Synthesis, Crystal Structure and Biological activity of *N*-(5-(o-tolyl)-1, 3, 4-thiadiazol-2-yl)cyclopropanecarboxamide

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Summary: A new 1, 3, 4-thiadiazole compound, *N*-(5-(o-tolyl)-1,3,4-thiadiazol-2-yl) cyclopropanecarboxamide, was synthesized and its structure was confirmed by ^{1}H NMR, MS and HRMS. The single crystal structure of the title compound was determined by X-ray diffraction. The preliminary biological test showed that the synthesized compound has moderate herbicidal activity against *Brassica campestris* and fungicidal activities against *Sclerotinia sclerotiorum*(Lib.) de Bary, *Rhizoctonia solanii*, *Fusarium oxysporum*, *Corynespora cassiicola*, and *Botrytis cinerea*.

Keywords: Crystal structure, synthesis, 1,3,4-thiadiazole, herbicidal activity, fungicidal activity.

Introduction

Recent years, heterocyclic compounds have received considerable attentions in medicinal and pesticidal fields [1-7]. So synthesis of broader spectrum and highly bioactive heterocyclic compounds become the hot spot in the agricultural and medicinal chemistry fields, especially 1, 3, 4-thiadiazoles. 1, 3, 4-Thiadiazoles displayed diverse functions due to it is a very useful materials for synthesis, such as biological activity [8, 9], complex synthesis [10, 11], polymer synthesis [12], analytical material [13] and so on. Many medicines or pesticides contain amide group and 1, 3, 4-thiadiazole moiety (Fig. 1).

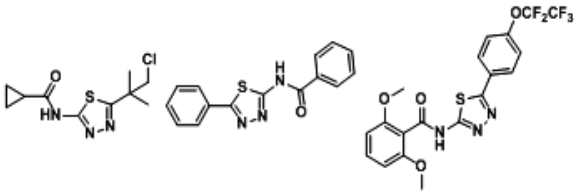

Fig. 1: The pesticides containing 1,3,4-thiadizole ring.

In view of these facts, and also as a part of our work on the development of bioactive heterocyclic compounds, herein a 1, 3, 4-thiadiazole compound was synthesized and its single crystal structure was determined. The biological activity was also determined.

Results and Discussion

Synthesis

The cyclopropane-1, 1-dicarboxylic acid, prepared from 1,2-dichlorethane and diethyl malonate was cyclized for 16 h at refluxing temperature. Microwave assisted irradiation was applied which shortened the reaction time to 40 minutes. If the 1, 2-dichlorethane changed to 1, 2-dibromoethane, the reaction time is short. The cyclopropane-1, 1-dicarboxylic acid was obtained from the hydrolysis of diethyl cyclopropane-1,1-dicarboxylate, but the yield of this step was low, about 50%. Cyclopropanecarbonyl chloride was prepared from the cyclopropane dicarboxylic acid and $SOCl_2$, without isolation further reacted with 5-o-tolyl-2-amino-1, 3, 4-thiadizole at room temperature [8] as shown in scheme-1.

Fungicidal Activities

The *in vivo* fungicidal activities of title compounds against *Sclerotinia sclerotiorum*(Lib.) de Bary, *Rhizoctonia solanii*, *Fusarium oxysporum*, *Corynespora cassiicola*, and *Botrytis cinerea* were evaluated. The primary bioassay showed the title compound exhibits a moderate inhibiting activity towards *Sclerotinia sclerotiorum*(Lib.) de Bary, *Rhizoctonia solanii*, *Fusarium oxysporum*, *Corynespora cassiicola*, and *Botrytis cinerea*. Its inhibition rates to *Sclerotinia sclerotiorum*(Lib.) de Bary, *Rhizoctonia solanii*, *Fusarium oxysporum*, *Corynespora cassiicola*, and *Botrytis cinerea* reach 37.50%, 36.00%, 21.00%, 50.79% and 75.09% at 50 μg/mL respectively. Among them, the compounds displayed the highest fungicidal activity against *Botrytis cinerea*.

