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# Discovery of Novel Compounds for Combating Rising Severity of Plant Diseases Caused by Fungi and Viruses

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**ABSTRACT:** In recent years, the severity of plant diseases caused by plant pathogenic fungi and viruses has been on the rise. However, there is a limited availability of pesticide chemicals in the market for effectively controlling both fungal and viral infections. To solve this problem, a series of novel pyrimidine derivatives containing a 1,3,4-oxadiazole thioether fragment were synthesized. Among them, compound **6s** exhibited remarkable in vivo protection activity against tobacco mosaic virus, demonstrating the superior 50% effective concentration ($EC_{50}$) value of 0.42 $\mu$M, outperforming ningnanmycin (0.60 $\mu$M). Meanwhile, compound **6s** exhibited remarkable antifungal activity against *Botrytis cinerea* Pers. in postharvest blueberry in vitro, with an $EC_{50}$ value of 0.011 $\mu$M, surpassing the inhibition rate of Pyrimethanil (0.262 $\mu$M). Additionally, compound **6s** also demonstrated remarkable curative and protection activities against blueberry fruit gray mold in vivo, with control efficiencies of 54.2 and 60.4% at 200 $\mu$g/mL concentration, respectively, which were comparable to those of Pyrimethanil (49.3 and 63.9%, respectively). Scanning electron microscopy showed that the compound **6s**-treated hyphae of *B. cinerea* Pers. in postharvest blueberry became abnormally collapsed and shriveled. Furthermore, the molecular docking simulation demonstrated that compound **6s** formed hydrogen bonds with SER-17, ARG-43, and SER-39 of succinate dehydrogenase (SDH), providing a possible explanation for the mechanism of action between the target compounds and SDH. This study represents the first report on the antiviral and antifungal activities of novel pyrimidine derivatives containing a 1,3,4-oxadiazole thioether fragment.

## 1. INTRODUCTION

The future direction of pesticide development will be oriented toward highly potent, low residual, and eco-friendly pesticides in order to meet the demands of sustainable development in modern agriculture. Compounds featuring diverse heterocyclic groups hold significant importance in the field of drug development.[1] The majority of current pesticide patents revolve around heterocyclic compounds, particularly those containing nitrogen (N) atoms that are widely present in natural products and frequently utilized in pesticides and pharmaceuticals due to their unique biological and pharmaceutical activities.[2] Thus, the incorporation of nitrogen heterocyclic structures into organic compounds often introduces novel functional properties and garners considerable attention.[3−5]

Pyrimidine and its derivatives, a class of heterocyclic compounds containing two N atoms, which are widely distributed throughout various organisms and play a crucial role in nucleic acid structures.[6] Pyrimidine has found extensive applications in the fields of medicine and pesticides, with many commercial pesticides incorporating a pyrimidine moiety (Figure 1).[7−11] Numerous studies have reported that a plethora of pyrimidine derivatives exhibit diverse biological activities, including antitubercular, antibacterial, antiviral, antitumor, anti-inflammatory, insecticidal, antifungal, and herbicidal properties.[12−24] Therefore, it has been extensively utilized in the fields of medicine and pesticides. Particularly in recent years, a large number of literature reports have documented the antifungal and antiviral activities of pyrimidine and its derivatives, garnering significant attention from researchers.[25] Meanwhile, pyrimidine derivatives have shown promise as a potential starting point for the discovery of novel inhibitors targeting succinate dehydrogenase (SDH).[26−28]

The 1,3,4-oxadiazole skeleton, a class of heterocyclic compounds containing two N atoms, is widely utilized as a fundamental building block in natural products and pharmaceutical molecules due to its remarkable biological and pharmacological activities.[29−34] Therefore, the development of synthetic methodologies for 1,3,4-oxadiazole frameworks has consistently been a focal point of research in the chemical community. Notable commercially available pesticides containing a 1,3,4-oxadiazole moiety include oxadiazon and metoxadiazone. Furthermore, several studies from the literature have also documented the remarkable antiviral and fungicidal activities exhibited by derivatives of 1,3,4-oxadiazoles.[35] Meanwhile, thioether compounds are abundantly found in natural products and possess significant research value in the fields of pharmaceutical chemistry and pesticides.

