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Experimental and theoretical investigations on the tautomerism of 1-phenyl-2-thiobarbituric acid and its methylation reaction

Gang Wang [a], Jian Wang [a], Han Nie [a], Shen-Peng Tan [a], Wei-Qun Shi [b], Dong-Mei Zhao [a], Mao-Sheng Cheng [a,*]

[a] Key Laboratory of Structure-Based Drugs Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China
[b] Key Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, PR China

HIGHLIGHTS

- Synthesis and structural identification of thiobarbituric derivatives are presented.
- Tautomerism form was investigated by experimental methods and quantum calculations.
- The methylation site was identified experimentally and theoretically.

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ABSTRACT

In the present study, 1-phenyl-2-thiobarbituric acid (1) was synthesized and the tautomerism of this compound was investigated by FT-IR spectroscopy, X-ray analysis and ^{1}H NMR study as well as quantum chemical calculations. It is found that compound 1 exists in triketo form in the solid state and in $CDCl_3$ solution while tautomerization was observed in DMSO-d_6, DMF-d_7 and CD_3OD solution. The geometry optimization of eight possible tautomers of 1-phenyl-2-thiobarbituric acid was performed in gas phase and in different solvents using COSMO method. The calculated results are in good accordance with experimental data. Additionally, the methylation reaction of 1-phenyl-2-thiobarbituric acid was investigated for the first time by combination of experimental and theoretical methods. The methylation product might be varied with three possible isomers because of the structural features of 1-phenyl-2-thiobarbituric acid. We identified the obtained methylation product by X-ray diffraction and FT-IR spectroscopy, and transition state search and energy calculation were conducted to elucidate experimental data, the results revealed that the theoretical calculations are consistent with experimental data.

1. Introduction

2-Thiobarbituric acid and its derivatives are a well known class of compounds which have many applications in pharmaceutical and chemical fields. They exhibit various biological activities such as anticonvulsant [1,2], anesthetic [3], antitumor [4] and antibacterial [5]. 2-Thiobarbituric acid is also applied in detecting aldehydes and lipid oxides in food based on the reaction of 2-thiobarituric acid with bifunctional aldehydes [6,7].

Additionally, 2-thiobarbituric acid has thioamide and β-diketone moieties which could conduct thiol-thione and keto–enol tautomerism simultaneously. Investigation on tautomerism of pharmacologically active compounds is important to rationalize their biological activity and to elucidate the mechanism of action as the relative stability of tautomers of a compound may be crucial for the ligand-receptor interaction [8–11]. In tautomerism research, density functional theory (DFT) calculations are widely used method in the determination of lowest energy structures of tautomers, especially when the calculated results are confirmed by experimental data [12–16]. Some theoretical and experimental studies on 2-thiobarbituric acid and its derivatives have been carried out due to their structure characteristic for tauomerism research. Millefiori and Millefiori [17] estimated the relative energies of thiobarbituric acid tautomers by means of AM1 method. Mendez et al. [18] investigated the molecular structure of 2-thiobarbituric acid in solid, in polar solutions and on gold nanoparticles finding that tautomerization is easy to occur in methanol solution. Chierotti et al. [19] reported the tautomeric polymorphs of 2-thiobarbituric acid demonstrating that 2-thiobarbituric acid family of crystal forms represents the richest collection of examples of tautomeric polymorphism so far.

* Corresponding author. Tel.: +86 24 23986419; fax: +86 24 23995043.
E-mail address: mscheng@syphu.edu.cn (M.-S. Cheng).

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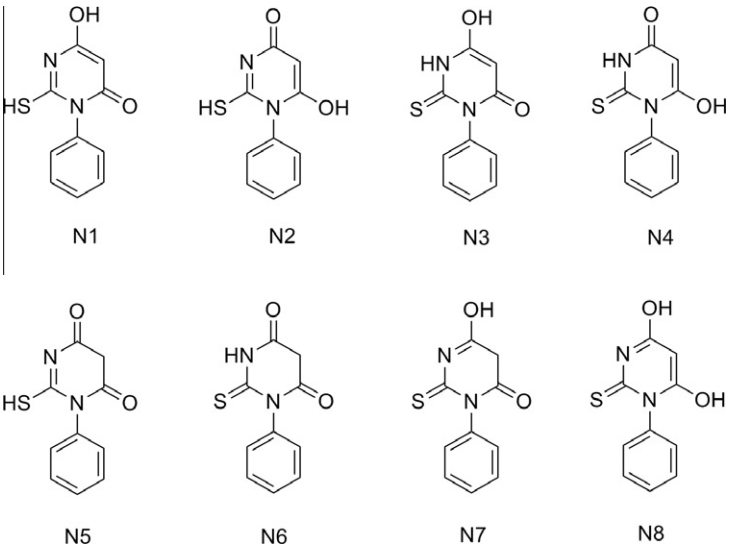

Fig. 1. The eight possible tautomers of 1-phenyl-2-thiobarbituric acid.

