Synthesis, Crystal Structure and Anti-TMV Activity of (*Z*)-4-[3-(4-Methyl-1,2,3-thiadiazol-5-yl)-3-(4-trifluoromethylphenyl)acryloyl]morpholine①

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ABSTRACT The target compound (*Z*)-4-[3-(4-methyl-1,2,3-thiadiazol-5-yl)-3-(4-trifluoromethylphenyl)acryloyl]morpholine was synthesized by the nucleophilic substitution, Horner-Emmons reaction, ester hydrolysis, and condensation. Its structure was characterized by NMR, H RMS and single-crystal X-ray diffraction. The crystal of the target compound belongs to monoclinic system, space group $P2_1$ with a = 11.5058(15), b = 6.6626(10), c = 23.184(3) Å, V = 1777.3(4) Å^3, Z = 8, D_c = 1.496 Mg/m^3, F(000) = 792 and μ = 0.229 mm^{-1}. X-ray analysis indicated C–H···O intermolecular H-bonds in this crystal structure. The target compound exhibited 53% curative activity against TMV.

Keywords: 1,2,3-thiadiazole, cinnamic amide, synthesis, crystal structure, anti-TMV activity;
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1 INTRODUCTION

Cinnamic acids and their derivatives exhibited good fungicidal activity[1-3]. By optimization of cinnamic amide as a lead scaffold, three cinnamic acid fungicides dimethomorph[4], flumorph, and pyrimorph have been launched for oomycete disease control[5-7].

The application of heterocyclic scaffold as active substructure was an important measure for novel fungicide discovery[8]. 1,2,3-Thiadiazole is an important class of five-membered aromatic heterocyclic ring structures with both S and N atoms, showing broad-spectrum of biological activities such as activating plant systemic acquired resistance[9-11], fungicidal[12-15] and antiviral activities[16]. The typical corresponding products are BTH, tiadinil and methiadinil[17].

This study was aimed at developing novel cinnamic amide fungicides by employing the active scaffold of cinnamic amide and 1,2,3-thiadiazole as substructures to the target molecule. (*Z*)-4-[3-(4-Methyl-1,2,3-thiadiazol-5-yl)-3-(4-trifluoromethylphenyl)acryloyl]morpholine was synthesized according to the description in Scheme 1, and its crystal structure and anti-TMV activity were determined.

2 EXPERIMENTAL

2. 1 Instruments and reagents

Melting point of new compound was determined on an X-4 melting point apparatus and uncorrected. A High-resolution mass spectra (HRMS) was detected by using a 6520 Q-TOF LC/MS instrument. ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were measured on an AV400 spectrometer in chloroform-*d* ($CDCl_3$). Crystal structure was recorded on a Bruker SMART 1000CCD diffraction meter.

2. 2 Synthetic procedure for the target compound

General procedure for the synthesis of compound 1 4-Methyl-1,2,3-thiadiazole-5-carboxylic acid (5.0 g, 18.7 mmol) was added to the thionyl chloride (50.0 mL) and the

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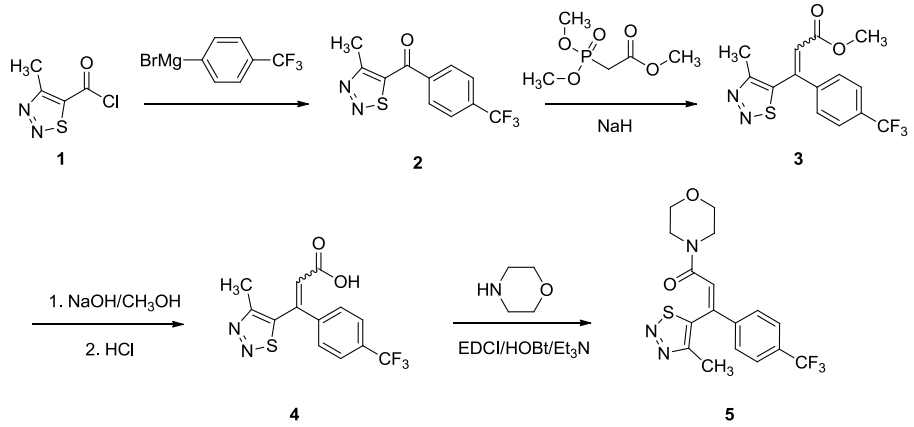
mixture was refluxed for 4 h. When the reaction was completed, compound **1** was obtained by the vacuum distillation in a yield of 85%.

