Synthesis, Crystal Structure and Biological Activity of 4-Cyclopropyl-3-[(3-fluorobenzyl)thio]-5-methyl-4H-1,2,4-triazole Monohydrate

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The compound 4-cyclopropyl-3-[(3-fluorobenzyl)thio]-5-methyl-4H-1,2,4-triazole monohydrate (C₁₃H₁₄N₃SF·H₂O) were synthesized and recrystallized from ethanol. The compound was characterized by ¹H NMR, elemental analyses and X-ray diffraction. The compound crystallized in the orthorhombic space group Pbca with a = 10.513(2), b = 8.4646(17), c = 30.965(6) Å, α = 90, β = 90, γ = 90°, V = 2755.6(10) Å³, Z = 8 and R = 0.0510 for 2542 observed reflections with I > 2σ(I). X-ray analysis reveals that not only intermolecular O-H...N interactions, but also C-H...π stacking interactions exist in the adjacent molecules. The biological activities results showed that it exhibited significant herbicidal activity against *Brassica napus*.

Key Words: 1,2,4-Triazole, Cyclopropane, Herbicidal activity, Synthesis.

INTRODUCTION

In recent years, heterocyclic compounds are commonly used as scaffolds on which pharmacophores are arranged to provide potent and selective medicines or pesticides¹. Usually, triazoles and their derivatives have been proved to be effective bactericides, pesticides and fungicides². Some of them had been developed as fungicides or antifungal agents, such as diniconazole, triadimefon, triadimenol, flusilazole, fluconazole, itraconazole. Also 1,2,4-triazoles exhibited diversity activity, such as antibacterial³, antifungal⁴, anti HIV⁵, antioxidant activity⁶, antiinflammatory activity⁷, etc. Furthermore, cyclopropane group displayed excellent biological activities⁸. Meanwhile, many bioactive molecules contain cyclopropane structure. For example, the insecticide allethrin was discovered from the pyrethrum.

In view of these facts and also as a part of our work on the synthesis of bioactive lead compounds for drug discover, the title compounds were designed by introducing cyclopropane pharmacophore into 1,2,4-triazole scaffold. A new 1,2,4-

triazole derivative *i.e.*, 4-cyclopropyl-3-[(3-fluorobenzyl)thio]-5-methyl-4H-1,2,4-triazole monohydrate was synthesized and characterized by ¹H NMR, elemental analysis and X-ray diffraction. The herbicidal activity of the title compound was also tested.

EXPERIMENTAL

Melting points were determined using an X-4 apparatus and uncorrected. ¹H NMR spectra were measured on a Bruker AV-400 instrument using TMS as an internal standard and CDCl₃ as the solvent. Elemental analyses were performed on a Vario EL elemental analyzer. Crystallographic data of the compound were collected on a rigaku saturn diffractometer. All the reagents are of analytical grade or freshly prepared before use.

General procedure: The title compounds were synthesized according to the route shown in **Scheme-I** and the yields were not optimized.

A mixture of ethyl acetate (8.8 g, 0.1 mol) and hydrazine hydrate (7.0 g, 85 %) was refluxed for 12 h in alcohol and