1-Phenyl-2-thiobarbituric acid, a derivative of 2-thiobarbituric acid, is also an important pharmacophore which presented in some compounds possessing different biological activities such as antimicrobial and antitumor [20–22]. On the other hand, this compound has an active methylene at 5-position which could react with aldehydes by Knoevenagel condensation and has been applied in analytical chemistry [23] and chemical modification of natural products [24].

Recently, we become interested in the biological activity of thiobarbituric acid derivatives. In the process of synthesizing novel thiobarbituric acid derivatives, we synthesized 1-phenyl-2-thiobarbituric acid and its methylated derivative. Similar to 2-thiobarbituric acid, tautomerism could also take place in 1-phenyl-2-thiobarbituric acid with 8 possible tautomeric forms (Fig. 1). Whereas the N-phenyl substitution reduces a mobile H atom compared with 2-thiobarbituric acid, so that the methylation reaction with excess amount of methylating agent generate dimethylated product at most in contrary to 2-thiobarbituric acid which results in trimethylated product [25]. But because of the structural features of 1-phenyl-2-thiobarbituric acid, the methylation site might be varied with three possible products (Fig. 2). In the current study, 1-phenyl-2-thiobarbituric acid was synthesized and its tautomerism equilibrium was examined experimentally in solid state (IR, X-ray) and solution phase (NMR), supported by quantum chemical calculations (DFT method). In addition, the methylation reaction of 1-phenyl-2-thiobarbituric acid was firstly investigated and the product was characterized by experimental data (IR, X-ray) along with theoretical calculations (DFT).

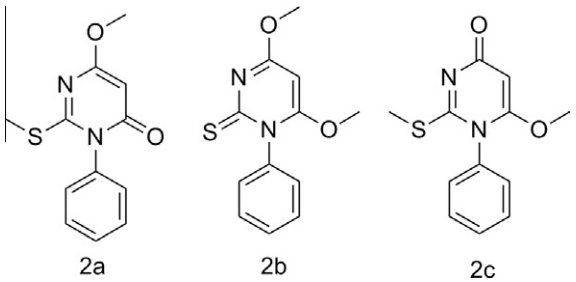

Fig. 2. Three possible methylation products.

2. Experimental

All synthetic materials and reagents were commercially available and were used without further purification. Fourier's transformation infrared (FT-IR) spectra were recorded on BRUKER IFS-55 FTIR spectrometer as KBr pellets with a resolution of 2 cm^{-1} in the 4000–400 cm^{-1} range. ^1H NMR spectra were recorded on Bruker ARX-300 spectrometer at 300 MHz using tetramethylsilane (TMS) as internal reference.

2.1. Synthesis

The synthetic route of 1-phenyl-2-thiobarbituric acid 1 and its methylated derivate 2 is shown in Fig. 3. For compound 1, we adopted a similar reaction condition as the synthesis of 2-thiobarbituric acid [26]. Phenylthiourea (5.0 g, 32.8 mmol) and diethyl malonate (5.2 g, 32.8 mmol) were refluxed in a freshly prepared sodium ethylate solution (Na 0.75 g, 32.8 mmol, in 100 mL of ethanol) for 5 h. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure. The residue was dissolved in 30 mL of water, then pH was adjusted to 7 by conc. HCl, the resulted precipitate was filtered and recrystallized from ethanol to obtain slightly yellow crystals (5.2 g, 72% yield).

6-Methoxy-2-(methylthio)-3-phenylpyrimidin-4(3H)-one 2 was prepared by methylation of 1 following a classical procedure [27] using dimethyl sulfate as methylating reagent. Potassium carbonate (4.7 g, 34 mmol) was added to a solution of 1 (3.0 g, 13.6 mmol) in 50 mL dimethylformamide, dimethyl sulfate (3.8 g, 29.9 mmol) was added dropwise and the solution was stirred for 2 h at room temperature. Then the reaction mixture was poured into cold water and the solid resulted was filtered, and then recrystallized from ethyl acetate to give white solid (2.5 g, 74% yield).