General procedure for the synthesis of compound 2 A solution of 4-trifluoromethyl phenyl magnesium bromide in THF (1 mol/L, 2.5 mL) was added to a solution of compound **1** (2.5 mmol, 0.4 g) in THF at –30 °C under N_2, and then the reaction mixture was stirred for 1 h. When the reaction was completed, it was quenched with sat ammonium chloride (NH_4Cl) aq solution. The organic layer was extracted with ethyl acetate, washed with water and saturated brine, and then dried over anhydrous sodium sulfate. After filtration, the solvent in organic layer was evaporated, and the residue was purified on a silica gel column eluted with a mixture of ethyl acetate/petroleum ether (bp 60～90 ℃) (1:15, v/v) to obtain compound **2** in a yield of 80%.

General procedure for the synthesis of compound 3 Triethyl phosphonoacetate (1.26 mmol) was added to a suspension of NaH (60 wt% in mineral oil, 2.52 mmol) in THF (25 mL) under 0 °C. When the reaction mixture had no air bladder generation, the solution of compound **2** (0.63 mmol) in THF was added and stirred at room temperature for 15 min. When the reaction was completed, water was added. The organic layer was extracted with ethyl acetate, washed with water and saturated brine, and dried over anhydrous sodium sulfate. After filtration, the solvent in organic layer was evaporated, and the residue was then purified on a silica gel column eluted with a mixture of ethyl acetate/petroleum ether (bp 60～90 °C) (1:10, v/v) to obtain compound **3** in a yield of 40%.

General procedure for the synthesis of compound 4 NaOH (2.0 equiv, 2 mmol) was added to the solution of compound **3** (1 mmol) in methanol, and the reaction mixture was stirred and refluxed for 1 h. When the reaction was completed, the solvent was evaporated. The residue was diluted with water, and its pH was adjusted to 2～3 by using dilute hydrochloric acid (2 mol/L). The aqueous layer was extracted with ethyl acetate. The solvent in combined organic layer was evaporated to obtain compound **4** in a yield of 95%.

General procedure for the synthesis of compound 5 1-Ethyl-3-(3-(dimethylamino)-propyl) carbodiimide hydrochloride (0.34 g, 1.80 mmol) and 1-hydroxybenzotriazole (0.22 g, 1.54 mmol) was added slowly to the solution of compound **4** (1.50 mmol) in dry CH_2Cl_2 under 0 °C. The reaction mixture was stirred for 15 min, and then the solution of morpholine (0.07 g, 0.79 mmol) in CH_2Cl_2 was added and followed by Et_3N (0.18 g, 1.80 mmol), and the reaction mixture was stirred at room temperature for 5 h. After completion of the reaction, the organic layer was successively washed with saturated brine for three times, and dried over anhydrous sodium sulfate. After filtration, the solvent in organic layer was evaporated, and the residue was then purified on a silica gel column eluted with a mixture of ethyl acetate/petroleum ether (bp 60～90 ℃) (1:1, v/v) to obtain compound **5** in a yield of 42%. Data for compound **5**: white crystal; yield, 42%; m.p.: 127～129 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.67 (d, J = 7.8 Hz, 1H, Ar-H), 7.65 (d, J = 7.8 Hz, 1H, Ar-H), 7.37 (d, J = 8.2 Hz, 1H, Ar-H), 7.35 (d, J = 8.2 Hz, 1H, Ar-H), 6.87 (s, 1H, C=CH), 3.67～3.55 (m, 6H, morpholine-H), 3.52～3.49 (m, 2H, morpholine-H), 2.44 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 164.01 (s), 157.67 (s), 145.05 (s), 141.28 (s), 135.08 (s), 131.66 (q, J = 32.9 Hz), 127.45 (s), 127.14 (s), 126.07 (s), 122.29 (s), 66.58 (s), 66.54 (s), 46.61 (s), 41.80 (s), 13.16 (s). H RMS (m/z) calcd. for $C_{17}H_{16}F_3N_3O_2S$ $(M+H)^+$: 384.0915, found: 384.0983.



Scheme 1. Synthetic route of the target compound

2. 3 Single-crystal structure determination

The colorless transparent crystal of compound **5** (Fig. 1) with a size of 0.20 mm × 0.18 mm × 0.12 mm was selected for X-ray diffraction analysis. The SHELXS-97 program was applied for the structure analysis according to reported methods[18]. All measurements were made on a Bruker SMART 1000 CCD diffractometer with graphite-monochromatic Mo$K\alpha$ radiation (λ = 0.71073 Å) at 113(2) K. A total of 23177 integrated reflections in the range of 3.17⩽θ⩽27.60 ° (index ranges: –14⩽h⩽14, –8⩽k⩽8, –30⩽l⩽30) were collected with 7905 unique ones (R_{int} = 0.0272). All of the nonhydrogen atoms were located with successive difference fourier syntheses by full-matrix least-squares and the final refinement gave R = 0.0271, wR = 0.0751 ($w = 1/[\sigma^2(F_o^2) + (0.0489P)^2 + 0.0740P]$, where $P = (F_o^2 + 2F_c^2)/3$ and S = 1.084 by using the SHELXL program[19], and the hydrogen atoms were added from difference Fourier map and refined freely.

