

Synthesis, X-Ray diffraction, theoretical and anti-bacterial studies of bis-thiourea secondary amine



Imran Fakhar ^a, Nasry Jassim Hussien ^{a,b}, Suhaila Sapari ^a, Anmar Hameed Bloh ^c, Siti Fairus Mohd Yusoff ^a, Siti Aishah Hasbullah ^{a,**}, Bohari Mohammad Yamin ^a, Sahilah Abdul Mutalib ^a, Mehdi Salih Shihab ^{d,*}, Emad Yousif ^d

^a School of Chemical Sciences and Food Technology, University Kebangsaan Malaysia, Bangi, 43600, Selangor, Malaysia

^b Department of Chemistry, College of Education for Pure Science, Diyala University, Baqubah, Iraq

^c School of Biosciences and Biotechnology, University Kebangsaan Malaysia, Bangi, 43600, Selangor, Malaysia

^d Department of Chemistry, College of Science, Alnahrain University, Baghdad, Iraq

ARTICLE INFO

Article history:

Received 20 October 2017

Received in revised form

13 January 2018

Accepted 14 January 2018

Available online 30 January 2018

Keywords:

Bis-thiourea

Anti-bacterial

PM3

Crystallographic study

ABSTRACT

N^1,N^4 -Bis[(2-hydroxyethyl)(methyl)carbamothioyl]terephthalamide (1A) was synthesized by reacting terephthaloyl chloride and ammonium thiocyanate and the product was reacted with 2-Methyl amino ethanol to afford the final product. The product was characterized by Infra Red, Nuclear Magnetic Resonance and Electrospray Ionization mass Spectrometric techniques. The crystal was obtained by recrystallization from DMSO by slow evaporation technique. The X-ray studies reveal that (1A) is crystallized in monoclinic system with space group P 21/n, $a = 6.9727(9)$, $b = 17.649(2)$, $c = 8.2629(11)$, $\alpha = 90^\circ$, $\beta = 112.329(4)$, $\gamma = 90^\circ$. $Z = 2$ and $V = 940.6(2)$. In the crystal structure, the molecules are linked by O(1) ... H(1) ... S(1), and O(1) ... H(1) ... O(2) intermolecular H-bonds forming a 3-D network. In addition, the antibacterial activities against four different strains of bacteria and theoretical evaluation for the stable geometries for (1A) has been performed using semi-empirical calculations of PM3 method.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

Thiourea is an emerging class of organic compounds, first synthesized by Nencki in 1873 [1]. Thiourea is the analogue compound to urea with replacement of oxygen atom in urea by sulfur atom. They are extremely versatile building blocks composed basically of C, S, N and H atoms. Thiourea linkage ($-\text{HN}-\text{CS}-\text{NH}-$) attributes chemical reactivity and biological properties to this class of compounds, resulting in an assortment of organometallic complexes and a wide spectrum of biological activities. Thiourea derivatives demonstrate an extensive employments in the field of pharmacy, agro-science, and analytical chemistry. They demonstrate a wide range of biological activities, for example, antiviral [2,3], anti-bacterial [4], fungicidal [5–7], analgesic, herbicidal [8,9], plant growth regulator [10], anti-aggregant [11], anti-arrhythmic sedate [12], local anesthesia [13], and anti-hyperlipidemic activity [14].

Some thiourea compounds show anticancer activities and show significant inhibition towards HIV reverse transcriptase [15].

Derivatives of bis-thiourea, comprised of two thiourea moieties, so their activities attributable to thiourea functionality demonstrate impressive enhancement [16,17]. Some of the Bis-thiourea derivatives display antitumor activities and found to exhibit cytotoxic inhibition towards various malignant growths [18].

In general, aromatic derivatives of thiourea are quite stable. The aromatic nuclei alongside thiourea fragment are coplanar and impart rigidity to the auxiliary structure. They additionally possess many sites for the substitution of many functional groups [19]. The C–O and C–N bond lengths of 1.26 (1) and 1.33 (1) Å [20,21] in urea, as well as the complete planarity of this molecule, including hydrogen, indicate a delocalized π molecular orbital involving all of the non-hydrogen atoms. For reference, the C–N bond in s-triazine [22] found to be 1.338 (1) Å and a normal C–N single bond would be 1.47 Å [23]. Thiourea compound in this study has C–S and C–N bond lengths of 1.659 (19) and 1.362 (3) Å, respectively. The paucity of data on C–S multiple bonds does not allow a quantitative assessment of multiple bonding in thiourea, but 1.659 Å is considerably shorter than the 1.81 Å expected from single-bond radii [23].

* Corresponding author.

** Corresponding author.

E-mail addresses: aishah80@ukm.edu.my (S.A. Hasbullah), mehdi_shihab@yahoo.com (M.S. Shihab).

The 1.36 Å C–N distances in both urea and thiourea compared with s-triazine indicate significant multiple C–N bonding in both of these molecules [24].

In the current study, symmetrical bis-thiourea derivative (**1 A**) was successfully synthesized from terephthaloyl chloride, which was used as a spacer and held the central position of the molecule, while, 2-methyl amino ethanol was incorporated as a side chain linker (Scheme 1). The synthesized derivative was characterized by FTIR, ¹H NMR, ¹³C NMR, ESI-MS, CHNS Elemental analysis and X-ray crystallographic techniques. The antibacterial activity of Bis-thiourea derivative **1A** was examined *in vitro* against bacteria *Lysinibacillus* sp. Gb01, *Vibrio owensii* Gb04, *Vibrio owensii* SS1, *Vibrio alginolyticus* SS17 by using the agar diffusion technique at 37 °C. The stable geometry of the compound was confirmed using PM3 method.