2.2. X-ray investigation

Single crystals of compound 1 and 2 suitable for X-ray diffraction analysis were grown by slow evaporation of ethanol solution (for 1) and a mixed solvent of n-hexane: ethyl acetate (3:1) (for 2). The X-ray diffraction data were collected on a Bruker APEX-II CCD diffractometer at 273 K using graphite-monochromated Mo Ka radiation (λ = 0.71073 Å). The crystal structure was solved by

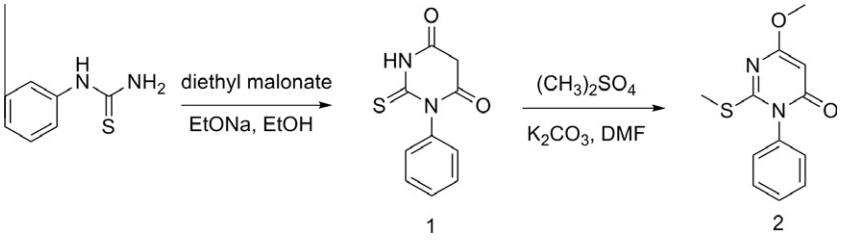

Fig. 3. Synthesis of compound 1 and 2.

direct method and refined by the full-matrix leastsquares procedure on F2 using SHELXL-97 program [28]. All non-hydrogen atoms were refined anisotropically, and hydrogen atoms were located by geometrical calculation. A summary of basic crystallographic data and structure refinement details are given in Table 1.

CCDC 901812 and CCDC 901813 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +441223 336033).

2.3. Computational methods

All computational calculations were performed by using Amsterdam Density Functional (ADF) program [29]. The gas phase geometry optimization for the eight possible tautomers N1—N8 and three possible methylation products were calculated by DFT method using Becke's three-parameters hybrid exchange–correlation functional (B3LYP) [30] employing TZP basis set. The solution phase geometry optimization of N1—N8 were performed by COSMO method for chloroform, ethanol and DMSO all of which have different dielectric constants. Similarly the geometry optimization of three possible methylation products in the reaction solvent N,N-dimethylformamide (DMF) was implemented at the same level of theory by COSMO method. The total energy and dipole moment were obtained from the optimization output. The transition state search for methylation reaction of 1-phenyl-2-thiobarbituric acid was carried out at the same DFT level as used in the geometry optimization.

3. Results and discussion

3.1. Description of crystal structure and optimized geometry

Single crystal X-ray analysis followed by quantum chemical calculations for compound 1 and 2 were carried out in order to identify the molecular structure and the tautomeric form in solid state. The selected geometrical parameters for 1 and 2 are given in Table 2. The displacement ellipsoid plot with numbering of the atoms is shown in Fig. 4 and the packing of molecules in the unit cell is shown in Fig. 5.

With regard to structural analysis of 1-phenyl-2-thiobarbituric acid 1, it crystallizes in the monoclinic $P2_1/n$ space group with one crystallographically independent molecular in the asymmetric unit. The C1—S1 bond length of 1.6342 (16) Å is typical for the C—S double bond. The bond lengths of C2—O2 and C4—O1 are 1.209 (2) Å and 1.2093 (19) Å which is typical for a carbonyl group [31]. These three features indicate that in the crystalline state compound 1 exists in the triketone form. The dihedral angle between the pyrimidone ring and phenyl ring is 87.165(49)° revealing that the two rings adopt a approximately perpendicular conformation. The bond lengths and angles in the pyrimidone ring are similar to 2-thiobarbituric acid [18]. In the crystal, intermolecular hydrogen bonds of N1—H1···O1 were found which stabilizing the

Table 1
Crystal data and structure refinement for compound 1 and 2.

	1	2
Empirical formula	$C_{10}H_8N_2O_2S$	$C_{12}H_{12}N_2O_2S$
Formula weight	220.25	248.31
Wavelength (Å)	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/n$
a (Å)	11.0748 (9)	7.7784 (15)
b (Å)	7.3546 (5)	16.972 (3)
c (Å)	13.3517 (10)	9.6375 (19)
β (°)	113.603 (1)	108.755 (2)
Volume (Å^3)	996.53 (13)	1204.7 (4)
Z	4	4
$F(000)$	456	520
Calculated density (Mg/m^3)	1.468	1.369
μ (mm^{-1})	0.30	0.26
T_{min}, T_{max}	0.941, 0.955	0.949, 0.962
θ	3.1–26.1	2.4–26.1
Index ranges h, k, l	−13/11, −5/9, −16/16	−6/9, −21/19, −11/11
No. of measured reflections	5065	6558
No. of independent reflections	1972 (R_{int} = 0.021)	2373 (R_{int} = 0.026)
Crystal size (mm)	0.2 × 0.18 × 0.15	0.2 × 0.18 × 0.15
Refinement		
Goodness-of-fit on F^2	1.02	1.12
R [$F^2 > 2\sigma(F^2)$]	0.034	0.043
$wR(F^2)$	0.093	0.113
Data/restraints/parameters	5065/0/136	6558/0/156

Table 2
Selected experimental and computed geometrical parameters for 1 and 2.