Scheme-I: Synthetic route of title compounds

evaporated to alcohol. Acetohydrazide **1** was obtained in 82.2 %, m.p. 62–63 °C ref. 67 °C. Compound **1** mixed with 1.5 g of isothiocyanatocyclopropane for refluxing *ca.* 3 h in alcohol. After cooling, a good amount of solid was obtained. The solid was filtered, drying, recrystallized from methanol to give intermediate **2**, white crystal, yield 88.5 %, m.p. 117–118 °C, ¹H NMR (400M, CDCl₃): 0.49–0.52(m, 2H, cyclopropane-CH₂), 0.57–0.64 (m, 2H, cyclopropane-CH₂), 1.79 (s, 3H, Me), 2.88–2.94 (m, 1H, cyclopropane-CH), 7.83 (br, 1H, NH), 9.15 (br, 1H, NH), 9.57 (br, 1H, NH). Compound **2** (10 mmol) mixed with 15 mL, 2 N NaOH for refluxing *ca.* 4 h. After cooling, 4 N HCl was added, a good amount of solid was obtained. The solid was filtered, drying, recrystallized from methanol to give intermediate **3**, white crystals, yield 86.3 %, m.p. 134–135 °C, ¹H NMR (400 MHz, CDCl₃): 0.94–0.99(m, 2H, cyclopropane-CH₂), 1.26–1.31 (m, 2H, cyclopropane-CH₂), 2.94 (s, 3H, CH₃), 3.05–3.10 (m, 1H, cyclopropane-CH), 10.86 (br, 1H, SH);. To a stirred solution of **3** (1.5 g, 5.1 mmol) and K₂CO₃ (0.2 g, 5.6 mmol) in DMF (15 mL), a mixture of 1-(chloromethyl)-3-fluorobenzene (5.6 mmol) was added dropwise. The resulting mixture was stirred at room temperature for overnight. The mixture was poured into water, The precipitate formed was filtered off and recrystallized from petroleum ether/acetone to give **4** in good yields. white crystal, yield, 85.6 %; m.p. 100–102 °C; ¹H NMR (400 MHz, CDCl₃), 1.14–1.18(m, 2H, cyclopropane-CH₂), 1.21–1.27(m, 2H, cyclopropane-CH₂), 2.57(s, 3H, Het-CH₃), 3.01–3.27(m, 1H, cyclopropane-CH), 4.96(s, 2H, CH₂), 7.01–7.13(m, 1H, Ph-H), 7.22–7.61(m, 3H, Ph-H); ESI-MS: 548.81[2M + Na]⁺, 526.68 [2M]⁺, 264.16 [M + H]⁺; Anal. calcd. (%) for C₁₃H₁₄N₃SF: C, 59.29; H, 5.36; N, 15.96. Found (%): C, 58.98; H, 5.44; N, 15.67.

Herbicidal activity assay: *In vivo* herbicidal activity of title compounds was determined by rape root and barnyardgrass cup tests according to the literature¹.

Inhibition of the root-growth of Rape (*Brassica campestris*): The evaluated compounds were dissolved in water and emulsified by 100 µL of N,N-dimethyl formamide with 0.1 µL of Tween 20. Rape seeds were soaked in distilled water for 4 h before being placed on a filter paper in a 6 cm Petri plate, to which 2 mL of inhibitor solution had been added in advance. Usually, 15 seeds were used per plate. The plate was placed in a dark room and allowed to germinate for 65 h at 28 ± 1 °C. The length of 10 rape roots selected from each plate was measured and the mean was calculated. The check test was carried out in distilled water only. The inhibition rate was calculated from the root length using the following equation:

$$\text{Relative inhibition rate (\%)} = \left[\frac{(\text{CK} - \text{PT})}{\text{CK}} \right] \times 100 \%$$

where CK is the average root length during the blank assay and PT is the average root length after treatment during testing.

Inhibition of the seedling-growth of Barnyard grass (*Echinochloa crusgalli*): The evaluated compounds were dissolved in water and emulsified if necessary. 10 barnyard grass seeds were placed into a 50 mL cup covered with a layer of glass beads and a piece of filter paper at the bottom, to which 5 mL of inhibitor solution had been added in advance. The cup was placed in a bright room and allowed to germinate for 65 h at 28 ± 1 °C. The height of seedlings of above-ground

plant parts from each cup was measured and the mean was calculated. The check test was carried out in distilled water only. The inhibition rate was calculated from the plant height using the following equation:

$$\text{Relative inhibition rate (\%)} = \left[\frac{(\text{CK} - \text{PT})}{\text{CK}} \right] \times 100 \%$$

where CK is the average plant height during the blank assay and PT is the average plant height after treatment during testing.