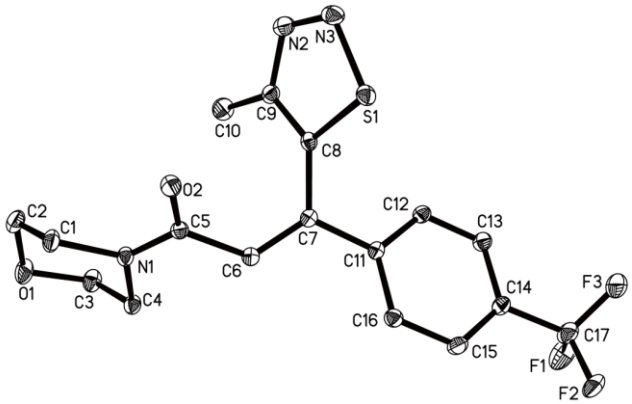


Fig. 1. Molecular structure for compound 5 by X-ray diffraction determination

2. 4 Anti-tobacco mosaic virus activity

The anti-tobacco mosaic virus (TMV) activity of compound **5** was evaluated at 100 μg/mL in the inactivation, curative, protection and induction modes according to the reported reference[20]. Dimethomorph and tiadinil were selected as the positive controls.

3 RESULTS AND DISCUSSION

3. 1 Synthesis of the title compound

The general procedure for the synthesis of compound **5** is shown in Scheme 1. Compound **1** acyl chloride could be obtained from the reaction between 4-methyl-1,2,3-thiadiazole-5-carboxylic acid and thionyl chloride. Compound **2** was prepared by the nucleophilic substitution reaction between compound **1** and 4-trifluoromethyl phenyl magnesium bromide, and then compound **2** reacted with trimethyl phosphonoacetate and NaH by the Horner-Emmons reaction to obtain compound **3**, which was hydrolyzed to give the corresponding acid **4**. Compound **5** was prepared in a moderate yield by a condensation reaction between the morpholine and compound **4**.

3. 2 Analysis of the crystallographic structure

The selected bond lengths, bond angles, and twist angles of compound **5** are shown in Tables 1 and 2 respectively. The molecular structure and crystal packing of compound **5** are shown in Figs. 1 and 2, respectively.

As shown in Table 1, bond lengths and bond angles within the thiadiazole ring agreed well with the values reported. The sum of C(5)–N(1)–C(4), C(5)–N(1)–C(1) and C(4)–N(1)–C(1) angles is 359.72 °, close to 360 °, indicating the sp^2 hybridization state of N(1) atom. The bond angles of C(16)–C(11)–C(12), C(16)–C(11)–C(7) and C(12)–C(11)–C(7) are 119.40(12) °, 120.90(12) ° and 119.69(11) ° in turn, showing the sp^3 hybridization state of C(11) atom. Besides, the torsion angles of C(6)–C(7)–C(8)–C(9) and C(6)–C(7)–C(8)–S(1) are –54.1 ° and –128.36 °, respectively, indicating that the carbon atom C(6) was not placed at the same plane with the thiadiazole ring. As shown in Table 2, the torsion angles of C(1)–N(1)–C(5)–O(2) and C(4)–N(1)–C(5)–O(2) are –5.1 and –178.60 °, respectively, so the oxygen atom O(2) was not placed at the same plane with the morpholine ring. The torsion angles of C(15)–C(14)–C(17)–F(2) and C(13)–C(14)–C(17)–F(2) are –21.8 and 158.45 °, respectively, and this results indicated that the fluorine atom F(2) was not placed at the same plane with the benzene ring. The torsion angles of C(3)–O(1)–C(2)–C(1) and N(1)–C(1)–C(2)–O(1) are –60.12(17) ° and 55.13(18) °, suggesting that the morpholine

ring is non-planar. The torsion angles of C(9)–N(2)–N(3)–S(1) and C(8)–S(1)–N(3)–N(2) are respectively –0.05(15) ° and –0.88(11) °, so the thiadiazole ring was similarly planar. The torsion angle of C(8)–C(7)–C(11)–C(12) is –32.53(18) °, implying the dihedral angle between the thiadiazole and benzene rings is 147.47 °. Besides, C–H···O intermolecular hydrogen bonds are found in the crystal structure of compound **5**, which stabilizes the solid state structure.

