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). ^{1}H-NMR (DMSO-d_6) δ(ppm): 1.66 (s, 12H, CH_3–); 9.53 (s, 2H, NH). ^{13}C-NMR (DMSO-d_6) δ(ppm): 25.7 (4C); 79.6 (2C); 169.4 (2C). Anal. Calcd. (%) for $C_8H_{14}N_4S_2$: C, 41.74; H, 6.09; N, 24.35; S, 27.83. Found: C, 37.96; H, 7.34; N, 23.51; S, 27.71. MS (ESI) m/z = 230.954 $[M]^+$ (100%).

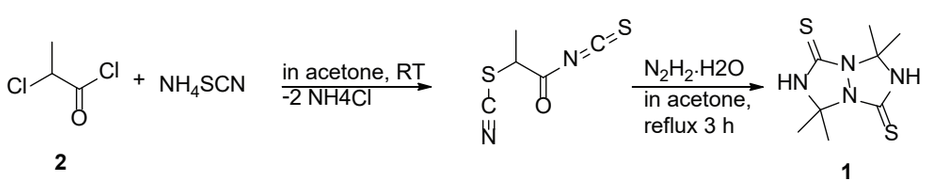
Biological Study

The antibacterial activity of **1** was screened against a series of Gram-positive bacteria: *S. epidermidis, S. aureus, B. subtilis, E. faecalis,* and Gram-negative bacteria: *E. aerogenes, K. pneumonia, P. vulgaris, E. coli,* and *Pseudomonas aeruginosa* broth dilution method (Figure 1). Chloramphenicol, cefotaxime and tobramycin were used as positive controls. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were calculated.

RESULT AND DISCUSSION

Chemistry

Synthesis of **1** was carried out in one-pot reaction involved a substitution reaction of 2-chloropropionyl chloride with a two-fold equimolar amount of ammonium thiocyanate in dry acetone as the solvent and followed by hydrazinolysis with half-fold equimolar of hydrazine hydrate. The white precipitate of 2-chloroisothiocyanate in the first step could be filtered or left in the flask during the reflux.



2 **1**

Scheme 1. Reaction scheme for the formation of **1**.

The infrared spectrum shows three important thiourea bands at 3084.7, 1217.9 and 587.2 cm^{-1} assigned to the stretching of υ(N-H), υ(C-N) and υ(C=S) functional groups respectively. However, the presence of $\upsilon(CH_3)$ at 2979.8 cm^{-1} indicated that the product was not the expected bis-thiourea.

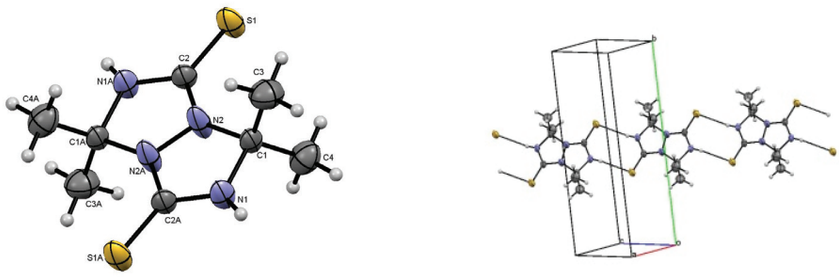
The ^{1}H NMR and ^{13}C NMR spectra were recorded in deuterated DMSO. The ^{1}H signal at 1.6 ppm corresponded to the protons of four symmetrical methyl groups whereas at 9.53 ppm was the amino protons. The ^{13}C NMR spectrum showed the presence of the methyl carbon at 25.7 ppm. The signals at 79.6 ppm corresponded to the quaternary carbon that bonded two methyl groups. The signal at 169.4 ppm was assigned for the thiono carbon of the -N(C=S) N- fragment.

The X-ray structure was studied to support the spectroscopic and elemental analysis data. The crystal system and refinements parameters (Table1) are in agreement with the previous report [12].

The two-fold symmetrically generated molecule has two triazol rings fused at N-N bond (Figure 2a). The fused rings S1/C1/C2/N1/N2/S1A/C1A/C2A/N1A/N2A are planar with the maximum deviation of 0.052(2)Å for N2 and N2A atoms from the least square plane. The two methyl groups with C3 and C4 atoms are sitting at the top and bottom of the ring are at an angle of 112.50(16)° about C1 atom. In the crystal structure, the molecule is stabilized by N1-H1A…S1 (1+x,y,1+z; N-H 0.86Å, H…A=2.59Å; D…A= 3.3669Å; D-H…A=171°) intermolecular hydrogen bonds to form one dimensional chain along a-axis (Figure 2b).

Table 1. The crystal system and refinement data.

