Synthesis, Crystal Structure and Biological Activity of A Novel 1,2,3-Thidiazole Compound

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Summary: A new 1,2,3-thiadiazole compound was synthesized and characterized by ^{1}H NMR, MS and HRMS. The crystal structure of the title compound ($C_{12}H_{11}ClN_2O_4S_2$, Mr = 346.80) has been determined by single-crystal X-ray diffraction. The crystal is of triclinic, space group P-1 with a = 8.4425(17) Å, b = 8.9801(18) Å, c = 9.859(2) Å, α = 84.36(3) °, β = 86.71(3)°, γ = 83.25(3)°, V = 737.9(3)$Å^3$, Z = 2, F(000) = 356, Dc = 1.561 g/cm^3, μ = 0.557 mm^{-1}, the final R1 = 0.0380 and wR2 = 0.0982 for 2160 observed reflections with $I > 2\sigma(I)$. A total of 12585 reflections were collected, of which 2601 were independent (Rint = 0.0364). The herbicidal activity of title compound was determined, the results showed the title compound displayed excellent herbicidal activity against *Brassica campestris*.

Keywords: 1,2,3-thiadiazole; synthesis; herbicidal activity; crystal structure.

Introduction

Recent years, heterocyclic compounds are becoming focus of study in medicinal and pesticidal fields [1-8]. They exhibited diverse activities, such as KARI activity, fungicidal activity, anticancer activity and antibacterial activity. 1,2,3-Thiadiazole, one of the five-member ring, had been widely used in organic synthesis, especially in medicinal or agricultural. For example, some 1,2,3-thiadiazoles had been developed as commercial agrochemicals, such as the plant inducers thiadinal, BTH. Also, 1,2,3-thiadiazoles exhibited fungicidal activity [9, 10], herbicidal activity [11, 12].

In view of these facts, and also as a part of our work on the development of bioactive heterocyclic compounds [13-16], herein a 1,2,3-thiadiazole compound was synthesized and its single crystal was determined. The structure of title compound was characterized and the preliminary biological activity was investigated.

Results and Discussion

Synthesis

The synthetic route of the novel 1,2,3-thiadiazole compound is presented in Scheme-1. Ethyl 4-methyl-1,2,3-thiadiazole-5-carboxylate was synthesized according the reference. The key intermediate ethyl 4-(bromomethyl)-1,2,3-thiadiazole-5-carboxylate **4** was prepared via two pathways using carbonic acid diethyl ester as the starting material. To a solution of ethyl 4-methyl-1,2,3-thiadiazole-5-carboxylate (0.04 mol) in CCl_4 (120 mL) was added NBS(7g) and a little AIBN, the mixture was refluxed for 5 h. After the reaction is completed, filtrated, washed with brine, dried over Na_2SO_4, and condensed under reduced pressure to afford the ethyl 4-(bromomethyl)-1,2,3-thiadiazole-5-carboxylate. This intermediate was not further purified.

Generally, the sulfone can be easily obtained using a stronger oxidating agent such as $KMnO_4$, *m*-CPBA, $K_2S_2O_8$ or H_2O_2/HOAc system. The combination of H_2O_2 and HOAc has been recognized as an efficiency system to oxidize sulfur into sulfone or sulfoxide. In our case, the sulfone was easily obtained at reflux condition for short time.

Spectra

In the ^{1}H NMR spectra of **6**, the CH_3 proton signals of title 1,2,3-thiadiazole was observed at δ1.43 ppm; the CH_2 were observed at δ 4.41 ppm and δ 5.32 ppm as OCH_2 and SCH_2 respectively. The other doublet peaks observed at δ 7.51 ppm and at δ 7.67 ppm which were protons of the phenyl ring. The ESI-MS of title compound showed molecular ion peak of $[M-H]^-$ clearly. With regard to the HRMS, the title compound showed $[M+H]^+$ signals. The measure data of HRMS results is accord with the theoretical values.