Cloning, expression and purification of rice KARI: The DNA sequence corresponding to mature KARI was amplified by PCR using the oligonucleotide primers 5'-aaaggatCCATGGTCGCGGCGC-3' and 5'-cccAaaTTgaagcttCTACG ATGACTGCCGGAG-3'. In these sequences, lower case represents mismatched bases, underlining indicates the location of introduced BamHI and HindIII restriction sites and italics show the Met-54 codon or the reverse complement of the TAG stop codon. The PCR product was digested with BamHI and HindIII and ligated into the pET-30a plasmid that had been digested with the same enzymes. The resultant expression plasmid was used to transform *Escherichia coli* BL21(DE3) cells.

A single colony of these cells was inoculated into 20 mL of LB medium containing 50 mg/mL kanamycin. The culture was incubated overnight at 37 °C and was used to inoculate each of two 500 mL volumes of LB medium containing 50 mg/mL kanamycin; the cultures were incubated at 37 °C with shaking. When an OD₆₀₀ of 0.8 was reached, expression was induced by adding 0.5 mM isopropyl β-D-thiogalactoside to each culture. These were then incubated at room temperature (22 °C) for a further 4 h with shaking and the cells were harvested by centrifugation.

The frozen cell pellet was thawed, suspended in ice-cold purification buffer [20 mM Tris-HCl (pH 7.9)/500 mM NaCl] containing 5 mM imidazole and then treated with lysozyme (10 mg/g of cells for 0.5 h at 0 °C). The cells were disrupted by sonication, insoluble material was removed by centrifugation and the supernatant was passed through a 0.45 mm filter. The cell extract was applied to a 7 mL column of His-Bind resin (Novagen) that had been charged by using 50 mM NiSO₄ then equilibrated with purification buffer containing 5 mM imidazole. The loaded column was washed with 23 mL of the same buffer, followed by 30 mL of purification buffer containing 25 mM imidazole and then KARI was eluted with 30 mL of purification buffer containing 400 mM imidazole. Fractions containing the enzyme were pooled, concentrated to 2.5 mL by ultrafiltration and exchanged into 20 mM Na-Hepes buffer, pH 8.0 using a Pharmacia PD-10 column. The eluate was snap-frozen in liquid nitrogen and stored at -70 °C⁹.

Enzyme and protein assays: Gerwick *et al.*¹⁰, reported that the inhibition of *Escherichia coli* KARI is time-dependent. To characterise the steady-state inhibition constant, *Escherichia coli* KARI was preincubated for 10 min with NADPH, Mg²⁺ and the title compound, then the reaction was initiated with hydroxypyruvate. Under these conditions, the change in A₃₄₀ was found to be linear with time.

Structure determination: The cube-shaped single crystal of the title compound was obtained by recrystallization from EtOH. The crystal with dimensions of 0.24 mm × 0.20 mm ×

0.18 mm was mounted on a Rigaku Saturn diffractometer with a graphite-monochromated MoK α radiation ($\lambda = 0.71073$ Å) by using a Phi scan modes at 113(2) K in the range of $2.34^\circ \leq \theta \leq 27.87^\circ$. A total of 21313 reflections were collected, of which 3277 were independent ($R_{\text{int}} = 0.0691$) and 2542 were observed with $I > 2\sigma(I)$. The calculations were performed with SHELXS-97 program¹¹ and the empirical absorption corrections were applied to all intensity data. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were determined with theoretical calculations and refined isotropically. The final full-matrix least squares refinement gave $R = 0.0510$ and $wR = 0.1242$ ($w = 1/[\sigma^2(F_o^2) + (0.0702P)^2 + 0.5372P]$ where $P = (F_o^2 + 2F_c^2)/3$, $S = 1.08$, $(\Delta/\sigma)_{\text{max}} = 0.002$, $\Delta\rho_{\text{max}} = 0.330$ and $\Delta\rho_{\text{min}} = -0.44$ e Å⁻¹. Atomic scattering factors and anomalous dispersion corrections were taken from international table for X-ray crystallography¹². A summary of the key crystallographic information were given in Table-1.