2. Experimental

2.1. Materials and measurements

Most of the chemicals used in this study were purchased from Sigma-Aldrich (St Louis, MO, USA) and Acros Organics (Geel, Belgium) and utilized as received. Solvents were further purified by distillation. The micro-elemental analysis for CHNS was performed on a Carlo Erba 1108 Elemental Analyzer (Milan, Italy). The infrared spectra (FTIR) of the products (KBr pellets) were recorded using a Perkin Elmer Spectrum GX spectrophotometer (Perkin Elmer, Waltham, MA, USA) in the range of 400 ~ 4000 cm⁻¹. NMR (¹H and ¹³C) experiments were performed on a Bruker 400 MHz instrument utilizing DMSO-d6 as a solvent. Single-crystal X-ray investigations were performed on a Bruker D-QUEST diffractometer (Bruker, AXS Inc., Madison, WI, USA) with graphite monochromated Mo-K α radiation ($\lambda = 0.71073 \text{ \AA}$). Measurements of the intensity data were recorded at room temperature by ω -scan. Precise cell parameters and orientation matrix were ascertained by the full-matrix least squares fit of 25 reflections. Intensity data were gathered for Lorentz and polarization effects. The empirical absorption amendment was completed utilizing multi-scan. The structure was solved by a direct method and least squares refinement of the structure was performed by the SHELXL-2007 program [25].

2.2. General procedure for the synthesis of **1A**

Terephthaloyl chloride (0.003 mol, 0.609 g), was dissolved in dry acetone (20 ml). A solution of Ammonium thiocyanate (0.006 mol, 0.456 g, antecedently dried (80 °C, 2 h), in dry Acetone (15 ml), was prepared. To the stirring solution of Terephthaloyl chloride, Ammonium thiocyanate was added slowly over a time period of 30 min, then reaction mixture was stirred at room temperature for an additional hour. White precipitates of Ammonium ammonium chloride was filtered off. 2-Methyl amino ethanol (0.006 mol, 0.450 g), in dry acetone (15 ml) was added to the filtrate containing Terephthaloyl isothiocyanate intermediate. The reaction mixture was then refluxed for 10 h. Excess of crushed ice was added to the

flask, Bis-thiourea analogue was collected as precipitates in good yield, 91.8% (Scheme 1). Precipitates were washed several times with water and dried in desiccator over Calcium sulfate. The product was further purified by flash chromatography using dry Acetone. Purified compound was recrystallized from Ethanol + DMSO, yellow colored crystals were obtained.

(0.784 g, 91.8%) as yellow solid, mp: 203–204 °C, [Found: C, 48.89; H, 5.71; N, 13.91; S, 15.83%. C₁₆H₂₂N₄O₄S₂ requires C, 48.22; H, 5.56; N, 14.06; S, 16.09%]; ν_{max} (KBr/cm⁻¹) 3366 (N–H), 3197 (C–H_{arom}), 3024 (C–H_{aliph}), 1678 (C=O), 1551 (C–N), 1536 (Ar–C), 995 (C=S), (S1); δ H (400 MHz, DMSO-d6), 3.22 (6H, s, 2 × CH₃), 3.78 (4H, t, J = 5.6 Hz, 2 × CH₂–O), 3.98 (4H, t, J = 5.6 Hz, 2 × CH₂–N), 4.97 (2H, s, 2 × OH), 8.00 (4H, s, Ar–H), 10.97 (2H, s, 2 × NH) (S2). δ C (150 MHz, DMSO-d6) 41.8 (CH₃), 57.8 (CH₂), 59.1 (CH₂), 128.0 (C_{arom}), 128.8 (C_{arom}), 164.1 (C=O), 181.0 (C=S), (S3); Expected M⁺ = 398.1; MS (EI): (m/z) = 421.1 [M+Na]⁺.

2.3. Theoretical method

The total energy calculation and the corresponding structure optimization for the most stable geometry was based on the semi-empirical molecular quantum calculations within the PM3 method [26] and molecular mechanics within MM + method as implemented in Hyperchem package version 7.52 [27]. No frozen core approximation used throughout the calculations. All calculations carried out in the gas phase at 25 °C.

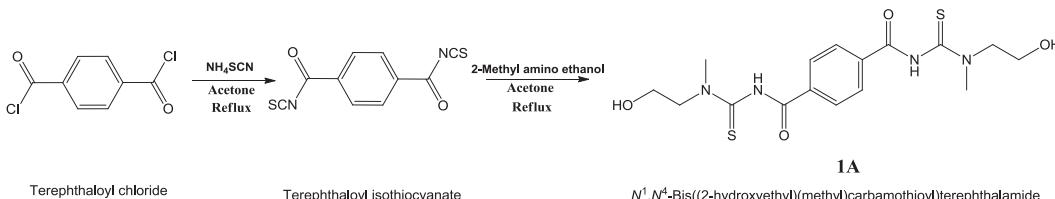
3. Results and discussions

3.1. Theoretical computational studies

The stable geometry of the compound was confirmed using the PM3 method that implemented in Hyperchem package (Fig. 1). Clearly, the intra H-bonding could be achieved between the hydrogen atom of N and the oxygen atom in both sides of the molecule which is in confirmation to ORTEP diagram in this study (Fig. 4). The total energy (−427280.0 kJ/mol) of the molecular modelling system was calculated after the geometry is fully relaxed. A non-flat conformation was the most stable geometry for the prepared compound due to the minimum angle and torsional strains.