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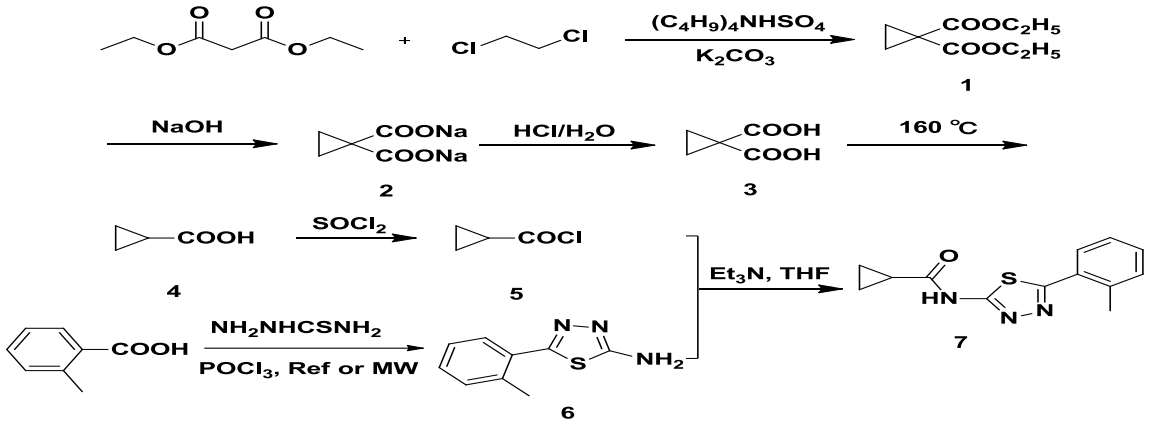

Scheme-1: The synthetic route of title compound.

Table-1: The fungicidal activities of compound 7 *in vivo* at 500 μg·mL$^{-1.}$

No.	*Sclerotinia sclerotiorum* (Lib.) de Bary	*Rhizoctonia solanii*	*Fusarium oxysporum*	*Corynespora cassiicola*	*Botrytis cinerea*
7	**37.50**	**36.00**	**21.00**	**50.79**	**75.09**
Dimethanchlon	**96.70**				
jinggangmycin		**92.10**			
Thiophanate methyl			**97.00**		
chlorothalonil				**86.43**	
pyrimethanil					**99.17**

Herbicidal Activities

The herbicidal activity resulted of the title compounds against *Echinochloa crusgalli* and *Brassica campestris* were determined. Its inhibition rates to *Echinochloa crusgalli* and *Brassica campestris* reach 0%, 41% at 100 μg/mL and 0%, 7.2% at 10μg/mL respectively. The title compounds exhibited moderate herbicidal activities against *Brassica campestris* at 100 ppm. On the other hand, the title compounds exhibited no herbicidal activity against *Echinochloa crusgalli.*

Crystal Structure

The selected bond lengths, bond angles and torsion angles are shown in Table (3 and 4). The molecular structure of the title compound is shown in Fig. 2. The molecular packing of the molecule is shown in Fig. 3.

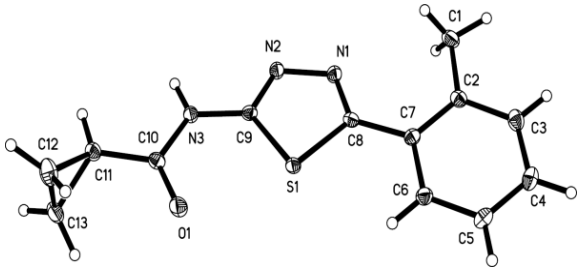

Fig. 2: Molecular structure of the title compound, showing displacement ellipsoids drawn at the 30% probability level.