1			2		
Parameter	Experimental	Calculated	Parameter	Experimental	Calculated
Bond length (Å)					
S1—C1	1.6342 (16)	1.648	S1—C7	1.7563 (17)	1.774
C1—N1	1.375 (2)	1.382	S1—C12	1.8006 (17)	1.819
C1—N2	1.3878 (19)	1.389	O1—C10	1.3463 (19)	1.350
N1—C2	1.370 (2)	1.380	O1—C11	1.432 (2)	1.428
N2—C4	1.386 (2)	1.410	O2—C8	1.224 (2)	1.219
N2—C5	1.451 (2)	1.451	N1—C7	1.300 (2)	1.297
O2—C2	1.209 (2)	1.213	N1—C10	1.366 (2)	1.358
O1—C4	1.2093 (19)	1.208	N2—C7	1.374 (2)	1.368
C5—C10	1.371 (2)	1.386	N2—C8	1.420 (2)	1.442
C5—C6	1.381 (2)	1.386	N2—C1	1.4485 (19)	1.440
C4—C3	1.491 (2)	1.513	C1—C6	1.384 (2)	1.389
C2—C3	1.484 (2)	1.505	C1—C2	1.386 (2)	1.389
			C2—C3	1.388 (2)	1.389
			C8—C9	1.425 (2)	1.433
			C9—C10	1.359 (2)	1.368
Bond angle (°)					
N1—C1—N2	116.33 (14)	115.92	C7—S1—C12	100.14 (8)	99.75
N1—C1—S1	120.69 (12)	119.89	C10—O1—C11	117.19 (14)	117.74
N2—C1—S1	122.98 (12)	124.19	C7—N1—C10	116.57 (14)	117.79
C2—N1—C1	127.34 (13)	129.03	C7—N2—C8	121.06 (13)	121.02
C4—N2—C1	124.35 (13)	124.58	C7—N2—C1	120.88 (13)	121.32
C4—N2—C5	116.51 (12)	116.50	C8—N2—C1	118.01 (13)	117.66
C1—N2—C5	118.71 (13)	118.91	C6—C1—C2	121.48 (15)	120.84
C10—C5—C6	122.02 (15)	121.21	C6—C1—N2	118.77 (14)	119.56
C10—C5—N2	119.06 (15)	119.47	C2—C1—N2	119.71 (14)	119.59
C6—C5—N2	118.87 (15)	119.31	C1—C2—C3	118.97 (16)	119.46
O1—C4—N2	120.54 (15)	121.13	C3—C4—C5	120.41 (16)	120.09
O1—C4—C3	122.13 (15)	121.63	N1—C7—N2	124.08 (14)	124.01
N2—C4—C3	117.30 (14)	117.22	N1—C7—S1	120.24 (12)	120.13
O2—C2—N1	121.49 (15)	121.33	N2—C7—S1	115.68 (11)	115.86
O2—C2—C3	122.46 (16)	123.77	O2—C8—N2	119.09 (15)	119.51
N1—C2—C3	116.05 (14)	114.89	O2—C8—C9	126.75 (15)	127.06
C5—C10—C9	118.69 (17)	119.27	N2—C8—C9	114.15 (14)	113.43
C2—C3—C4	117.86 (15)	117.49	C10—C9—C8	119.72 (15)	120.08
			O1—C10—C9	125.53 (15)	124.86
			O1—C10—N1	110.08 (14)	111.46
			C9—C10—N1	124.39 (15)	123.67
Torsion angle (°)					
C4—N2—C5—C10	−89.94 (19)	−89.46	C7—N2—C1—C2	−101.2(2)	91.93
C1—N2—C5—C10	97.35 (18)	89.20	C7—N2—C1—C6	80.8(2)	−88.66
C4—N2—C5—C6	87.65 (19)	89.34	C8—N2—C1—C2	81.5(2)	−88.14
C1—N2—C5—C6	−85.07 (19)	−89.46	C8—N2—C1—C6	−96.6(2)	91.27

molecular structure and the crystal packing. The hydrogen bonding geometries are as follows: d (N1—H1) = 0.859 Å, d (H1···O1) = 2.296 Å, d (N1···O1) = 3.063 Å.

The methylation product 2 also crystallizes in monoclinic $P2_1/n$ space group with one independent molecular in the asymmetric unit. The crystal structure demonstrated that the methylation occurred on sulfur atom and the oxygen atom which is far from phenyl ring (isomer 2a). The C7—S1 bond length of 1.7563 (17) Å is in accordance with S-Csp^2 single bond [32], whereas the C12—S1 bond length of 1.8006 (17) Å is slightly longer than that of C7—S1. The O1—C10 and O1—C11 bond lengths are 1.3463 (19) Å and 1.432 (2) Å which is similar to a methoxyl group attached to phenyl ring [33]. The bond lengths of C7—N1 and C9—C10 are 1.300 (2) Å and 1.359 (2) Å respectively, showing the double bond character of these two bonds. The pyrimidine ring and phenyl ring adopt similar conformation to that in compound 1 with the dihedral angle be 81.162(56)°. The torsion angles of C11—O1—C10—C9 and C12—S1—C7—N1 are 9.807(249)° and −0.091(155)° demonstrating the orientation of the two methyl groups.