(Fig. 2) shows the electrostatic potential energy maps of the molecular modeling system. It illustrates the charge distributions of three dimensional molecules. These maps assist in visualization of the variable charged regions of the molecule. The variation in distribution of electronic density in the molecule leads to have a dielectric constant (3.43 D).

There is a difference in the distribution of electronic density and energy levels (HOMO = −8.8897 eV and LUMO = −1.4047 eV). Such difference could lead to the interaction with other reactive sites. The frontier molecular orbitals related to the electronic transition between molecular orbitals HOMO → LUMO is shown in (Fig. 3).



Scheme 1. Synthesis of Bis-thiourea **1 A**.

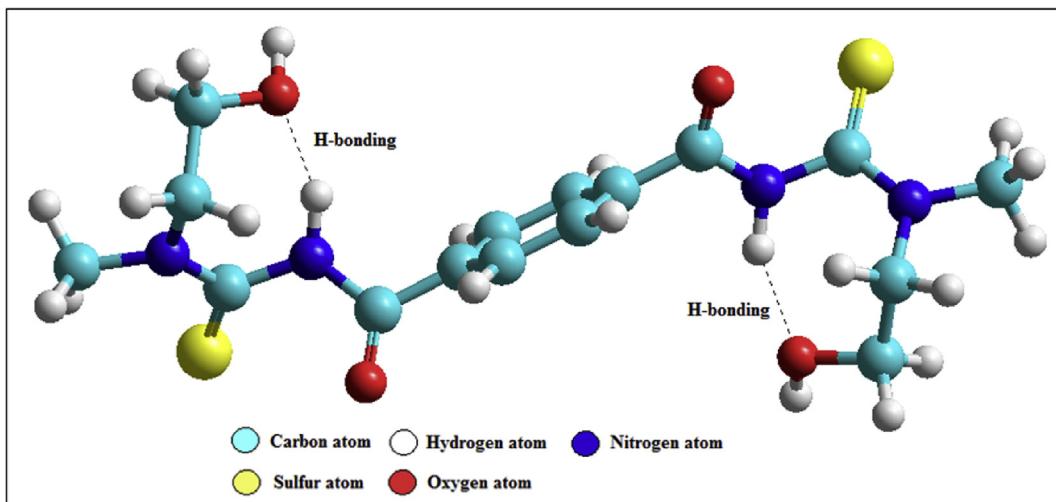


Fig. 1. Conformation structure for 1A using PM3 method. H-bonding length on both sides is 182 pm.

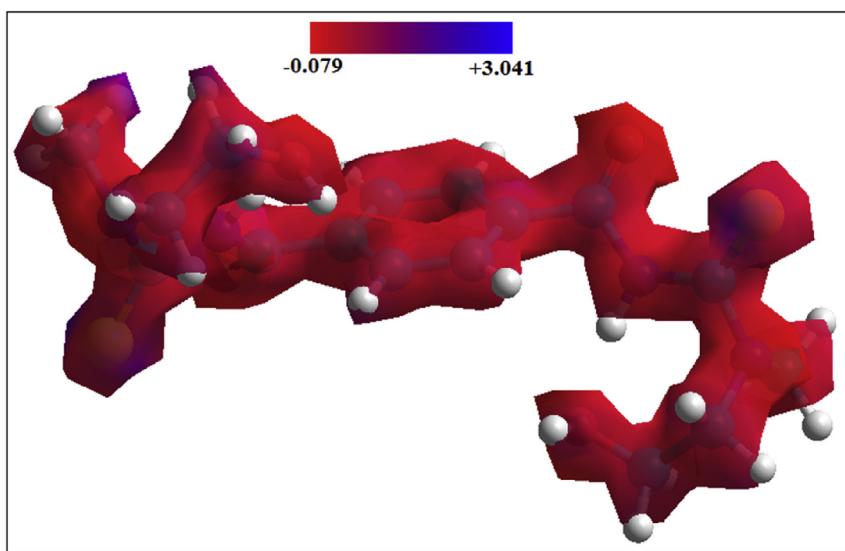


Fig. 2. Electrostatic potential maps for 1A using PM3 method.

3.2. X-ray crystallography

The CCDC No. of the deposited structure is 1542140. The isomer 1B crystallized in monoclinic system with space group P 21/n, $a = 6.9727(9)$, $b = 17.649(2)$, $c = 8.2629(11)$, $\alpha = 90^\circ$, $\beta = 112.329(4)$, $\gamma = 90^\circ$. $Z = 2$ and $V = 940.6(2)$. The given crystal state and refinement parameters are given in Table 1.

The molecule adopt cis-trans configuration with respect to the position of the 2-methyl amino ethanol group relative to the O(2) atom across the C(5)-N(2) bonds (Fig. 4). shows the molecular structure of the molecule with numbering scheme.

The benzene ring is essentially planar with maximum deviation of 0.002 Å. The thiourea moiety along with benzoyl ring O(2)-C(5)-N(2) makes a dihedral angle of 125.43(18)°, with methyl group C(3)-N(1)-C(4) 119.76(18)°, and ethanol moiety C(2)-N(1)-C(4) 124.40(16)°. The bond lengths and angles in the molecule is in normal ranges (Table 2).

In the molecule there is only one intra molecular H-bond, N(2) ... H(2c) ... O(1) could be seen (Table 3). In the crystal structure, the molecules are linked by O(1) ... H(1) ... S(1), and O(1) ... H(1) ...

O(2) intermolecular H-bonds forming a 3-D network (Fig. 5). Complete details of crystal data can be seen in Tables S1–S7.