Table-2: The herbicidal activities of compound **7.**

No.	*Echinochloa crusgalli*		*Brassica campestris*	
	10 μg/mL	100 μg/mL	10 μg/mL	100 μg/mL
7	**0**	**0**	**7.2**	**41.0**
CPD	**0**	**17.2**	**10.6**	**27.7**

Table-3: Selected bond lengths (Å) and angles (°) for the title compound.

Bond lengths	Å	Bond angles	°
S(1)-C(9)	**1.7195(15)**	**C(9)-S(1)-C(8)**	**86.43(7)**
S(1)-C(8)	**1.7563(14)**	**C(8)-N(1)-N(2)**	**112.56(12)**
O(1)-C(10)	**1.2223(18)**	**C(9)-N(2)-N(1)**	**112.55(12)**
N(1)-C(8)	**1.3014(19)**	**C(10)-N(3)-C(9)**	**123.62(12)**
N(1)-N(2)	**1.3802(17)**	**C(3)-C(2)-C(7)**	**117.37(14)**
N(2)-C(9)	**1.3058(19)**	**C(4)-C(3)-C(2)**	**122.85(15)**
N(3)-C(10)	**1.3680(19)**	**C(5)-C(4)-C(3)**	**119.24(15)**
N(3)-C(9)	**1.3736(19)**	**C(6)-C(5)-C(4)**	**119.45(15)**
C(1)-C(2)	**1.511(2)**	**C(5)-C(6)-C(7)**	**121.73(15)**
C(2)-C(3)	**1.393(2)**	**C(6)-C(7)-C(8)**	**117.48(13)**

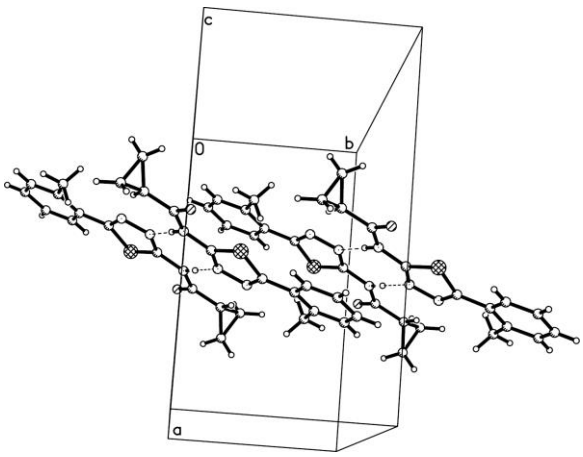

Fig. 3: The pack of title compound.

Table-4: Torsion angles [°] for title compound.

Torsion angles	°
C(6)-N(1)-C(5)-C(4)	-147.14(18)
C(1)-N(1)-C(6)-O(1)	-4.7(3)
C(1)-N(1)-C(6)-C(7)	178.75(16)
C(8)-N(2)-C(7)-C(6)	-2.7(3)
O(1)-C(6)-C(7)-N(2)	-92.4(2)
N(1)-C(6)-C(7)-N(2)	84.5(2)
N(1)-C(6)-C(7)-C(15)	-104.59(19)
C(7)-N(2)-C(8)-O(2)	81.7(3)
C(7)-N(2)-C(8)-C(9)	-101.6(2)
O(2)-C(8)-C(9)-C(10)	-20.3(3)
N(2)-C(8)-C(9)-C(14)	-15.6(3)
N(2)-C(7)-C(15)-C(16)	79.6(2)
C(6)-C(7)-C(15)-C(16)	-91.92(19)
N(2)-C(7)-C(15)-Cl(2)	-43.37(19)
C(6)-C(7)-C(15)-Cl(2)	145.08(13)
N(2)-C(7)-C(15)-Cl(1)	-160.35(14)
C(7)-C(15)-C(16)-S(1)	9.9(2)
Cl(2)-C(15)-C(16)-S(1)	131.34(12)
Cl(1)-C(15)-C(16)-S(1)	-110.25(14)
N(4)-S(1)-C(16)-C(17)	0.62(15)
N(4)-S(1)-C(16)-C(15)	177.60(16)
S(1)-C(16)-C(17)-N(3)	-0.5(2)
C(17)-N(3)-N(4)-S(1)	0.4(2)
C(16)-S(1)-N(4)-N(3)	-0.61(18)