TABLE-1
CRYSTAL STRUCTURE AND DATA
REFINEMENT PARAMETERS

Empirical formula	C ₁₃ H ₁₆ N ₃ OSF
Formula weight	281.35
Crystal system/space group	Orthorhombic, Pbc _a
a (Å)	10.513(2)
b (Å)	8.4646(17)
c (Å)	30.965(6)
α (°)	90
β (°)	90
γ (°)	90
V (Å ³)	2755.6(10)
Z	8
D _{calcd.} (g/cm ³)	1.356
μ (mm ⁻¹)	0.2400
Crystal size (mm)	0.24 x 0.20 x 0.18
Color/shape	Colorless/cube
Temp. (K)	113(2)
Theta range for collection	2.34 to 27.87°
Reflections collected	21313
Independent reflections	3277
Data/restraints/parameters	3277/1/181
Goodness of fit on F ²	1.076
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0510$, $wR_2 = 0.1242$
R indices (all data)	$R_1 = 0.0705$, $wR_2 = 0.1349$
Largest difference peak/hole	0.334 and -0.440

RESULTS AND DISCUSSION

Synthesis and spectra: The synthesis procedures for title compound were shown in **Scheme-I**. 1,2,4-Triazole sulfide intermediate **3** reacted with 3-chloro benzyl chloride at room temperature for 24 h to give title compound. In order to optimize the reaction condition and reaction times, microwave irradiation was employed. The title compound **4** was successfully obtained in good yield and short time under microwave irradiation (90 °C, 50 W). The proton magnetic resonance spectra of the triazoles have been recorded in CDCl₃. The triazole intermediates can exist either as a thione or the thiol tautomeric forms or as an equilibrium mixture of both forms, because they have a thioamide, -NH-C(=S) function. The chemical shift at δ 10.86 as a singlet may be due to SH proton, indicating that **3** existed not as thione but as the thiol tautomeric forms in solution. The

signal of CH₂ protons was observed at δ 4.96 ppm as a singlet. The chemical shifts at 2.57 ppm are the methyl of triazole. The mass of title compound showed that $[2M + Na]^+$, $[2M]^+$, $[M + H]^+$; The elemental analysis results is according with the calculated results.

Crystal structure: The selected bond lengths, bond angles and torsion angles are shown in Table-2. The molecular structure of the title compound is shown in Fig. 1. The molecular packing of the molecule is shown in Fig. 2. The hydrogen-bond distances (Å) of the title compound are listed in Table-3.

TABLE-2
SELECTED BOND LENGTHS (Å) AND BOND ANGLES (°)

Bond	Dist.	Angle	(°)
S(1)-C(3)	1.745(2)	C(3)-S(1)-C(7)	100.62(9)
S(1)-C(7)	1.822(2)	C(2)-N(1)-C(3)	104.75(16)
F(1)-C(12)	1.361(2)	C(2)-N(1)-C(4)	129.04(16)
N(1)-C(2)	1.370(2)	C(3)-N(1)-C(4)	126.12(16)
N(1)-C(3)	1.373(2)	C(2)-N(2)-N(3)	107.72(16)
N(1)-C(4)	1.444(2)	N(2)-C(2)-N(1)	110.21(17)
N(2)-C(2)	1.308(3)	N(2)-C(2)-C(1)	125.49(19)
N(2)-N(3)	1.406(2)	N(1)-C(2)-C(1)	124.29(18)
N(3)-C(3)	1.309(2)	N(3)-C(3)-N(1)	110.96(17)
C(1)-C(2)	1.489(3)	N(3)-C(3)-S(1)	128.19(14)
C(4)-C(6)	1.503(3)	N(1)-C(3)-S(1)	120.84(15)
C(4)-C(5)	1.505(3)	N(1)-C(4)-C(6)	118.05(17)
C(5)-C(6)	1.501(3)	N(1)-C(4)-C(5)	117.49(18)
C(7)-C(8)	1.508(3)	C(6)-C(4)-C(5)	59.87(14)
C(8)-C(9)	1.391(3)	C(5)-C(6)-C(4)	60.15(14)
C(8)-C(13)	1.399(3)	C(8)-C(7)-S(1)	105.47(13)
C(9)-C(10)	1.395(3)	C(9)-C(8)-C(13)	119.16(19)
C(10)-C(11)	1.381(3)	C(9)-C(8)-C(7)	120.54(18)
C(11)-C(12)	1.380(3)	C(8)-C(9)-C(10)	120.3(2)
C(12)-C(13)	1.375(3)	C(12)-C(11)-C(10)	117.4(2)
O(1)-H(1A)	0.91(3)	F(1)-C(12)-C(13)	118.5(2)
O(1)-H(1B)	0.89(3)	H(1A)-O(1)-H(1B)	108(2)