ADF software was used to perform the geometric optimization of compound 1 and 2 on the level of B3LYP with TZP basis set. Comparisons of the calculated geometric parameters with the experimental values are shown in Table 2. The results indicate that the calculated bond lengths and angles are in good accordance with those observed in X-ray diffraction. The calculated energy minimized molecules for 1 and 2 were aligned with the X-ray structures with root mean square deviation (RMSD) of 0.1684 Å for 1 and 0.1856 Å for 2 (Fig. 6).

3.2. IR spectra

IR spectroscopy provides recognizable adsorption bands for functional groups so that it is a useful tool for molecular structure identification [34]. The solid state IR spectra of the two compounds is given in Supplemental material (Fig. S1). In the spectrum of compound 1, a peak of medium intensity at 3258 cm^{-1} could be assigned to the N—H stretching frequency. And there are two strong absorptions at 1730 cm^{-1} and 1703 cm^{-1} corresponding to carbonyl stretching mode. These three absorption bands mainly characterize the compound 1 to be the triketone form which is validated by X-ray analysis. The absorptions at 1596 cm^{-1} and 1491 cm^{-1} are due to C—C stretching vibration of aromatic ring [35]. The stretching and bending vibrations of the CH_2 group at pyrimidone ring were observed at 2920 cm^{-1} and 1472 cm^{-1}. The absorption bands appear in 1100–1400 cm^{-1} should be

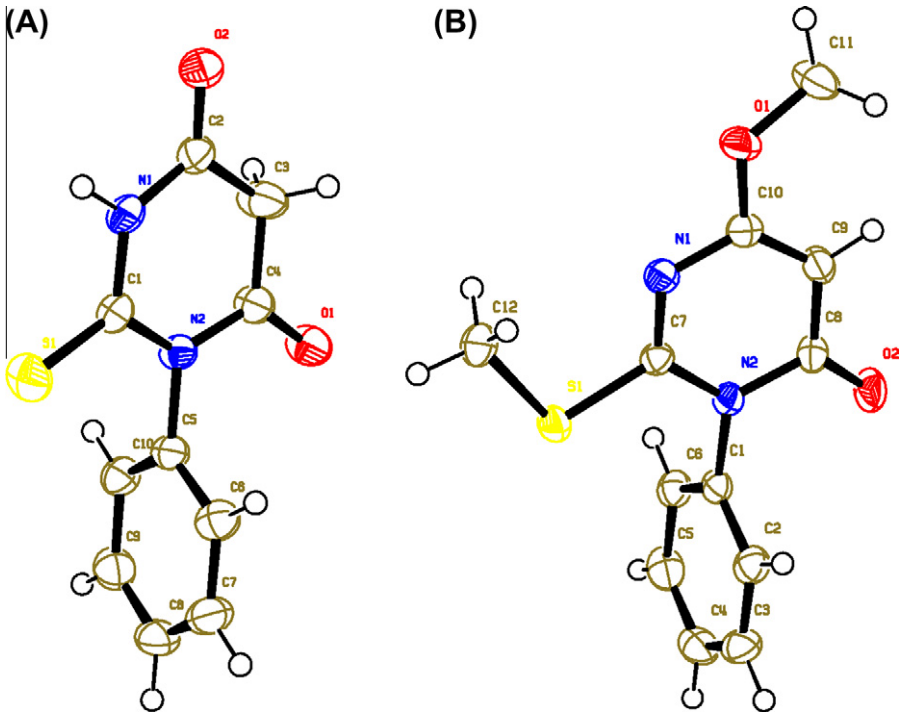

Fig. 4. ORTEP plot of compound 1 and 2 (ellipsoids are shown at the 50% probability level).