Generally, the average bond lengths and bond angles of ring system (phenyl and 1, 3, 4-thiadiazole) are normal ranges [14, 15]. However, the C8=N1 bond [1.3014(19) Å] and C9=N2 [1.3058(19) Å] are similar with the general C=N double bond length of 1.27 Å. As shown in Fig. 2, the 1,3,4-thiadiazole ring (N1, N2, C9, S1, C8) and phenyl rings (C2, C3, C4, C5, C6, C7) are fairly planar with mean deviations of 0.0049 nm and 0.0013 nm, respectively. Meanwhile, the 1,3,4-thiadiazole ring is parallel with the phenyl ring (C2~C7) with the angles of 7.2 º. As shown in Fig. 2, intermolecular N-H···N hydrogen bonds stabilize the solid-state structure (Table-5). The title compound has an extensive network of hydrogen bonding involving the two acceptor atoms, N. In the *bc* plane, they were linked together by N3-H3A···N2 hydrogen bonds. This hydrogen-bonding sequence is repeated to form a ring. The ring is shaped like a decagon and has two N1 and two H1 atoms at the vertices, leading to a hydrogen-bond network defining cyclic motifs denoted R_2^2 (6) [16]. The intermolecular edge-to-face π-π stacking appears between the thiadiazole ring and the methyl group in another adjacent molecule (Fig. 4), in which the distance of H1B and the centroid of thiadiazole ring is 2.697 Å. These interactions can help to further stabilize the crystal structure.

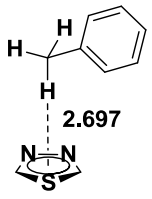

Fig. 4: Edge-to-face π-π stacking.

Table-5: Hydrogen Bond Lengths (Å) and Bond Angles (°).

D–H···A	d(D–H)	d(H···A)	d(D···A)	∠DHA
N(3)-H(3)...N(2)#1	0.893(9)	1.937(10)	2.8272(18)	175.3(17)

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

Experimental

Materials and Methods

All reagents are analytical grade. Melting points were determined using a X-4 apparatus and were uncorrected. ^{1}H NMR spectra were measured on a Bruker AC-P500 instrument (300MHz) using TMS as an internal standard and $CDCl_3$ as solvent. HRMS data was obtained on a FTICR-MS instrument (Ionspec 7.0T). Crystallographic data of the compound were collected on a Rigaku MM-07 Saturn 724 CCD diffractometer.

Synthesis

Preparation of title compound **7**: Mixture of 40 mmol of o-toluic acid and the equimolar amount of thiosemicarbazide and phosphorous oxychloride (30 mL) was refluxed gently for 40 min under microwave. The reaction mixture was cooled, then ice cold water (100 mL) was added to the flask. The solution was neutralized with NaOH solution. The precipitate was filtered, washed with water, dried and recrystallized from ethanol-water to yield 5-o-tolyl-2-amino-1,3,4-thiadizole **6**. The acid chloride was prepared according the reference [8]. Dropwised the acid chloride to 5-o-tolyl-2-amino-1,3,4-thiadizole (7.50 mmol), then vigorously stirred at ambient temperature for 4 h. The corresponding amide **7** precipitated immediately. The product was filtered, washed with THF, dried, and recrystallized from EtOH-H_2O to give the title compounds **7**. White crystal, yield 82.6%, m.p.216-218 ºC; ^{1}H NMR($CDCl_3$)δ: 1.04-1.25(m, 4H, cyclopropane-CH_2), 2.39-2.45(m, 1H, cyclopropane-CH), 2.56(s, 3H, CH_3), 7.29-7.65(m, 4H, Ar-H), 13.07(bs, 1H, NH); FTICR-MS for $C_{13}H_{13}N_3OS$: found 260.0841, calcd. 260.0852.