Fig. 1. Molecular structure of the title compound

The title compound consists of 1,2,4-triazole ring, benzene ring, cyclopropane ring and a molecule of water according to X-ray single-crystal structure determination. Generally, the average bond lengths and bond angles of ring system (phenyl, cyclopropane and 1,2,4-triazole) are normal ranges. However, the C2=N2 bond [1.308(3) Å] and C3=N3 [1.309(2) Å] are longer than the general C=N double bond length of 1.27 Å. The bond angle of C-S-C is 100.62(9)°. In the cyclopropane ring and phenyl ring, the C-C bond lengths range from

Fig. 2. Molecular packing of the molecule

1.375(3)-1.505(3) Å, almost equal to the values of typical bonds of aromatic structure²⁰⁻²³ and alkyl structure. The bond angles of phenyl ring vary from 118.0(2)-121.3° with the average of 120° and the bond angles of the other cyclopropane ring change from 59.87(14)-60.15(14)°, also with the average of 60°. The torsion angle of thioether group C3-S1-C7-C8 is -174.60(13)°.

TABLE-3

HYDROGEN-BOND DISTANCE (Å) OF THE TITLE COMPOUND

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1B)...N(3)	0.89(3)	2.08(3)	2.954(3)	167(3)
O(1)-H(1B)...N(2)	0.89(3)	2.63(3)	3.410(2)	147(3)

TABLE-4

IN VITRO AND IN VIVO INHIBITION RATE (%) OF THE TITLE COMPOUNDS AT 100 ppm

No	KARI	<i>Brassica napus</i>	<i>Echinochloa crusgalli</i>
4	0	69.6	10.0
CPD	100	17.2	27.7

As shown in Fig. 1, the 1,2,4-triazole ring is vertical with phenyl ring with a quite small dihedral angle (θ) of 98.4°. The triazole ring (N1, C2, N2, N3, C3) (cyclopropane ring (C4, C5, C6) is fairly planar with plane equation $6.813x + -5.226y + -13.809z = -5.2471$ ($-0.345x + 8.356y + -4.846z = -3.7176$) and the largest deviation from the least squares plane is 0.0036 nm (0.0000 nm). The cyclopropane ring is nearly vertical with phenyl ring and 1,2,4-triazole ring with the dihedral angle of 33.5 and 124.1°.

An interesting feature is the intermolecular edge-to-face π - π stacking, (Fig. 2), which exists in the pyrazole ring and the C-H of cyclopropane group, two phenyl ring of the adjacent molecule. The distance of H13 and the centroid of phenyl ring is 3.154 Å and the angle of C11-H13 and the phenyl ring centroid is 82.21°. Also distance of H6B and the centroid of pyrazole ring is 2.638 Å and the angle of N2-H6B and the pyrazole ring centroid is 76.30°. These interactions are estimated to play a role in stabilizing the crystal structure.

The title compound has an extensive network of hydrogen bonding. In the *ac* plane, they are linked together by N-H...O, C-H...O hydrogen bonds. The vertices are shared with

neighbouring water to form an infinite two-dimensional network of hydrogen bonds in the *ac* plane. The slight discrepancy of crystal structures is probably the consequence of the weakness of this hydrogen bond and van der Waals interactions in the solid-state structure.

Biological activity: To investigate the KARI inhibitory activity and herbicidal activity of these synthesized title compounds, cyclopropane-1,1-dicarboxylic acid (CPD), a potent inhibitor of KARI *in vitro*, was used as the control. We found that title compound **4** showed higher inhibition ability (69.6 %) of rape root than the control CPD at 100 µg/mL. It displayed the weak activity against *Echinochloa crusgalli* (10 %) at 100 µg/mL, it had no activity target KARI.

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