Indian Journal of Chemistry
Vol. 48B, November 2009, pp. 1565-1570

Spectral and single crystal X-ray structure of 4-(4′-methyl) benzylidene amino-5-phenyl-3-mercapto-1,2,4-triazole and its antimicrobial activity

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Received 1 October 2008; accepted (revised) 22 April 2009

4-(4′-Methyl) benzylidene amino-5-phenyl-3-mercapto-1,2,4-triazole (MBPMT) has been synthesized and characterized by elemental analyses, IR, UV, ^{1}H and ^{13}C NMR, DEPT, mass and XRD studies. The study of single crystal X-ray diffraction pattern of 4-(4′-methyl) benzylidene amino-5-phenyl-3-mercapto-1,2,4-triazole (MBPMT) reveals the presence of N—H· · ·S and C—H· · ·S intermolecular hydrogen bonding interactions. Equilibrium studies have been carried out with the MBPMT to determine the dissociation constant in 70% v/v dioxan-water medium at 303 K and 0.1 *M* (KNO_3) ionic strength which indicate the presence of one dissociable proton corresponding to thiol group. The antimicrobial activity of MBPMT in DMSO medium is also assessed.

Keywords: 4-(4′-Methyl) benzylidene amino-5-phenyl-3-mercapto-1,2,4-triazole, equilibrium studies, hydrogen bonding, antimicrobial activity

The ligand 3-mercapto-1,2,4-triazole[1] (MT) and its derivatives are known for their useful biological activities such as antibacterial, antifungal, hypoglycemic, antihypertensive and analgesic properties[2-6]. The scientific literature also states that the antiviral[7] and antibacterial[8,9] activities of thiourea derivatives are due to the presence of –NH-C(S)-NH- function in the molecule and the changes in the activity depend on the nature of its substituents[10]. The 1,2,4-triazole-3-thiones disubstituted in positions 4 and 5 are useful as bactericides, fungicides and pesticides[11]. A survey of literature revealed that no work has been reported till date on spectral studies, proton-dissociation constant and antimicrobial activity of the triazole selected for the present study. As the determination of dissociation constants is a prerequisite to understand basicity of the compound, an attempt has also been made to determine its value by employing *p*H-metry technique[12,13]. The compound was tested for its antibacterial effect against *Escherichia coli* and *Bacillus subtilis*.

Experimental Section

All chemicals used were of AR grade. The MBPMT was prepared in our laboratory following the reported procedure[14]. The solutions used in potentiometric titrations were prepared in double distilled water. Solutions of metal nitrates were estimated by titrating with the disodium salt of EDTA[15].

Synthesis of 4-amino-5-phenyl-3-mercapto-1,2,4-triazole

Synthesis of MBPMT involves four steps (i) Preparation of phenyl carboxy hydrazide: A mixture of hydrazine hydrate, ethyl benzoate in ethanol was refluxed on a water bath for 4-6 hr. The product separated out on cooling was recrystallised from ethanol (m.p. 103-105°C). (ii) Preparation of potassium-3-phenyl dithiocarbazate: A solution of KOH, phenyl carboxy hydrazide and CS_2 in absolute ethanol was stirred for 12-16 hr. A white coloured solid separates out. (iii) Preparation of 4-amino-5-phenyl-3-mercapto-1,2,4-triazole(APMT). A suspension of potassium salt obtained above, hydrazine hydrate and water was refluxed for 1 hr followed by acidification with conc. HCl yielded APMT (m.p. 202-205°C).

Synthesis of 4-(4′-methyl) benzylidene amino-5-phenyl-3-mercapto-1,2,4-triazole

4-Amino-5-phenyl-3-mercapto-1,2,4-triazole(APMT) was refluxed with 4-methyl benzaldehyde in 1:1 molar