Crystal Structure Determination

The prism-shaped single crystal of the title compound was obtained by recrystallization from EtOH. The crystal with dimensions of 0.20mm ×0.18mm × 0.20mm was mounted on a Rigaku MM-07 Saturn 724 CCD diffractometer with a graphite-

monochromated MoKα radiation (λ = 0.71073Å) by using a Phi scan modes at 113(2) K in the range of 3.06°≤θ≤25.01°. A total of 16930 reflections were collected, of which 2190 were independent (R_{int} = 0.0441) and 2048 were observed with I > 2σ(I). The calculations were performed with SHELXS-97 program [17] 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 R1 = 0.0315 and wR2 = 0.0793 ($w = 1/[\sigma^2(F_o^2) + (0.0366P)^2 + 1.1001P]$ where $P = (F_o^2 + 2F_c^2)/3$], S = 1.06, $(\Delta/\sigma)_{max} < 0.000$, $\Delta\rho_{max} = 0.22$ and $\Delta\rho_{min} = -0.23$ e Å^{-3}. Atomic scattering factors and anomalous dispersion corrections were taken from International Table for X-Ray Crystallography.[18] A summary of the key crystallgraphic information were given in Table-6.

Table-6: Crystal data of the title compound.

Empirical Formula	$C_{13}H_{13}N_3OS$
Formula weight	259.32
T/K	113(2)
λ/nm	0.071073
Crystal system, space group	Orthorhombic, Pbca
Unit cell dimensions	*a* = 14.643(3) Å α = 90° *b* = 7.9817(16) Å α = 90° *c* = 21.312(4) Å α = 90°
V/nm^3	2490.9(9)
Z	8
Calculated density/(g*cm^{-3})	1.383 Mg m^{-3}
Absorption coefficient/ mm^{-1}	0.251
F(000)	1088
Theta range for data collection	3.06 to 25.01 deg
Reflections collected / unique	16930 / 2190 [R(int) = 0.0441]
Final R indices [I>2sigma(I)]	R1 = 0.0315, wR2 = 0.0793
R indices (all data)	R1 = 0.0340, wR2 = 0.0809

Biological Activity

Fungicidal Activities Assay

A potted plant test method was adopted. Germination was conducted by soaking cucumber seeds in water for 2 h at 50 °C and then keeping the seeds moist for 24 h at 28 °C in an incubator. When the radicles were 0.5 cm, the seeds were grown in plastic pots containing a 1:1 (v/v) mixture of vermiculite and peat. Cucumber plants used for inoculations were at the stage of two seed leaves. Tested compounds were sprayed with a hand spray on the surface of the seed leaves on a fine morning, at the standard concentration of 500 μg/mL. After 2 h, inoculations of *Fusarium oxysporum*, *Corynespora cassiicola* were carried out by spraying a conidial suspension, and inoculation of *Rhizoctonia solanii*, *Sclerotinia sclerotiorum*(Lib.)de Bary, *Botrytis cinerea* was carried out by spraying a mycelial suspension. The experiment was repeated 4 times. After inoculation, the plants were maintained at 18-30 °C. The fungicidal activity were evaluated when the non-treated cucumber plant (blank) fully developed symptoms. The area of inoculated treated leaves covered by disease symptoms was assessed and compared to that of non-treated ones to determine the average disease index. The relative control efficacy of compounds compared to the blank assay was calculated via the following equation:

relative control efficacy (%)=*(CK-PT)/CK* × 100%

where CK is the average disease index during the blank assay and PT is the average disease index after treatment during testing.

Herbicidal Activity Assay

Inhibition of the Root Growth of Rape (*Brassica campestris*).

The evaluated compounds were dissolved in water and emulsified 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 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.

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