3.3. IR vibrational spectra

The IR stretching frequencies of 1A was in accordance with the vibrational frequencies of the functional groups as found in the literature [28,29]. The disappearance of $\nu(\text{NCS})$ vibrations of isothiocyanate moiety at 2000–2500 cm⁻¹ and the emergence of new vibrational frequency at 3366 cm⁻¹ which is attributed to $\nu(\text{NH})$ is a strong evidence of thiourea formation. The $\nu(\text{O-H})$ stretching frequencies of the hydroxyl group overlapped by (N-H) stretching peak hence a relatively broad peak is observed. The $\nu(\text{C-H})$ stretching vibrations for sp^2 carbon of aromatic ring was observed at 3197 cm⁻¹ [30] whereas, the $\nu(\text{C-H})$ stretching vibrations for sp^3 mode of the alkyl chain was observed at 3024 cm⁻¹ [31]. The lineament frequency of $\nu(\text{C=O})$ was observed 1678 cm⁻¹ [32]. The $\nu(\text{C-N})$ and $\nu(\text{C=C}_{\text{aromatic}})$ vibrational frequencies were observed at 1551 and 1536 cm⁻¹. The $\nu(\text{C=S})$ vibrational frequency was observed at 995 cm⁻¹. The lowering in the vibrational frequency of

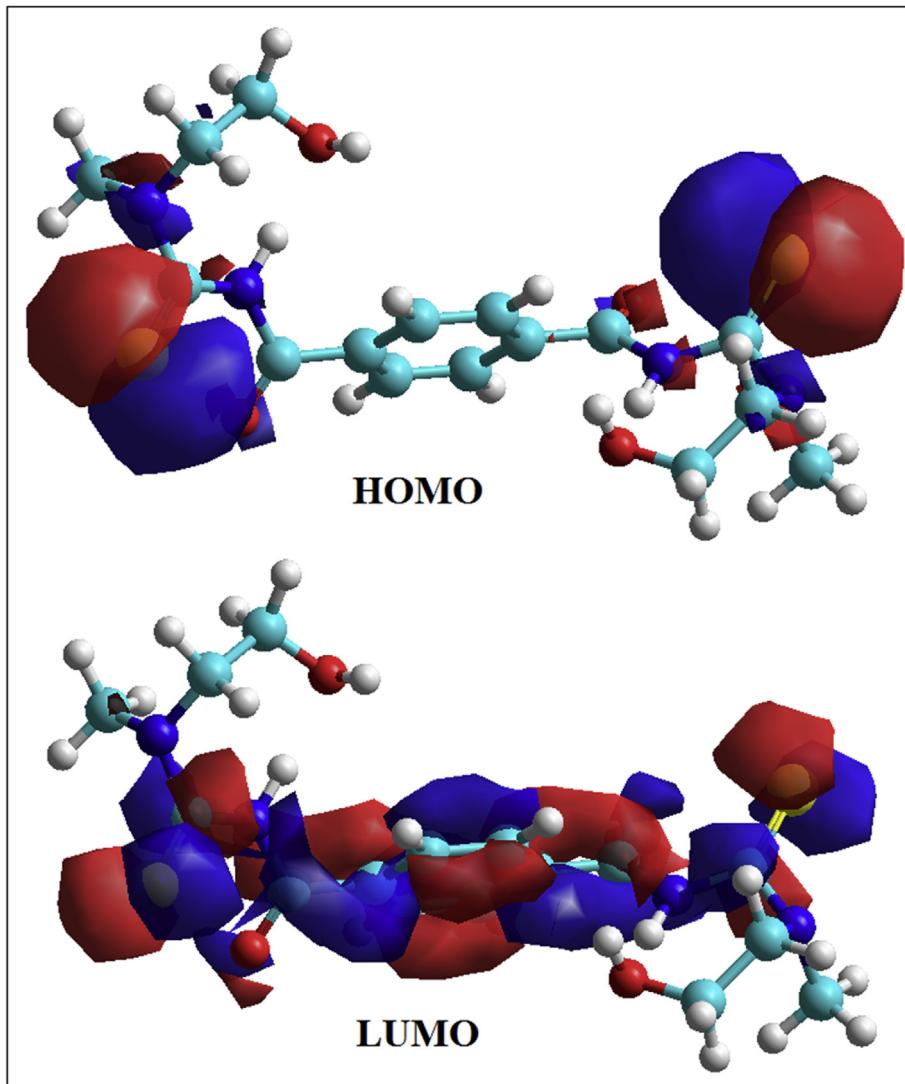


Fig. 3. The frontier molecular orbital density distributions for 1A using PM3 level.

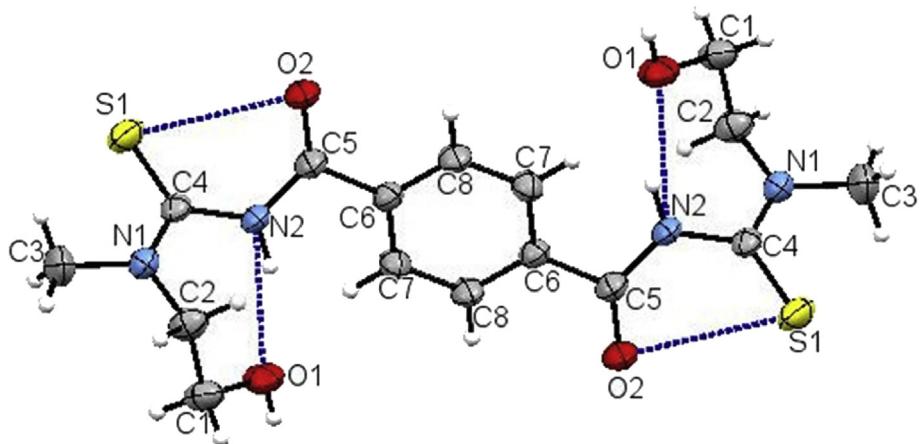


Fig. 4. ORTEP diagram of the N^1,N^4 -bis((2-hydroxyethyl)(methyl)carbamothioyl)terephthalamide (1 A) was drawn at 50% probability displacement ellipsoids. The dashed line indicates the intramolecular H-bond.