Table I — Crystal data and structure refinement for MBPMT at 298K

Empirical formula	$C_{16}H_{14}N_4S$
Formula weight	294.37
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/n$
Unit cell dimensions	a = 11.1062(12) Å α = 90°
	b = 7.3211(8) Å β = 102.510(2)°
	c = 19.108(2) Å γ = 90°
Volume	1516.7(3) A^3
Z, Calculated density	4, 1.289 Mg/m^3
Absorption coefficient	0.212 mm^{-1}
F(000)	616
Crystal size	0.42 × 0.30 × 0.22 mm
Theta range for data collection	1.96 to 24.99°
Limiting indices	-13<=h<=13, -8<=k<=8, -22<=l<=22
Reflections collected/ unique	14025 / 2677 [R (int) = 0.0221]
Completeness to theta = 24.99	100.0 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2677 / 0 / 195
Goodness-of-fit on F^2	1.031
Final R indices [I>2σ(I)]	R1 = 0.0437, wR2 = 0.1147
R indices (all data)	R1 = 0.0505, wR2 = 0.1209
Largest diff. peak and hole	0.291 and -0.204 $e.A^{-3}$

ratio in acidified ethanol medium for one hour. On cooling, pale yellow pluffy product separated out, m.p. 223-225°C. Anal. found (calc) for $C_{16}H_{14}N_4S$ (294): C, 65.17 (65.30); H, 4.53 (4.76), N, 19.23 (19.04).

Elemental analyses, IR, NMR, mass spectra and XRD studies were used for characterization. IR spectra (KBr) were recorded on a Perkin-Elmer 435 spectrophotometer. ^1H and ^{13}C NMR spectra on a Bruker WH (270 MHz) spectrometer, DEPT spectra on a ACF 200 Bruker 200 MHz superconductivity magnet spectrometer and mass spectra on a Micro Mass VG70-70H spectrometer operating at 70 eV using direct inlet system, were recorded. X-ray diffraction data were measured on a Bruker Smart Apex with CCD area detector. The equilibrium studies were carried out on a DIGISUN DI-707 *p*H-meter with an assembly of combined glass and calomel electrode. The dissociation constant of the MBPMT under study was calculated using Irving-Rossotti titration technique[12].

Crystal growth

The saturated solution of MBPMT was prepared in absolute ethanol and the crystals were developed on slow evaporation by diffusion method in ether.

Single crystal X-ray diffractometry

High resolution single crystal X-ray diffraction data were collected at 298K on a Bruker SMART APEX CCD diffractometer, equipped with a graphite monochromator and a fine focus sealed tube [λ(Mo-Kα,) = 0.71073Å]. A single crystal was mounted in a Lindmann capillary and 2400 frames were recorded with scanning angle ω of 0.3°, each for 5 sec exposure with 0.5 mm collimated X-ray. The crystal to detector distance was kept 60 mm. The collected data were reduced by SAINTPLUS[16]. An empirical absorption correction was applied to the collected reflections with SADABS[17]. The structure was solved by direct methods using SHELXS-97 (ref 18) and the refinement was carried out against F^2 using SHELXL-97 (ref 19). The molecular-packing diagram was generated by mercury1.4.1 of CCDC. All non-hydrogen atoms were refined anisotropically and hydrogens were introduced on calculated positions and included in the refinement riding on their respective parent atoms. All the hydrogen atoms were located from difference Fourier maps.

Results and Discussion

Spectral characterization

IR (KBr): 3111 (ν_{N-H}), 3036, 2989, 2936 (ν_{C-H}), 1603 ($\nu_{C=N}$), 1477, 1446 ($\nu_{C=C}$), 1276 ($\nu_{C=S}$), 766, 690 cm^{-1} (1,4-disubstituted); ^1H NMR ($CDCl_3$; TMS): δ 13.50 (s, 1H, SH), 9.85 (s, 1H, azomethine carbon), 7.16-7.86 (m, 9H, arom-CH), 2.31(s, 3H, CH_3). The signals corresponding to SH readily exchanged with D_2O. ^{13}C NMR and DEPT chemical shifts are reported in **Table I**. ^{13}C NMR showed signals corresponding to aromatic quaternary, tertiary and azomethine (3˚) and Methyl (1˚) carbon atoms at δ: ($CDCl_3$+DMSO-d_6) 164.13, 162.63, 149.02, 143.12, 130.32, 129.61, 128.33, 125.76 and 21.55. The DEPT 45, 90 and 135 recorded signals corresponding to aromatic carbon (3˚) azomethine carbon (3˚) and