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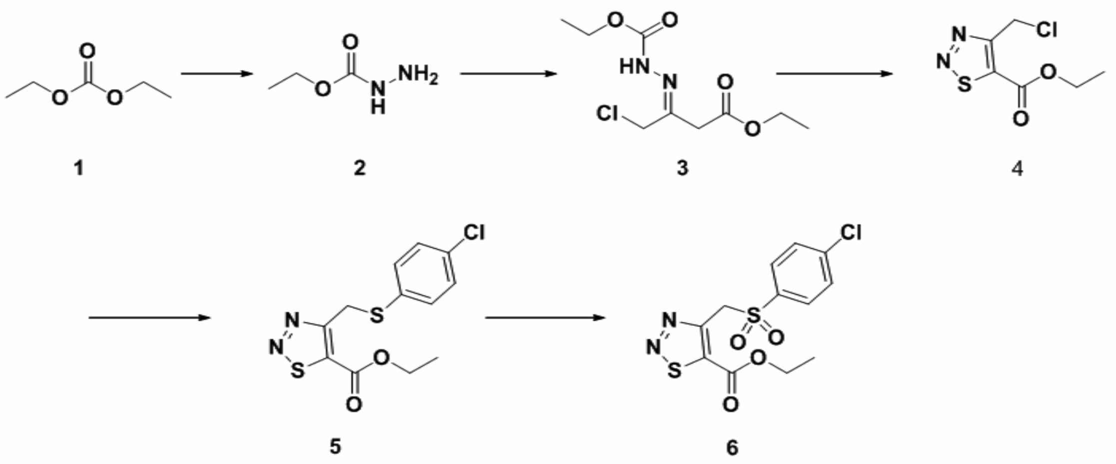

Scheme-1: The synthetic route of the title compounds.

Crystal Structure

The selected bond lengths, bond angles are shown in Table-1. The molecular structure of the title compound is shown in Fig. 1. The molecular packing of the molecule is shown in Fig. 2.

Table-1: Selected Bond Lengths (Å) and Bond Angles (°).

Bond	Dist.	Angle	(°)
S(2)-O(4)	1.4347(16)	O(4)-S(2)-O(3)	119.41(11)
S(2)-O(3)	1.4352(17)	O(4)-S(2)-C(7)	107.87(10)
S(2)-C(7)	1.762(2)	O(3)-S(2)-C(7)	108.96(10)
S(2)-C(6)	1.793(2)	O(4)-S(2)-C(6)	105.79(11)
S(1)-N(1)	1.661(2)	O(3)-S(2)-C(6)	108.73(10)
S(1)-C(4)	1.686(2)	C(7)-S(2)-C(6)	105.17(10)
Cl(1)-C(10)	1.732(2)	N(1)-S(1)-C(4)	93.21(10)
O(1)-C(3)	1.194(2)	C(3)-O(2)-C(2)	116.82(17)
O(2)-C(3)	1.338(2)	N(2)-N(1)-S(1)	111.99(14)
O(2)-C(2)	1.471(3)	N(1)-N(2)-C(5)	113.66(17)
N(1)-N(2)	1.297(2)	O(2)-C(2)-C(1)	110.6(2)
N(2)-C(5)	1.369(3)	O(1)-C(3)-O(2)	125.2(2)
C(1)-C(2)	1.472(4)	O(1)-C(3)-C(4)	124.14(19)
C(3)-C(4)	1.475(3)	O(2)-C(3)-C(4)	110.62(18)
C(4)-C(5)	1.378(3)	C(5)-C(4)-C(3)	127.73(19)
C(5)-C(6)	1.492(3)	C(5)-C(4)-S(1)	108.00(15)
C(7)-C(8)	1.386(3)	C(3)-C(4)-S(1)	124.18(16)
C(7)-C(12)	1.389(3)	N(2)-C(5)-C(4)	113.13(18)
C(8)-C(9)	1.381(3)	N(2)-C(5)-C(6)	119.12(18)
C(9)-C(10)	1.385(3)	C(4)-C(5)-C(6)	127.73(18)
C(10)-C(11)	1.375(3)	C(5)-C(6)-S(2)	114.07(15)
C(11)-C(12)	1.371(3)	C(8)-C(7)-S(2)	120.26(15)