(C=S) bond was due to mesomeric electron releasing nitrogen bonded to thiocarbonyl group (N—C=S) as in the case of thioureas

and also due to the presence of intramolecular hydrogen bond between the hydrogen atom of thioamide group H—N—C=S and

Table 1
Crystal data and structure refinement for 1 A.

CCDC deposition number	1542140
Empirical formula	C16 H22 N4 O4 S2
Formula weight	398.49
Temperature	305(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2 ₁ /n
Unit cell dimensions	a = 6.9727(9) Å b = 17.649(2) Å c = 8.2629(11) Å
Volume	940.6(2) Å ³
Z	2
Density (calculated)	1.407 Mg/m ³
Absorption coefficient	0.313 mm ⁻¹
F(000)	420
Crystal size	0.500 × 0.200 × 0.160 mm ³
Theta range for data collection	2.904–28.485°.
Index ranges	-9 ≤ h ≤ 8, -23 ≤ k ≤ 23, -10 ≤ l ≤ 11
Reflections collected	18603
Independent reflections	2365 [R(int) = 0.0580]
Completeness to theta = 25.242°	99.9%
Refinement method	Full-matrix least-squares on F ²
Data/restraints/parameters	2365/1/124
Goodness-of-fit on F ²	1.054
Final R indices [I > 2sigma(I)]	R1 = 0.0478, wR2 = 0.1189
R indices (all data)	R1 = 0.0797, wR2 = 0.1345
Extinction coefficient	n/a
Largest diff. peak and hole	0.288 and -0.289 e.Å ⁻³

the oxygen atom of carbonyl group. This lowering of C=S stretching frequency is also due to acquiring partial polar character [33,34].

3.4. ¹H NMR and ¹³C NMR spectroscopy

Further 1A was characterized and confirmed by ¹H, and ¹³C NMR. Bis-thiourea 1A was found to have a plane of symmetry, so one half of the compound was exactly similar to the other half which can be clearly seen in the integration values (S1). The proton chemical shifts of the amide functionality appeared as singlet at δ 10.97 ppm. The downfield signal of amide protons are due to the formation of H-bonding between the amino proton and the oxygen/sulfur atoms of carbonyl/thiocarbonyl group and also due to anisotropic effect [35]. All aromatic protons were identical and appeared as a singlet at δ 8.00 ppm. The hydroxyl proton appeared as broad peak at δ 4.97 ppm. The methylene (CH₂) protons of ethanol functionality were found as triplet at δ 3.78 ppm for (CH₂—O) and 3.98 ppm for (CH₂—N). Whereas, the methyl (CH₃) protons were observed as singlet at δ 3.22 ppm. These alkyl protons were observed downfield due to deshielding effect of electron with drawing amino group and hydroxyl groups.

The ¹³C NMR spectrum showed all the chemical shifts as reported in the literature [36]. The carbon chemical shifts of C=S, and C=O were found at δ 181.0 and 164.1 ppm. The aromatic carbons were observed at δ 128.8 and 128.0 ppm. The chemical shifts of two (CH₂) groups were observed at δ 59.1 and 57.8 ppm, respectively. Whereas the signal for methyl carbon at δ 41.8 ppm.

3.5. Elemental analysis and ESI-Mass spectroscopy

The CHNS analysis was found in close accordance with the theoretical values.

The ESI-MS spectrum showed pseudo sodium molecular ion peak at *m/z* 421.1, which is in accordance with the expected molecular ion peak value.

Table 2
Bond lengths [Å] and angles [°] for 1 A.

Bond Lengths (Å)	Bond Angles (°)
S(1)—C(4)	1.6591(19)
O(1)—C(1)	1.412(3)
O(1)—H(1)	0.82
O(2)—C(5)	1.213(2)
N(1)—C(4)	1.337(3)
N(1)—C(3)	1.468(3)
N(1)—C(2)	1.468(3)
N(2)—C(5)	1.362(3)
N(2)—C(4)	1.402(2)
N(2)—H(2C)	0.852(10)
C(1)—C(2)	1.511(3)
C(1)—H(1A)	0.97
C(1)—H(1B)	0.97
C(2)—H(2A)	0.97
C(2)—H(2B)	0.97
C(3)—H(3A)	0.96
C(3)—H(3B)	0.96
C(3)—H(3C)	0.96
C(5)—C(6)	1.500(3)
C(6)—C(8)	1.389(3)
C(6)—C(7)	1.390(3)
C(7)—C(8)#1	1.379(3)
C(7)—H(7)	0.93
C(8)—C(7)#1	1.379(3)
C(8)—H(8)	0.93
C(1)—C(2)—C(1)	114.55(17)
C(2)—C(1)—H(1B)	110
C(2)—C(1)—H(1A)	110
O(1)—C(1)—H(1B)	110
H(1A)—C(1)—H(1B)	108.4
N(1)—C(2)—H(2A)	108.6
C(1)—C(2)—H(2A)	108.6
N(1)—C(2)—H(2B)	108.6
C(1)—C(2)—H(2B)	108.6
H(2A)—C(2)—H(2B)	107.6
N(1)—C(3)—H(3A)	109.5
N(1)—C(3)—H(3B)	109.5
H(3A)—C(3)—H(3B)	109.5
N(1)—C(3)—H(3C)	109.5
H(3A)—C(3)—H(3C)	109.5
H(3B)—C(3)—H(3C)	109.5
N(1)—C(4)—N(2)	113.35(16)
N(1)—C(4)—S(1)	124.07(14)
N(2)—C(4)—S(1)	122.47(15)
O(2)—C(5)—N(2)	125.43(18)
O(2)—C(5)—C(6)	120.99(18)
N(2)—C(5)—C(6)	113.49(16)
C(8)—C(6)—C(7)	119.08(17)
C(8)—C(6)—C(5)	117.97(16)
C(7)—C(6)—C(5)	122.94(17)
C(8)#1—C(7)—C(6)	120.10(18)
C(8)#1—C(7)—H(7)	119.9
C(6)—C(7)—H(7)	119.9
C(7)#1—C(8)—C(6)	120.82(17)
C(7)#1—C(8)—H(8)	119.6
C(6)—C(8)—H(8)	119.6