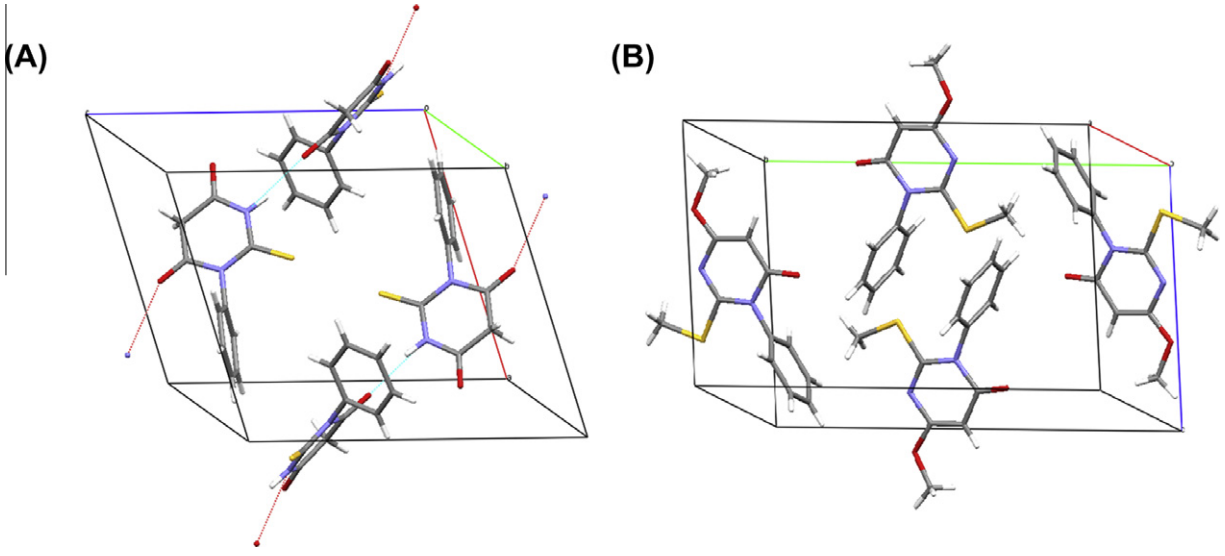

Fig. 5. The molecular packing of compound 1 and 2. Hydrogen bonds are shown as dashed lines.

attributed to aromatic C—H bending vibrations, N—H bending vibrations and C—N stretching vibrations.

In the spectrum of compound 2, the N—H band and one of the carbonyl absorptions in the spectra of compound 1 disappear result from methylation. The remaining C=O stretching vibration shifts to lower frequency at 1674 cm^{-1} compared with compound 1, this might because the formation of C10=C9—C8=O2 α,β-unsaturated ketone moiety causes conjugation effect which decrease the force constant of C8=O2 carbonyl group. The absorptions at 1582 cm^{-1} and 1523 cm^{-1} could be assigned to aromatic ring stretching mode, the absorption intensity of this two peaks is much stronger than those in compound 1, this might due to the formation of C7=N1 and C9=C10 double bonds by methylation increases the conjugation effect between the pyrimidine ring and phenyl ring. The C—H stretching and bending vibrations of methyl group appear in 2939 cm^{-1}, 1441 cm^{-1} and 1388 cm^{-1}, respectively. The strong absorption peak at 1241 cm^{-1} accounts for the C—O stretching mode of methoxy group similar to a literature data [36].

3.3. ^1H NMR study of 1-phenyl-2-thiobarbituric acid

The ^1H NMR spectra of 1-phenyl-2-thiobarbituric acid were taken in deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD), DMSO-d_6 and DMF-d_7 respectively in order to determine the tautomerism tendency in different solvents. The spectra are shown in Fig. S2. In $CDCl_3$ (Fig. S2a), there is a singlet at