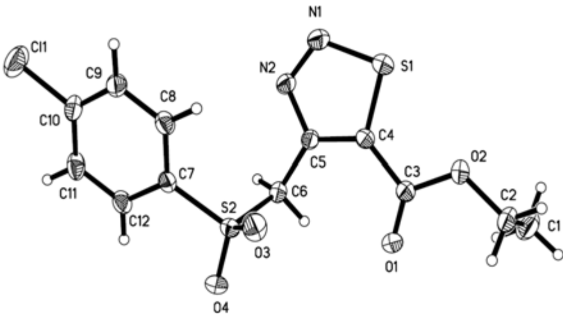

Fig. 1: Molecular structure of the title compound, showing displacement ellipsoids drawn at the 30% probability level.

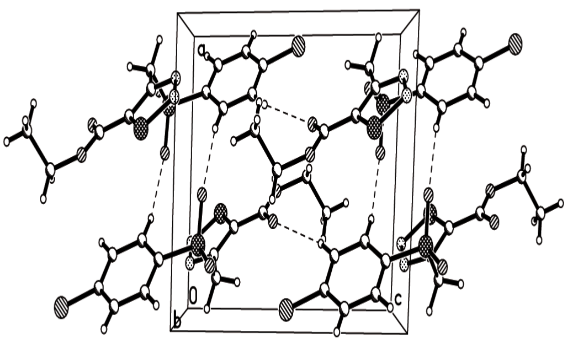

Fig. 2: The molecular packing of the molecule.

Generally, the average bond lengths and bond angles of ring system (phenyl and 1,2,3-thiadiazole) are normal ranges [17-24]. However, the C5=N2 bond [1.369(3) Å] is longer than the general C=N double bond length of 1.28 Å. The C4–C5 bond lengths of 1,2,3-thiadiazole group is 1.378(3) Å. In the phenyl ring, the C–C bond lengths range from 1.371(3) to 1.389(3) Å, almost equal to the values of typical bonds of aromatic structure. The bond angles of phenyl ring vary from 119.0(2) to 121.7(2)° with the average of 120°. Also, the bond angles of 1,2,3-thiadiazole ring are range from 93.21(10) to 113.66(17)°. The torsion angle of thioether group C7–S2–C6–C5 is -74.44(19)°.

As shown in Fig. 1, the 1,2,3-thiadiazole ring is nearly planar with phenyl ring with a quite small dihedral angle (θ) of 14.7º. The 1,2,3-thiadiazole ring (N2, N1, C1, C4, C5) and phenyl ring (C7, C8, C9, C10, C11, C12) is fairly planar with plane equation 7.045x + 3.549y + -3.791z

= 2.3985 and 6.993x + 5.176y + -1.887z = 5.4319 respectively, and the largest deviation from the least squares plane is 0.0014 nm and 0.0120 nm. Meanwhile, the ester group is also planar with the 1,2,3-thiadiazole ring with the dihedral angles of -3.38 °. Also, it is nearly that the CH_2SO_2 group is vertical with 1,2,3-thiadiazole ring and phenyl ring with the bond angle of 114.1°, 86.81° respectively. Meanwhile, intermolecular edge-to-face π-π stacking appear between the phenyl ring C7~C12 and C6-H6B in another adjacent molecule (Fig. 3), in which the distance of H6B and the centroid of phenyl ring C7~C12 is 3.534 Å, with the angle of C6–H6B and the phenyl centroid being 70.11°. These interactions can help to further stabilize the crystal structure.

The title compound has an extensive network of hydrogen bonding involving the two acceptor atoms O. In the *ac* plane, they are linked together by C-H···O hydrogen bonds. This hydrogen-bonding sequence is repeated to form a ring. The ring has two O atoms at the vertices, leading to a hydrogen-bond network defining cyclic motifs denoted $R_2^2(10)$. The vertices are shared with neighbouring decagon to form an infinite two-dimensional network of hydrogen bonds in the *ac* plane.