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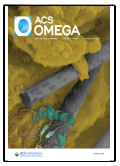


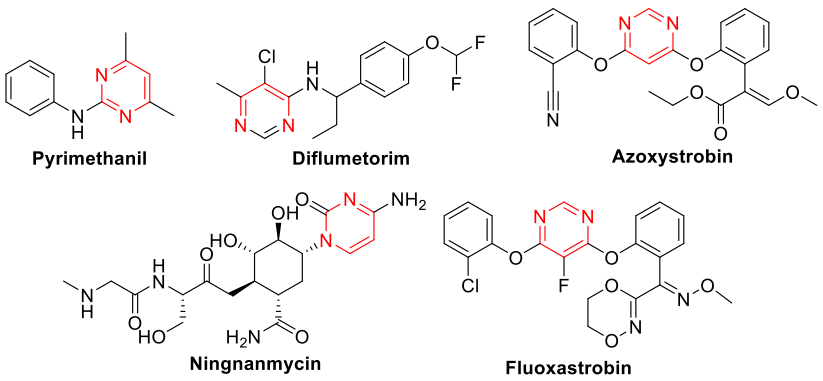
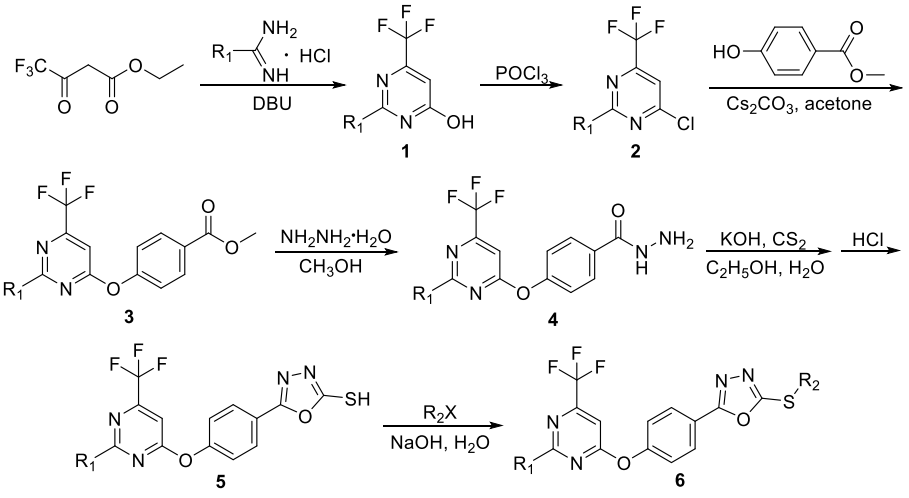