Parameter	Data
Empirical Formula	$C_8H_{14}N_4S_2$
Formula weight	230
Crystal system	Monoclinic
Space group	P21/c
Unit cell dimensions	a = 5.7342(4) Å α = 90°
	b = 15.2734(9) Å β = 107.369(2)°
	c = 6.9099(4) Å γ = 90°
Volume	577.58(6) $Å^3$
Z, calculated density	4, 1.290 Mg/m^3
F(0 0 0)	236
Crystal size	0.500 × 0.310 × 0.240 mm^3
Theta range for data collection	3.365 to 25.498°
Index indices	-6<=h<=6
	-18<=k<=18
	-8<=l<=7
Reflections collected/unique	11891
Completeness to theta = 25.242°	99.7%
Refinement method	Full-matrix least-squares on F2
Data/restraints/parameters	1066 / 0 / 69
Goodness-of-fit on F2	0.657
Final R indices	R_1= 0.0323, wR_2 = 0.0991
R indices	R_1 = 0.0338, wR_2 = 0.1013
Largest diff. peak and hole	0.190 and -0.224 e.Å-3

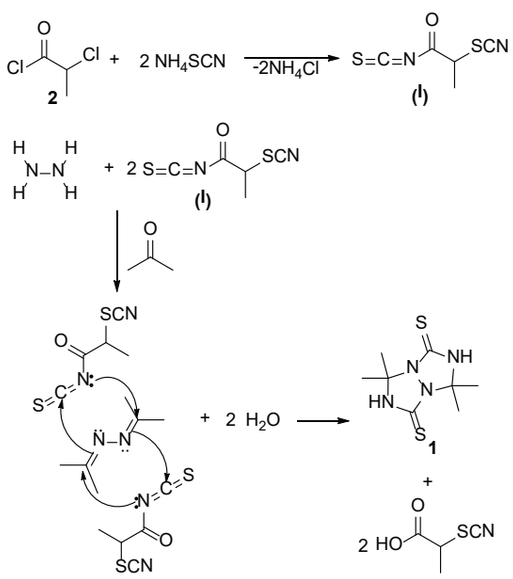


(a) (b)

Figure 2. (a) Molecular structure of compound **1** drawn at 50% probability ellipsoids; (b) The molecular packing of **1**.

The bond lengths and angles were in normal ranges with C2-S1 and N2-C2 bond length of 1.6722(16) and 1.328(2) Å, respectively. The N1-C2-N2, N1-C2-S1 and N2-C2-S1 bond angles were 106.34(14), 127.68(13) and 125.99(13)°, respectively.

It was now possible to suggest the mechanism of the reaction. The first step was the formation of 2-thiocyanato-propionyl isothiocyanate **(I)** based on the fact the ipso chlorine atom would be replaced by isothiocyanato and the α-chloro by thiocyanato group. Unlike in the case organo-isothiocyanato that would react directly with hydrazine, here the hydrazine reacted with two acetone molecules to form aldazine which would then react with two equivalents of **(I)** via criss-cross cycloaddition reaction and hydrolysis to form compound **1** as shown in Scheme **2**.



Scheme 2. Proposed reaction mechanism of the synthesis of **1**.

Biological Study

The MIC and the MBC of **1** were obtained. MIC was the lowest concentration of antimicrobial agent required to inhibit the growth of bacteria, while MBC was the lowest concentration of antimicrobial needed to kill the bacteria. The value of MIC and MBC of the synthesized compound are shown in Table 2. This compound showed activity against all the tested bacteria with high values of MIC and MBC against *S. epidermis* and *E. coli*. However, their selectivity index was low (<10) indicating not the highly potential therapeutic antibacterial agent.

CONCLUSION

The reaction of 2-chloropropionylchloride with ammonium thiocyanate and hydrazine did not give the expected bis-thiourea but instead, 3,3,7,7-Tetramethyl-tetrahydro-[1,2,4]triazolo [1,2-a][1,2,4]triazole-1,5-dithione was formed in high yield. The mechanism was proposed based on the formation of mixed-isothiocyanato and thiocyanato intermediate that reacted with aldazine and underwent criss-cross addition and followed by hydrolysis.

The compound showed significant activity against the selected gram-positive and Gram-negative bacteria with high MIC and MBC values against *Staphylococcus epidermis* and *Escherichia coli*. However, the selectivity index values were less the 10 indicating not a potential therapeutic antibacterial agent. The fact that compound **1** is not toxic, further study on other biological activities including anti-virus and anticancer would be of interest.

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Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (in mg/ml).

Bacteria	MIC	MBC
Staphylococcus epidermidis	1.563	1.563
Staphylococcus aureus	3.125	6.25
Bacillus subtilis	1.563	>25
Enterococcus faecalis	3.125	25
Enterobacter aerogenes	3.125	3.125
Klebsiella pneumoniae	3.125	3.125
Proteus vulgaris	3.125	3.125
Escherichia coli	1.563	1.563
Pseudomonas aeruginosa	3.125	25

Selectivity index = CC_{50}/MIC (CC_{50} for 1 was predetermined against Vero cell = 0.3416)

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