Bioactivity

To investigate the KARI inhibitory activity and herbicidal activity of the synthesized 1,2,3-thiadiazole compounds, cyclopropane-1,1-dicarboxylic acid (CPD), a potent inhibitor of KARI *in vitro*, was used as the reference. The bioactivities of the 1,2,3-thiadiazole compound as well as CPD are shown in Table-2. With regard to the KARI inhibitory assay, since that the 1,2,3-thiadiazole compound is not soluable in the test system**.** To investigate the herbicidal activities of the synthesized 1,2,3-thiadiazole compound, the bioactivities showed that 1,2,3-thiadiazole compound exhibited low herbicidal activities against *Brassica napus* and *Echinochloa crusgalli* (about 30%) at 100 ppm.

Table-2: The *in vitro* and *in vivo* inhibition rate (%) of the title compounds at 100ppm.

No	KARI	*Brassica napus*	*Echinochloa crusgalli*
6	-	27.9	30.0
CPD	100	17.2	27.7

Note: "-" indicates that the compound is not soluablee in our test system, thus no data obtained.

Experimental

Materials and Methods

All the reagents are analytical grade. Melting points were determined using an X-4 apparatus and uncorrected. ^1H NMR spectra were measured on a Bruker AC-P500 instrument using TMS as an internal standard and $CDCl_3$ as solvent. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage LC/mass detector apparatus. Crystallographic data of the compound were collected on a rigaku saturn diffractometer. HRMS data was obtained on a FTICR-MS instrument (Ionspec 7.0T).

Synthesis

The title compound was synthesized according to the route shown in Scheme-1, and the yield was not optimized.

Carbonic acid diethyl ester (11.8 g, 0.1 mol) and hydrazine hydrate (5.6 mL, 0.095 mol, 85%) were added into a 250 mL round-bottom flask equipped with a condenser. The reaction mixture was heated to 50 °C and stirred for 20 min, and then cooled down to room temperature and further stirred for 30 h. Net, water, ethanol, and the unreacted carbonic acid diethyl ester were distilled off under reduced pressure. After drying, a white crystal **2** (9.88 g) was obtained with a yield of 95%. To a stirred solution of compound **2** (6.36 g, 0.06 mol) in ethanol (16.7 mL), a solution of ethyl 4-chloro-3-oxobutanoate (9.84 g, 0.06 mol) in ethanol (3.7 mL) was added at room temperature. The reaction mixture was stirred for 6 h. Then, the solvent was removed in vacuo and the crude product **3** was directly used in the next step without further purification. Compound **3** (15.0 g, 0.06 mol) was dissolved in dry dichloromethane (25 mL), and thionyl chloride (25 mL) was subsequently added into the stirred reaction mixture drop wise at 0 °C for 1 h. Next, the reaction mixture was permitted to stand for 20 h at room temperature. The excess thionyl chloride and dichloromethane were distilled off, and the resulting crude product was subjected to fractional distillation under reduced pressure. An umber oil **4** was obtained with yield of 75.4%. To a stirred solution of **4** (1.05 g, 5.1 mmol) and K_2CO_3 (0.2 g, 5.6 mmol) in DMF (15 mL), a mixture of a 4-chloro benzenethiol (5.1 mmol) was added drop wise. The resulting mixture was stirred at room temperature overnight. Next, the mixture was poured into water. The precipitate formed was filtered off. The crude product was purified by chromatography over silica gel to give **5** (PE:EA = 5:1). To a stirred solution of compound **5** (1 mmol) in HOAc (10 mL) was added H_2O_2 (2 mmol), then the mixture was stirred at 80 °C for 1 h. During this course, a large amount of white solid was formed. After filtration, the solid was purified by chromatography over silica gel to give the pure **6**, white solid, yield 83.2%, m.p. 114-115°C; ^{1}H NMR($CDCl_3$)δ: 1.43(t, *J*=7.16Hz, 3H, CH_3), 4.41(q, *J*=7.16Hz, 2H, CH_2), 5.32(s, 2H, SCH_2), 7.51(d, *J*=8.57Hz, 2H, Ar-H), 7.67(d, *J*=8.59Hz, 2H, Ar-H); ESI-MS: 344.83$[M-H]^-$; HRMS for $C_{12}H_{11}ClN_2O_3S_2$: found 346.9916, calcd. 346.9922 $[M+H]^+$.