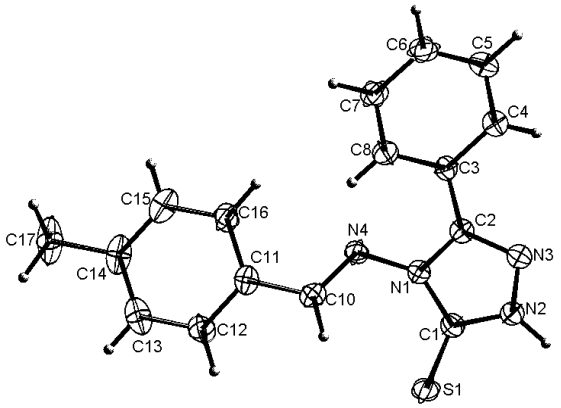

Figure 1 — Thermal ellipsoidal plot with 50% probability for non-H atoms

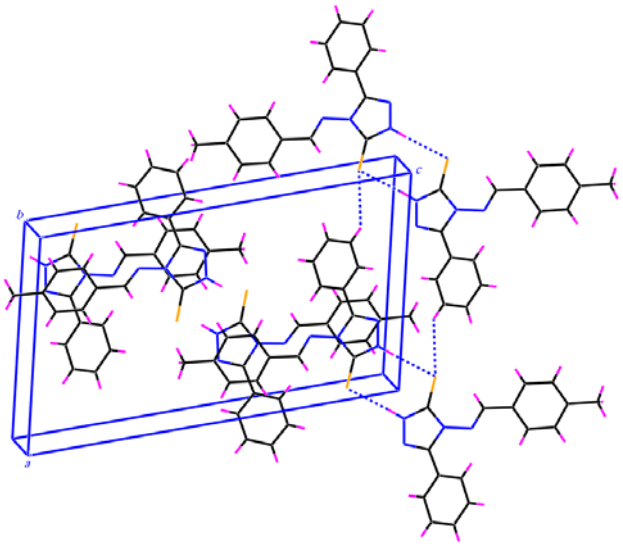

Figure 2 — Packing diagram with an extended chain along crystallographic *a* axis.

methyl (1˚) carbon at δ: 164.13, 130.26, 129.58, 128.66, 128.27 and 21.55. In DEPT 90 spectrum only tertiary carbon are recorded. The comparison of ^{13}C NMR and DEPT 90 spectra with DEPT 45 and 135 clearly revealed the chemical shifts of 1˚, 2˚, 3˚ and 4˚ carbons.

Description of the crystal structure

The thermal ellipsoidal plot of MBPMT with labeling of non-hydrogen atoms is shown in **Figure 1**. The unit cell parameters are listed in **Table I**. MBPMT crystallizes in the cetrosymmetric monoclinic $P2_1/n$ space group with all atoms located at general positions (**Figure 2**) and there are four such molecules in the unit cell (Z = 4). The analysis of bond angles and bond lengths, as given in **Table II,**

Table II — Bond lengths [Å] and angles [deg] for MBPMT

Bond lengths	
C(1)-N(2)	1.325(2)
C(1)-N(1)	1.378(2)
C(1)-S(1)	1.674(2)
C(2)-N(3)	1.299(2)
C(2)-N(1)	1.380(2)
C(2)-C(3)	1.467(2)
C(10)-N(4)	1.273(2)
C(10)-C(11)	1.457(3)
C(10)-H(10)	0.9300
C(14)-C(17)	1.518(3)
C(17)-H(17A)	0.9600
C(17)-H(17B)	0.9600
C(17)-H(17C)	0.9600
N(1)-N(4)	1.400(2)
N(2)-N(3)	1.363(2)
N(2)-H(2N)	0.93(2)
Bond angles	
N(2)-C(1)-N(1)	102.80(16)
N(2)-C(1)-S(1)	127.97(15)
N(1)-C(1)-S(1)	129.23(15)
N(3)-C(2)-N(1)	110.25(16)
N(3)-C(2)-C(3)	123.84(16)
N(1)-C(2)-C(3)	125.90(16)
C(4)-C(3)-C(2)	118.02(17)
C(8)-C(3)-C(2)	122.94(17)
N(4)-C(10)-C(11)	120.00(18)
N(4)-C(10)-H(10)	120.0
C(11)-C(10)-H(10)	120.0
C(12)-C(11)-C(10)	119.15(19)
C(16)-C(11)-C(10)	122.24(19)
C(13)-C(14)-C(17)	120.9(3)
C(15)-C(14)-C(17)	120.8(3)
C(14)-C(17)-H(17A)	109.5
C(14)-C(17)-H(17B)	109.5
H(17A)-C(17)-H(17B)	109.5
C(14)-C(17)-H(17C)	109.5
H(17A)-C(17)-H(17C)	109.5
H(17B)-C(17)-H(17C)	109.5
C(1)-N(1)-C(2)	108.16(15)
C(1)-N(1)-N(4)	128.33(15)
C(2)-N(1)-N(4)	122.07(15)
C(1)-N(2)-N(3)	114.51(16)
C(1)-N(2)-H(2N)	124.5(14)
N(3)-N(2)-H(2N)	120.5(14)
C(2)-N(3)-N(2)	104.24(15)
C(10)-N(4)-N(1)	115.55(16)