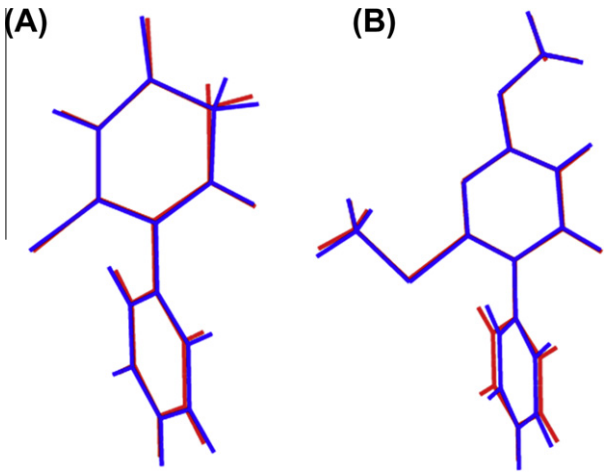

Fig. 6. Superimposition of X-ray structure (red) and gas phase optimized (blue) structure of compound 1 and 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.88 ppm integrated to 2H which should be the 5-CH_2 methylene proton. And the signal at 9.42 ppm should be assigned to the N—H proton of thioamide moiety. These two signals indicate that compound 1 is presented in triketo form (2-C=S, 4,6-C=O) in $CDCl_3$ solution which is in accordance with the X-ray crystal structure. From the spectrum in DMSO-d_6 (Fig. S2b), a polar but aprotic solvent, it is observed that the signal at 3.86 ppm become a broad singlet, and a new broad singlet appeared at 5.14 ppm compared with the spectrum in CDCl3. The signal at 3.86 ppm should be assigned to 5-CH_2 methylene proton. The new appearing singlet at 5.14 ppm should belong to the vinylic hydrogen at C-5 of the enol form of 1-phenyl-2-thiobarbituric acid [37], and the very broad singlet between 11.11 ppm and 11.62 ppm probably belong to O—H proton signal of enol form. Additionally the signals at 7.15–7.46 ppm and 12.68 ppm should be assigned to aromatic hydrogen and N—H proton respectively. The coexistence of 5-CH_2 methylene and 5-CH vinylic proton signals indicates that the tautomerization of compound 1 occurred in DMSO-d_6. From the integral values of 5-CH_2 and 5-CH vinylic proton it is concluded that the ratio of keto:enol tautomers is 38%:62%. The spectrum in another polar and aprotic solvent DMF-d_7 is shown in Fig. S2c, the singlet at 3.99 ppm should be assigned to 5-CH_2 methylene protons and the singlet at 5.25 ppm should belong to the 5-CH vinylic hydrogen. Besides the aromatic hydrogen signal at 7.28–7.49 ppm, there are two active hydrogen signals at 12.55 and 12.89 ppm which should be assigned to O—H proton of enol form and N—H proton respectively. Therefore, we could conclude that tautomerism of compound 1 also occurred in DMF-d_7. The ratio of keto:enol form is 44%:56% according to the integral values. But only according to ^{1}H NMR spectra, we could not identify which enol form exists in DMSO-d_6 and DMF-d_7 solution (It could be tautomer N3 or N4 which is shown in Fig. S3). In protic solvent CD_3OD, we observed that both the 5-CH_2 methylene protons and the N—H proton signals disappeared, only the aromatic hydrogen signals at 7.15–7.48 ppm remained. This could be interpreted by the exchange process of the H's on C-5 and N—H with D's on deuterated methanol. The exchange of the 5-CH_2 protons could take place through the O—H proton of one of the enol forms of 1-phenyl-2-thiobarbituric acid. Because of this, both the 5-CH_2 proton and the O—H proton of enol form disappear in ^{1}H NMR spectrum taken in CD_3OD. The possible tautomers and possible H–D exchange process of compound 1 in CD_3OD are shown in Fig. S4. From the ^{1}H NMR spectra in different solvents, it is concluded that tautomerization of compound 1 occurred more easily in polar solvent than in apolar solvent.

3.4. Theoretical calculations on 1-phenyl-2-thiobarbituric acid 1

The geometry optimization for the eight tautomers of 1-phenyl-2-thiobarbituric acid 1 was performed in gas phase and different solvent media at B3LYP/TZP level. We chose three types of solvent with different dielectric constants (Chloroform = 4.9, Ethanol = 24.3, Dimethyl sulfoxide = 46.7). The total energy and dipole moment of N1—N8 were obtained from the optimization output and are listed in Table 3. From the result it can be seen that tautomer N6 is the most energetically stable one both in gas phase and solution phase. The energy gap between different tautomeric forms and the most stable form N6 is listed in Table 4. The energy gap is in the range of 8.16–33.07 kcal/mol for all investigated tautomers in different phases. The second stable tautomer is N4 according to calculated energy with the ΔE to be 8.16–10.29 kcal/mol in gas and different solvents. The calculated energy support the experimental data obtained from IR spectra in KBr pellets and X-ray single crystal diffraction for the solid state and from ^{1}H NMR spectrum for the $CDCl_3$ solution because it has shown that in this two phases 1-pheny-2-thiobarbituric acid exists in the most energetically stable tautomer N6. It is found that with the increasing of the solvent dielectric constants, the calculated dipole moment value also increase for most of the 1-phenyl-2-thiobabituric acid tautomers. Depending on the increase of solvent polarity, the molecules with higher dipole moment are more easily stabilized by solvent. This may be one of the reasons of why the tautomerization occurs easier in DMSO-d_6 and CD_3OD solution.

3.5. Theoretical calculations on the methylation of 1-phenyl-2-thiobarbituric acid

To the best of our knowledge, the methylation reaction of 1-phenyl-2-thiobarbituric acid 1 has not been investigated till now. We carried out the methylation reaction of 1-phenyl-2-thiobarbituric acid using 2.2 eq of dimethyl sulfate as methylating reagent. The molecular weight of the obtained product is 248 detected by ESI-MS, concluding that its a dimethylated product. This result is

Table 3
The calculated energy and dipole moments of N1—N8.