Symmetry transformations used to generate equivalent atoms: #1 -x, -y, -z.

3.6. Anti-bacterial studies

The antibacterial activity of bis-thiourea derivative 1A was examined *in vitro* at concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml against bacteria *Lysinibacillus* sp. Gb01, *Vibrio owensii* Gb04, *Vibrio owensii* SS1, *Vibrio alginolyticus* SS17 by using agar diffusion technique at 37 °C [37,38]. The results of bacterial growth inhibition at 5 different concentrations is shown in (Fig. 6).

The various effects of the newly synthesized compound 1A at different concentrations can be expressed by their minimum inhibitory concentration (MIC). The MIC value was determined by extrapolating the concentration at the zero growth rate of different bacteria [39] MIC values of 1A against different bacteria is shown in Table 4.

The presence of C=S, C=O, and N—H functional groups in thiourea derivatives are reported to give good antibacterial activity

Table 3
Hydrogen bonds for compound 1 A [(Å) and (°)].

D-H ... A	d(D-H)	d(H ... A)	d(D ... A)	<(DHA)
O(1)—H(1) ... S(1)	0.82	2.84	3.4532(5)	133
O(1)—H(1) ... O(2)	0.82	2.08	2.8183(4)	149
N(2)—H(2c) ... O(1)	0.86	1.93	2.7524(4)	159

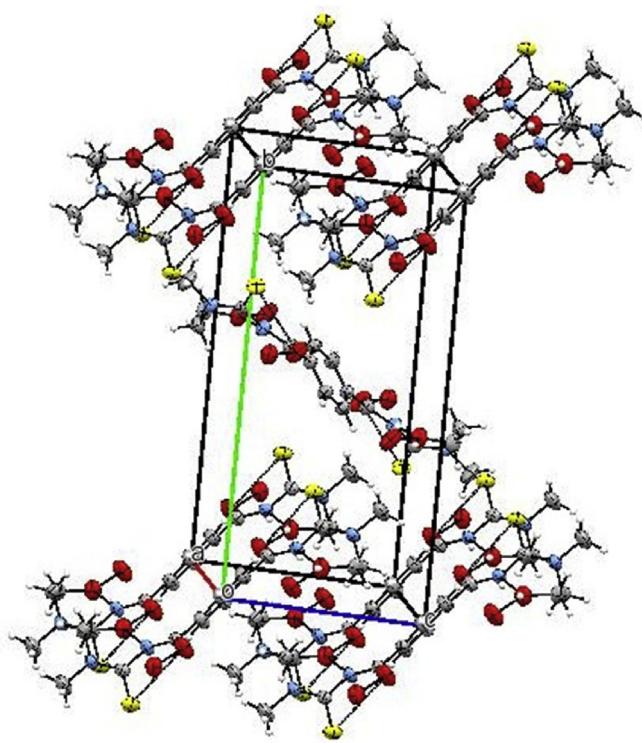


Fig. 5. Molecular packing viewed down the axis a. Dashed lines denote C–H···O, O–H···O and N–H···O hydrogen bonds.

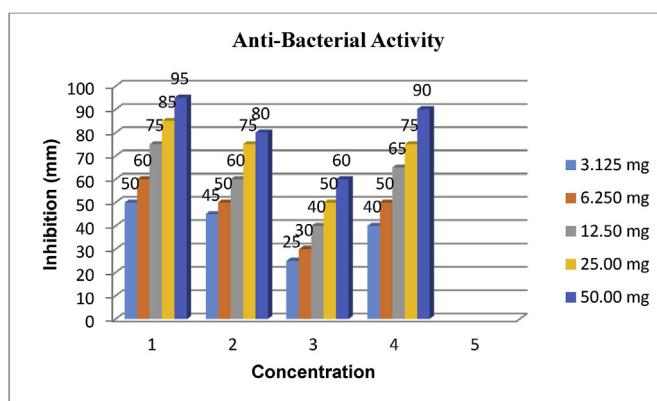


Fig. 6. Anti-bacterial activities of 1 A against different bacteria.

as they react with carboxyl and phosphate groups of the bacterial surface [40]. Derivative 1A, however, showed no excellent biological activity against various bacteria. It is evident from the structure of bis-thiourea derivative (1 A) that the thiourea moiety is not planar because of intermolecular attractions (Fig. 1) hence offering steric hindrance. The occurrence of steric hindrance creates a force that obstructs the contact between active sites in the compound

Table 4
Minimum inhibition concentration (MIC) of compound 1 A.

Bacteria	<i>Lysinibacillus</i> sp. Gb01	<i>Vibrio owensii</i> Gb04	<i>Vibrio owensii</i> SS1	<i>Vibrio alginolyticus</i> SS17
MIC (mg/ml)	0.74	0.70	0.85	0.50

with receptor site of the bacteria [16].