reveals that the two-phenyl moieties are coplanar with the triazole ring. The compound MBPMT forms a dimer by N—H· · ·S and C—H· · ·S hydrogen bonding interactions[20,21] (**Figure 3**). These dimers further extend along crystallographic *a*-axis as shown in **Figure 4**. Interaction matrices at 298 K are given in **Table III**. The data of the bond lengths indicate that the bond strengths of hydrogen bonds as: N—H· · ·S > C—H· · ·S which is due to high electronegativity of nitrogen than carbon. The analysis of C(5)—H(5)· · ·S(1) and N(2)—H(2)· · ·S(1) interactions indicate that these are intermolecular hydrogen bonds.

Equilibrium studies

The potentiometric titrations with MBPMT were carried out in 70% (v/v) dioxin-water medium at

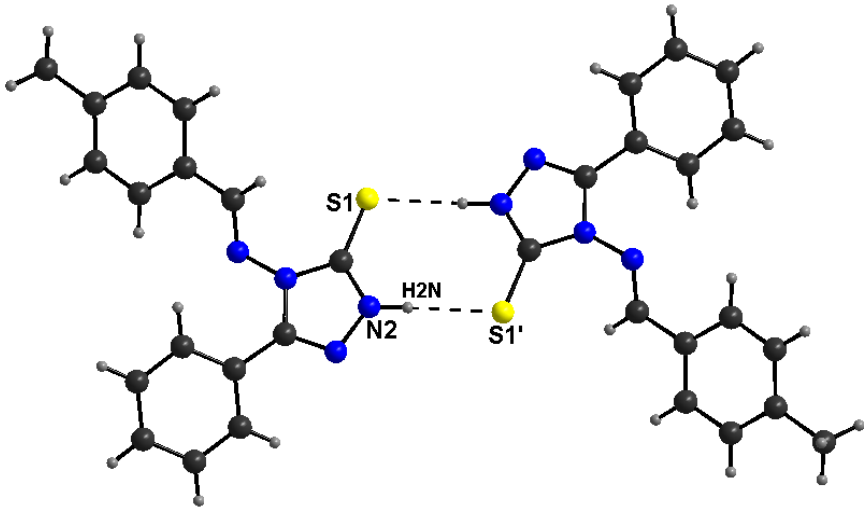

Figure 3 — Dimer formed by N–H···S hydrogen-bonding interactions with symmetry codes i) 1-x,-y,1-z;