Tautomers	Gas		$CHCl_3$		EtOH		DMSO	
	Total energy (a.u)	μ (D)	Total energy (a.u)	μ (D)	Total energy (a.u)	μ (D)	Total energy (a.u)	μ (D)
N1	−1043.8478	2.2135	−1043.8499	5.7530	−1043.8379	3.1495	−1043.8459	7.0865
N2	−1043.8327	7.9322	−1043.8166	15.5452	−1043.8200	11.8284	−1043.8182	14.9991
N3	−1043.8530	3.5041	−1043.8476	5.6359	−1043.8427	6.4249	−1043.8424	6.5193
N4	−1043.8596	5.8736	−1043.8556	8.8855	−1043.8508	8.5776	−1043.8503	8.6705
N5	−1043.8510	5.4896	−1043.8459	7.3248	−1043.8425	7.9617	−1043.8422	8.0471
N6	−1043.8726	1.7823	−1043.8693	1.6748	−1043.8666	1.5705	−1043.8667	1.5796
N7	−1043.8487	1.9663	−1043.8443	3.6574	−1043.8384	4.3997	−1043.8375	4.4808
N8	−1043.8417	6.2191	−1043.8395	7.8788	−1043.8336	8.8953	−1043.8340	9.0293

Table 4
The energy gap between N6 and other tautomers (kcal/mol).

Tautomers	Gas	$CHCl_3$	EtOH	DMSO
N1	15.56	12.17	18.01	13.05
N2	25.04	33.07	29.24	30.43
N3	12.30	13.62	15.00	15.25
N4	8.16	8.60	9.91	10.29
N5	13.55	14.68	15.12	15.37
N6	0	0	0	0
N7	15.00	15.69	17.69	18.32
N8	19.39	18.70	20.71	20.52

Table 5
The energy of possible methylated products in gas phase and DMF solution.

	Gas (a.u)	DMF (a.u)
2a	−1122.4382	−1122.4219
2b	−1122.3992	−1122.3796
2c	−1122.4217	−1122.4092

as expected because the N-phenyl substitution reduces a mobile H atom consequently reduces a methylation site. But 1-phenyl-2-thiobarbituric acid has three potential methylation site (sulfur atom and two oxygen atoms). And as observed in the ^1H NMR spectrum in DMF-d_7, we found that tautomerization of compound 1 occurs but the exact tautomer (N3 or N4) could not be identified. Accordingly we conclude that there might be three possible methylated products (Fig. 2) of 1-phenyl-2-thiobarbituric acid using DMF as reaction solvent. But we only observed one product after the reaction so that a problem arise that which two sites had been methylated. In order to determine the methylation site, theoretical calculations were carried out to assist experimental data. First we searched the transition state of the methylation reaction using ADF software at DFT level finding that there is no transition state in the reaction process, the energy kept decreasing during the whole process. Consequently the methylation reaction will generate the most energetically stable product. Therefore, we calculated the energy for the three possible methylated products 2a–2c in gas phase and in its reaction solvent (DMF). The calculated energy is presented in Table 5. It can be seen from Table 5, that 2a is the most energetically stable one among the three isomers both in gas phase and in DMF solution. The energy sequence of the three isomers is 2a < 2c < 2b. So that 2a should be the theoretical product for the methylation reaction of 1-phenyl-2-thiobarbituric acid. As described in Section 3.1, the molecular structure of methylation reaction product was experimentally identified by X-ray diffraction to be 2a which verified the calculated results. And no other isomers were observed. In summary, the methylation reaction of 1-phenyl-2-thiobarbituric acid was investigated theoretically (DFT) and experimentally (X-ray, IR) and we found that the calculated results are in good accordance with experimental data.

4. Conclusions

In this study, 1-phenyl-2-thiobarbituric acid and its methylated derivative were synthesized and their molecular structure and tautomeric characteristic were investigated by experimental and computational techniques. FT-IR and X-ray diffraction studies indicate that in solid state 1-phenyl-2-thiobarbituric acid (compound 1) exists in the triketo form. ^1H NMR study shows that in $CDCl_3$ solution compound 1 also exists in triketo form whereas in DMSO-d_6, DMF-d_7 and CD_3OD solution tautomerization occurs, indicating that the tautomerization in polar solvents occurs easier than in apolar solvents. The geometric optimization and energy calculation of compound 1 at DFT level agree well with the experimental data. On the other hand, we explored the methylation reaction of 1-phenyl-2-thiobarbituric acid for the first time. There are three possible dimethylation products for compound 1 and we just observed one product in the reaction. The methylated site was identified experimentally (X-ray, FT-IR) and theoretically (transition state search, energy calculation), the results revealed that theoretical calculations are in good agreement with experimental data. In summary, this work demonstrates the combination of experimental and theoretical methods to improve knowledge of tautomerism equilibrium and reactivity of 1-phenyl-2-thiobarbituric acid and may help in synthesizing novel thiobarbituric acid derivatives.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molstruc.2012.12.017.

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