KARI assay

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'-cccAaaTTtgaagcttCTACG ATGACTGCCGGAG-3'. In these sequences, lower case represents mismatched bases, underlining indicates the location of introduced *Bam*HI and *Hind*III restriction sites, and italics show the Met-54 codon or the reverse complement of the TAG stop codon. The PCR product was digested with *Bam*HI and *Hind*III 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℃ 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℃ with shaking. When an OD_{600} 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℃) 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 30 min at 0℃). 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 $NiSO_4$ 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 ultrafltration 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℃ [25].

Enzyme and Protein Assays

Gerwick [26] *et al* reported that the inhibitor (100ppm) of *Escherichia coli* KARI is time-dependent. To characterise the steady-state inhibition constant, *Escherichia coli* KARI was preincubated for 10 min with NADPH, Mg^{2+} and the title compound, then the reaction was initiated with hydroxypyruvate. Under these conditions, the change in A_{340} was found to be linear with time.

Herbicidal Activities Assay

Inhibition of the Root Growth of Rape (Brassica campestris)

The evaluated compounds were dissolved in water and emulsified (100ppm) if necessary. 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 2mL of inhibitor solution had been added in advance. Usually, 15 seeds were used on each plate. The plate was placed in a dark room and allowed to germinate for 65 h at 28±1 °C. The lengths of 10 rape roots selected from each plate were measured, and the means were calculated. The check test was carried out in distilled water only. The percentage of the inhibition was calculated.

Inhibition of the Seedling Growth of Barnyard grass (Echinochloa crusgalli)

The evaluated compounds were dissolved in water and emulsified (100ppm) if necessary. A total of 10 barnyard grass seeds were placed into a 50mL 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 heights of seedlings of above-ground plant parts from each cup were measured, and the means were calculated. The check test was carried out in distilled water only. The percentage of the inhibition was calculated.

Crystal Structure Determination

The cube-shaped single crystal of the title compound was obtained by recrystallization from EtOH. The crystal with dimensions of 0.20 mm × 0.18 mm × 0.12 mm was mounted on a Rigaku Saturn diffractometer with a graphite-monochromated MoKα radiation ($\lambda = 0.71073$Å) by using a Phi scan modes at 293(2) K in the range of $1.61° \leq \theta \leq 25.01°$. A total of 12585 reflections were collected, of which 2601 were independent ($R_{\text{int}} = 0.0364$) and 2160 were observed with $I > 2\sigma(I)$. The calculations were performed with SHELXS-97 program [27] 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 = 0.0380 and wR = 0.0982 ($w = 1/[\sigma^2(F_o^2) + (0.0635P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$), S = 1.11, $(\Delta/\sigma)_{\max} < 0.0001$, $\Delta\rho_{\max} = 0.24$ and $\Delta\rho_{\min} = -0.34$ e Å^{-1}. Atomic scattering factors and anomalous dispersion corrections were taken from International Table for X-Ray Crystallography [28]. A summary of the key crystallographic information were given in Table-**3**.

Table-3: Crystal Structure and Data Refinement Parameters.

Empirical Formula	**$C_{12}H_{11}ClN_2O_4S_2$**
Formula Weight	**346.80**
Crystal System / Space Group	**Triclinic, P-1**
a / Å	**8.4425 (17)**
b / Å	**8.9801 (18)**
c / Å	**9.859 (2)**
α / °	**84.36 (3)**
β / °	**86.71 (3)**
γ / °	**83.25 (3)**
V / Å^3	**737.9 (3)**
Z	**2**
D_{calc} (g/cm^3)	**1.516**
μ (mm^{-1})	**0.5600**
Crystal size (mm)	**0.20 × 0.18 × 0.12**
Color / Shape	**Colorless/Cube**
Temp (K)	**113(2)**
Theta range for collection	**2.08–25.02°**
Reflections collected	**12585**
Independent reflections	**2601**
Data/restraints/parameters	**2601 / 0 / 191**
Goodness of fit on F^2	**1.115**
Final R indices [$I > 2\sigma(I)$]	**R1 = 0.0380, wR2 = 0.0982**
R indices (all data)	**R1 = 0.0469, wR2 = 0.1040**
Largest difference peak/hole	**0.237 and -0.343**

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