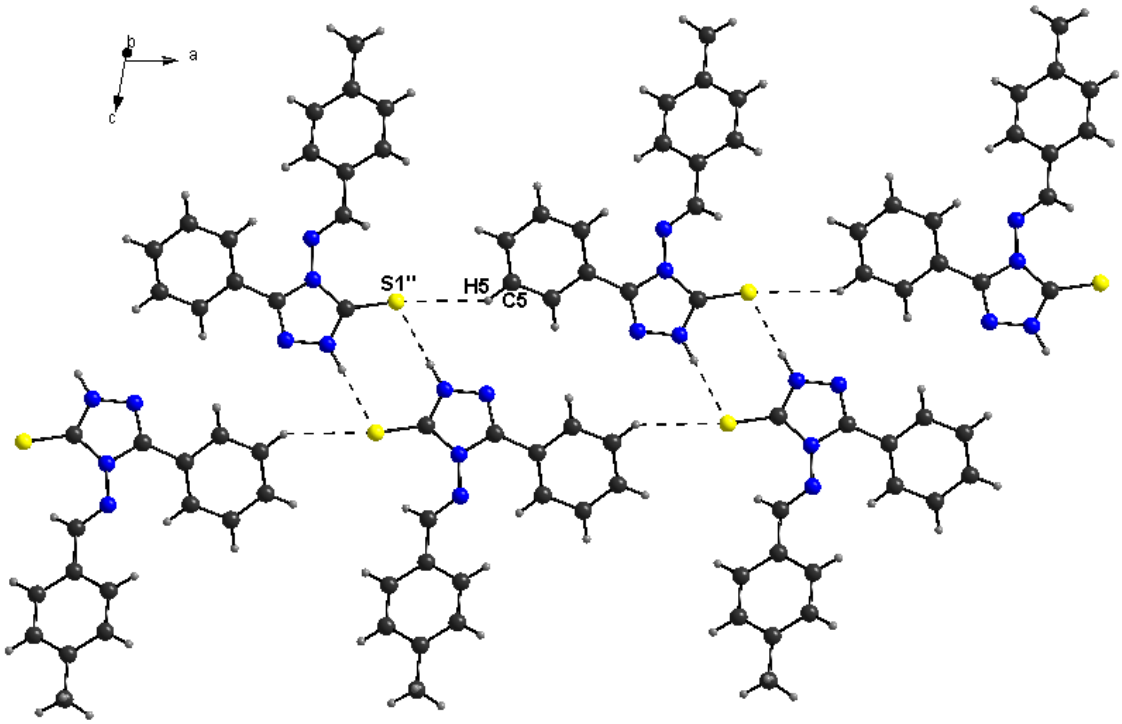

Figure 4 — Dimer extended along crystallographic *a* axis due to C–H···S hydrogen-bonding interactions with symmetry codes ii) -1+ x,

Table III — Hydrogen bonding parameters (Å, deg) of compound MBPMT

D–H···A	*d*(D–H)	*d*(H···A)	D(D···A)	∠DHA
N2–H2···S1′	0.928(3)	2.372(2)	3.294(4)	172(2)
C5–H5···S1″	0.930(3)	2.927(3)	3.716(2)	143(1)

Symmetry transformations used to generate equivalent atoms: i) 1-x,-y,1-z; ii) -1+ x, y, z.

Table IV — Antibacterial activity of MBPMT

E. coli (Gram–ve)	*Bacillus subtilis* (Gram +ve)
+	+++

+ > 5mm slightly active, ++ >7mm moderately active, +++ > 9mm highly active

303 K and 0.1 M (KNO_3) ionic strength. The dissociation constant value is calculated using Irving-Rossotti titration technique[12]. The results indicated the presence of one dissociable proton corresponding to thiol group of MBPMT (pK_a =9.46). The comparison of pK_a in MBPMT with 4-amino-5-phenyl-3-mercapto-1,2,4-triazole (APMT) (pK_a = 6.38) and 4-benzylidine amino-5-phenyl-3-mercapto-1,2,4-triazole (BPMT) (pK_a = 9.13) reported earlier[22] reveals the following order of basicity: MBPMT > BPMT > APMT. The higher pK_a in MBPMT than in APMT is due to conversion of amino nitrogen *(sp^3)* in APMT to imino nitrogen *(sp^2)* in MBPMT. While the higher pK_a in MBPMT than BPMT is due to the presence of electron releasing methyl group at para position of the ring in MBPMT. Thus the change of hybridization at nitrogen and the presence of electron releasing group influence the basicity parameter.

Biological testing

The broth dilution method[23,24] was employed for the study of antibacterial effects against *Escherichia coli* and *Bacillus subtilis*[10] of MBPMT. The tested compound was dissolved in DMSO to obtain a 1 mg/mL solution. The minimum inhibition concentration (MIC) was determined using spread plate method. The inhibitory effects of compound against these organisms are given in **Table IV**. The data indicated that the compound MBPMT exhibited weak inhibitory activity against Escherichia coli and high inhibitory activity against Bacillus subtilis.

Conclusions

The crystal system of 4-(4′-methyl) benzylidene amino-5-phenyl-3-mercapto-1,2,4-triazole is monoclinic and forms a dimer by N—H· · ·S hydrogen bonding interactions which further extends along crystallographic a axis. Single crystal X-ray diffraction studies confirm that the sulphur in mercapto triazole moiety is in thione form in crystalline state. Biological studies of MBPMT reveal its slight activity against *E. coli* and moderate antibacterial activity against *Bacillus subtilis*.

Supplementary material

Crystallographic data for the reported structure (MBPMT) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. 644949. Copies of the data can be obtained, free of charge on application to CCDC, 12 union Road CB2 1EZ, UK (Fax:+44(0)1223-336033 or email deposit@ccdc.cam.ac.uk).

Acknowledgements

We thank National Single Crystal Diffractometer Facility, Indian Institute of Chemical Technology, Hyderabad, for X-ray crystallographic studies.

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