The pictures of the bacterial culture showing inhibition can be seen in S4–S7.

4. Conclusion

New bis-thiourea derivative (1A) with secondary amine has been successfully synthesized and fully characterized by using micro elemental analysis, IR, ¹H and ¹³C NMR spectroscopic techniques. The molecular structure of the compound was determined using X-ray crystallography technique. The total energy calculation and the corresponding structure optimization for the most stable geometry of the synthesized derivative was performed by PM3 method and molecular mechanics within MM + method as implemented in Hyperchem package. The antibacterial results of the synthesized derivative showed weak antibacterial activities.

Acknowledgements

The authors wish to thank the School of Chemical Sciences and Food Technology, and the Universiti Kebangsaan Malaysia (DIP-2015-015) for providing necessary facilities. We greatly appreciate the Ministry of Higher Education for providing the funding of the project under Grants GUP-2017-086 and FRGS/1/2015/ST01/UKM/02/2 (Project leader Dr. Siti Aishah Hasbullah). Mr. Imran Fakhar would also like to thank Mr. Kamran Fakhar for providing financial assistance, his parents for providing moral support and Mr. Hasanuddin for providing the technical assistance.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.molstruc.2018.01.032>.

References

- [1] M. Nencki, Zur Kenntniss des Sulfoharnstoffs, Ber. Dtsch. Chem. Ges. 6 (1873) 598–600.
- [2] J. Sun, S. Cai, H. Mei, Molecular docking and QSAR studies on substituted acyl(thio)urea and thiadiazolo [2,3- α] pyrimidine derivatives as potent inhibitors of influenza virus neuraminidase, Chem. Biol. Drug Des. 76 (2010) 245–254.
- [3] C. Sun, X. Zhang, H. Huang, P. Zhou, Synthesis and evaluation of a new series of substituted acyl(thio)urea and thiadiazolo [2,3- α] pyrimidine derivatives as potent inhibitors of influenza virus neuraminidase, Bioorg. Med. Chem. 14 (2006) 8574–8581.
- [4] Z. Zhong, R. Xing, S. Liu, L. Wang, S. Cai, P. Li, Synthesis of acyl thiourea derivatives of chitosans and their antimicrobial activities in vitro, Carbohydr. Res. 343 (2008) 566–570.
- [5] F.H. Wang, Z.L. Qin, Q. Front, Synthesis and fungicidal activity of 1,3,4-oxadiazole substituted acylthiourea, Front. Chem. China 1 (2006) 112.
- [6] S.Y. Ke, S.J. Xue, Synthesis and herbicidal activity of N(o)thioureas derivatives and related fused heterocyclic compounds, Arkivoc 10 (2006) 63–68.
- [7] S.J. Xue, S.Y. Ke, T.B. Wei, L.P. Duan, Y.L. Guo, Ultrasonic irradiated synthesis of N(5-aryl-2-furyl)thiourea derivatives containing substituted pyrimidine ring under phase transfer catalysis, J. Chin. Chem. Soc. 51 (2004) 1013–1018.
- [8] L. Xiao, C.J. Liu, Y.P. Li, Ultrasound promoted synthesis of bis(substituted pyrazol-4-ylcarbonyl)-substituted thioureas, Molecules 14 (2009) 1423–1428.
- [9] J.H. Hua, L.C. Wang, H. Liu, T.B. Wei, Biological activities studies and phase transfer catalysts promoting the one-pot synthesis of N-Aryl-N’-(4-ethoxybenzoyl)-thiourea derivatives, Phosphorus, Sulfur Silicon Relat. Elem. 181 (2006) 2691–2698.
- [10] A. Ranise, F. Bondavalli, O. Bruno, et al., 1-acyl-3-acyl- and 1,3-diacyl-3-furfuryl-1-phenylthioureas with platelet antiaggregating and other activities, Farmaco 46 (1991) 1203–1216.
- [11] A. Ranise, A. Spallarossa, O. Bruno, et al., Synthesis of N-substituted-N-acylthioureas of 4-substituted piperazines endowed with local anaesthetic, antihyperlipidemic, antiproliferative activities and antiarrhythmic, analgesic, antiaggregating actions, Il Farmaco 58 (2003) 765–780.
- [12] S. Claridge, F. Raeppe, M.-C. Granger, et al., Discovery of a novel and potent series of thieno[3,2-b]pyridine-based inhibitors of c-Met and VEGFR2 tyrosine kinases, Bioorg. Med. Chem. Lett 18 (2008) 2793–2798.
- [13] S.N. Manjula, N. Malleshappa Noolvi, K. Vipan Parihar, et al., Synthesis and