Table 1. Selected Bond Lengths (Å) and Bond Angles (°) for Compound 5

Bond	Dist.	Bond	Dist.	Bond	Dist.
O(2)–C(5)	1.2315(18)	N(1)–C(4)	1.4653(19)	S(1)–N(3)	1.6826(14)
O(1)–C(2)	1.419(2)	N(1)–C(1)	1.4616(18)	S(1)–C(8)	1.7001(15)
O(1)–C(3)	1.4229(19)	C(1)–C(2)	1.510(2)	N(2)–N(3)	1.304(2)
N(1)–C(5)	1.3524(18)	C(3)–C(4)	1.513(2)	N(2)–C(9)	1.364(2)
Angle	(°)	Angle	(°)	Angle	(°)
N(3)–S(1)–C(8)	93.28(7)	C(4)–N(1)–C(1)	113.44(12)	O(1)–C(2)–C(1)	111.75(13)
C(2)–O(1)–C(3)	110.33(12)	N(2)–N(3)–S(1)	110.75(11)	C(16)–C(11)–C(12)	119.40(12)
C(5)–N(1)–C(4)	125.65(12)	N(3)–N(2)–C(9)	114.66(12)	C(16)–C(11)–C(7)	120.90(12)
C(5)–N(1)–C(1)	120.63(12)	N(1)–C(1)–C(2)	109.96(12)	C(12)–C(11)–C(7)	119.69(11)

Table 2. Selected Torsional Angles (°) for the Target Compound 5

Angle	(°)	Angle	(°)
C(9)–N(2)–N(3)–S(1)	–0.05(15)	O(1)–C(3)–C(4)–N(1)	–55.43(17)
C(8)–S(1)–N(3)–N(2)	–0.88(11)	C(1)–N(1)–C(5)–O(2)	–5.1(2)
C(6)–C(7)–C(8)–C(9)	–54.1(2)	C(4)–N(1)–C(5)–O(2)	–178.60(13)
C(6)–C(7)–C(8)–S(1)	128.36(12)	C(15)–C(14)–C(17)–F(2)	–21.8(2)
C(3)–O(1)–C(2)–C(1)	–60.12(17)	C(13)–C(14)–C(17)–F(2)	158.45(15)
N(1)–C(1)–C(2)–O(1)	55.13(18)	O(2)–C(5)–C(6)–C(7)	–37.25(19)
C(2)–O(1)–C(3)–C(4)	60.41(18)	N(1)–C(5)–C(6)–C(7)	145.12(13)
C(8)–C(7)–C(11)–C(12)	–32.53(18)	C(5)–C(6)–C(7)–C(8)	–9.2(2)
C(1)–N(1)–C(4)–C(3)	51.71(16)	C(5)–C(6)–C(7)–C(11)	172.13(12)

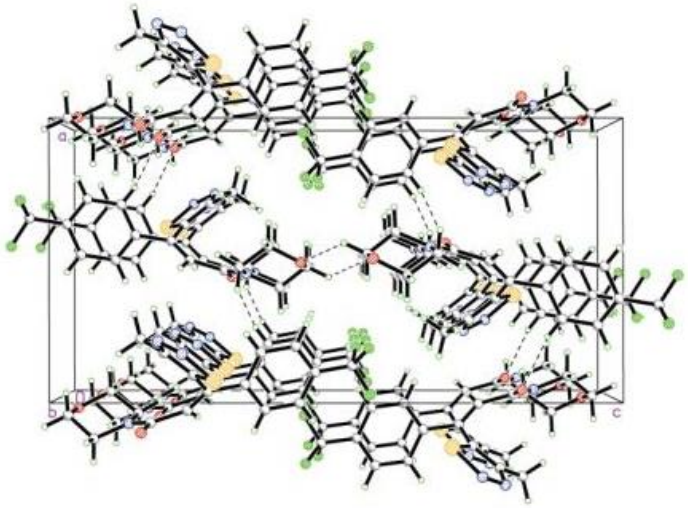


Fig. 2. Crystal packing of compound 5

3. 3 Anti-TMV activity of the target compound

The bioassay results are shown in Table 3. In the curative mode, compound **5** had an inhibition rate of 53% anti-TMV activity at 100 μg/mL, which was better than that of the controls dimethomorph and tiadinil. However, compound **5** showed no anti-TMV activity in the modes of inactivation, protection and induction. The above results indicated that compound **5** had excellent efficacy on TMV in the curative mode.

Table 3. In vivo anti-TMV Activity of Compound 5

Compound	Anti-TMV activity (% inhibition) at 100 μg/mL			
	Inactivation	Curative	Protection	Induction
Compound **5**	26 ±1	53 ±3	13 ±2	8 ±2
Dimethomorph	20 ±2	20 ±2	20 ±3	0
Tiadinil	30 ±1	25 ±2	30 ±1	65 ±3

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