- antitumor activity of optically active thiourea and their 2-aminobenzothiazole derivatives: a novel class of anticancer agents, *Eur. J. Med. Chem.* 44 (2009) 2923–2929.
- [14] R. Vig, C. Mao, T.K. Venkatachalam, L. Tuel-Ahlgren, E.A. Sudbeck, F.M. Uckun, Rational design and synthesis of phenethyl-5-bromopyridyl thiourea derivatives as potent non-nucleoside inhibitors of HIV reverse transcriptase, *Bioorg. Med. Chem.* 6 (1998) 1789–1797.
- [15] H. Peng, Y. Liang, L. Chen, L. Fu, H. Wang, H. He, Efficient synthesis and biological evaluation of 1,3-benzenedicarbonyl dithioureas, *Bioorg. Med. Chem. Lett.* 21 (2011) 1102–1104.
- [16] E.R. Fernandez, J.L. Manzano, J.J. Benito, R. Hermosa, E. Monte, J.J. Criado, Synthesis and characterization of bis-thiourea having amino acid derivatives, *J. Inorg. Biochem.* 99 (2005) 1559–1572.
- [17] B. Phetsuksiri, M. Jackson, H. Scherman, M. McNeil, G.S. Besra, A.R. Baulard, R.A. Slayden, A.E. DeBarber, C.E. Barry, M.S. Baird, D.C. Crick, P.J. Brennan, Unique mechanism of action of the thiourea drug isoxyl on *Mycobacterium tuberculosis*, *J. Biol. Chem.* 278 (2003) 53123–53130.
- [18] Y.-M. Zhang, T.-B. Wei, L.-M. Gao, Synthesis and biological activity of N-aryloyl-N'-substituted thiourea derivatives, *Synth. Commun.* 31 (2001) 3099–3105.
- [19] K.E. Koenig, G.M. Lein, P. Stucker, T. Kaneda, D.J. Cram, Host-guest complexation. 16. Synthesis and cation binding characteristics of macrocyclic polyethers containing convergent methoxyaryl groups, *J. Am. Chem. Soc.* 101 (1979) 3553–3566.
- [20] J.E. Worsham, H. Levy, S.W. Peterson, The positions of hydrogen atoms in urea by neutron diffraction, *Acta Cryst.* 10 (1957) 319–323.
- [21] P. Vaughan, J. Donohue, The structure of urea. Interatomic distances and resonance in urea and related compounds, *Acta Cryst.* 5 (1952) 530–535.
- [22] J.E. Lancaster, B.P. Stoicheff, High resolution Raman spectroscopy of gases: vii. Rotational spectra of s-triazine and s-triazine-d3, *Can. J. Phys.* 34 (1956) 1016–1021.
- [23] L. Pauling, *The Nature of the Chemical Bond*, third ed., Cornell University Press, Ithaca, New York, U. S. A., 1960.
- [24] E.A. Vizzini, I.F. Taylor, E.L. Amma, Electron-deficient bonding with sulfur atoms. III. Crystal and molecular structure of bis(thiourea)silver(I) chloride, *J. Inorg. Chem.* 7 (1968) 1351–1357.
- [25] G.M. Sheldrick, *SHELXTL Ver. 6.14. Program for Crystal Structure Determination*, University of Gottingen, Gottingen, Germany, 1997.
- [26] J.J.P. Stewart, Optimization of parameters for semiempirical methods I, Method, *J. Comput. Chem.* 10 (1989) 209–220.
- [27] HyperChemTM Professional 7.52, Hypercube, Inc., 1115 NW 4th Street, Gainesville, Florida 32601, USA, 2002.
- [28] 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.* 189 (2014) 832–838.
- [29] A.T. Kabbani, H. Ramadan, H.H. Hammud, A.M. Ghannoum, Y. Mouneimne, Synthesis of some metal complexes of N-[((benzoylamino)-thioxomethyl]-aminoacid (HL), *J. Univ. Chem. Technol. Metall.* 40 (2005) 339–344.
- [30] Y. Wen, Z. Weiqun, Z. Zhengjiang, *J. Mol. Struct.* 828 (2007) 46–53.
- [31] A. Hakan, F. Ulrich, K. Nevzat, B. Gün, The molecular structure and vibrational spectra of 2-chloro-N-(diethylcarbamothioyl)benzamide by Hartree–Fock and density functional methods, *Spectrochim. Acta Mol. Spectros.* 68 (2007) 1347–1355.
- [32] R. Sitti, B. Bunbun, Kinetics of the oxidation of vitamin C, in: Prosiding Seminar Kimia Bersama UKM-ITB. VIII 9–11 June, 2009, pp. 535–546.
- [33] M.L. Shankaranrayana, C.C. Patel, The electronic spectra of some derivatives of xanthic, dithiocarbamic and trithiocarbonic acids, *Acta Chem. Scand.* 19 (1965) 1113–1119.
- [34] H.M. Abosadiya, S.A. Hasbullah, B.M. Yamin, Synthesis, characterisation and X-ray structures of N-(4-bromobutanoyl-N'-methylphenyl)thioureas, *Chin. J. Struct. Chem.* 34 (2015) 379–385.
- [35] H.M. Abosadiya, E.H. Anouar, S.A. Hasbullah, B.M. Yamin, Synthesis, X-ray, NMR, FT-IR, UV/vis, DFT and TD-DFT studies of N-(4-chlorobutanoyl)-N'-(2-, 3- and 4-methylphenyl) thiourea derivatives, *Spectrochim. Acta Part A: Mol. Spectrosc.* 144 (2015) 115–124.
- [36] R.A. Freeman, *Handbook of Nuclear Magnetic Resonance*, Secd. Ed., Longman, London, UK, 1997.
- [37] R.C. Shank, D.G. Marmion, *Microbiology*, twelveth ed., Churchill Living Stone Edinbrg, London, 1979.
- [38] G.R. Larry, W.S. Chatles, R. Barth, *Antimicrob. Agents Chemother.* 19 (1981) 1050, <https://doi.org/10.1128/AAC.19.6.1050>.
- [39] M.A. Alvarez, V.E.P. Zarelli, N.B. Pappano, N.B. Debattista, Antimicrobial activity and synergism of some substituted flavonoids, *Folia Microbiol.* 53 (2008) 23–28.
- [40] H. Arslan, N. Duran, G. Borekci, C.K. Ozer, C. Akbay, Antimicrobial activity of some thiourea derivatives and their nickel and copper complexes, *Molecules* 10 (2009) 519–527.