**Figure 1.** Structures of some commercial pesticides containing a pyrimidine moiety.

**Scheme 1. Synthetic Route of the Target Compounds 6a−6y**




**6a**: $R_1$ = H, $R_2$ = $CH_3$
**6b**: $R_1$ = H, $R_2$ = $CH_2CH_3$
**6c**: $R_1$ = H, $R_2$ = 2-$CH_3C_6H_4CH_2$
**6d**: $R_1$ = H, $R_2$ = 2-$FC_6H_4CH_2$
**6e**: $R_1$ = H, $R_2$ =4-$FC_6H_4CH_2$
**6f**: $R_1$ = H, $R_2$ = 2-$ClC_6H_4CH_2$
**6g**: $R_1$ = H, $R_2$ = 3-$ClC_6H_4CH_2$
**6h**: $R_1$ = H, $R_2$ = 4-$ClC_6H_4CH_2$
**6i**: $R_1$ = H, $R_2$ = 2-$CF_3C_6H_4CH_2$
**6j**: $R_1$ = H, $R_2$ = 2,4-di$ClC_6H_3CH_2$
**6k**: $R_1$ = H, $R_2$ = 3,4-di$ClC_6H_3CH_2$
**6l**: $R_1$ = $CH_3$, $R_2$ = $CH_3$
**6m**: $R_1$ = $CH_3$, $R_2$ = $CH_2CH_3$
**6n**: $R_1$ = $CH_3$, $R_2$ = 2-$CH_3C_6H_4CH_2$
**6o**: $R_1$ = $CH_3$, $R_2$ = 4-$CH_3C_6H_4CH_2$
**6p**: $R_1$ = $CH_3$, $R_2$ = 2-$FC_6H_4CH_2$
**6q**: $R_1$ = $CH_3$, $R_2$ = 4-$FC_6H_4CH_2$
**6r**: $R_1$ = $CH_3$, $R_2$ = 2-$ClC_6H_4CH_2$
**6s**: $R_1$ = $CH_3$, $R_2$ = 3-$ClC_6H_4CH_2$
**6t**: $R_1$ = $CH_3$, $R_2$ = 4-$ClC_6H_4CH_2$
**6u**: $R_1$ = $CH_3$, $R_2$ = 2-$CNC_6H_4CH_2$
**6v**: $R_1$ = $CH_3$, $R_2$ = 4-$CNC_6H_4CH_2$
**6w**: $R_1$ = $CH_3$, $R_2$ = 4-$CF_3C_6H_4CH_2$
**6x**: $R_1$ = $CH_3$, $R_2$ = 2,4-di$ClC_6H_3CH_2$
**6y**: $R_1$ = $CH_3$, $R_2$ = 3,4-di$ClC_6H_3CH_2$

These compounds exhibit diverse pesticidal activities including antiviral, antibacterial, antifungal, and herbicidal properties.[36−39] Additionally, research has demonstrated that the incorporation of an electron-donating S atom to simultaneously connect multiple heterocyclic active centers in the molecule, specifically through a thioether structure, is conducive to enhancing the receptor−ligand affinity and effectively improving the compound's biological activity.[40−42] Recently, there have been many researchers focusing on the 1,3,4-oxadiazole thioether derivatives with diverse biological activities.[43−51]

Motivated by the above findings and to find novel lead compounds with good biological activity, the aims of this study are to (1) introduce the active structure of 1,3,4-oxadiazole thioether group into the pyrimidine ring to build a series of novel pyrimidine derivatives containing a 1,3,4-oxadiazole thioether fragment; (2) investigate the in vivo antiviral activity against tobacco mosaic virus (TMV) using the half-leaf method; (3) investigate the in vitro antifungal activities against *Botryosphaeria dothidea* (Moug.) Ces. De Not. in postharvest kiwifruit, *Phomopsis* sp. in postharvest kiwifruit, *Botrytis cinerea* Pers. in postharvest blueberry, *B. cinerea* Pers. in postharvest cucumber, *Sclerotinia sclerotiorum* (Lib.) de Bary in oilseed rape, *B. cinerea* Pers. in postharvest tobacco, *B. cinerea* Pers. in postharvest strawberry, *Colletotrichum* sp. in postharvest blueberry, and *Magnaporthe grisea* (Hebert) Barr in rice; (4) investigate the in vivo activity against blueberry fruit gray mold; (5) molecular docking study of the target compounds to SDH.

## 2. MATERIALS AND METHODS

**2.1. Instruments and Chemicals.** All chemical reagents were purchased from Aladdin Reagent (Shanghai, China) and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Energy Chemical (Shanghai, China), respectively. The melting points (m.p.) of all the target compounds were determined on an X-4B microscope m.p. apparatus and were uncorrected (Shanghai electrophysics optical Instrument Co., LTD, China). All $^{1}H$ NMR and $^{13}C$ NMR spectra data were recorded on a Bruker NEO-600 spectrometer. High-resolution mass spectrometry (HRMS) data of the target compounds were obtained using a Thermo Scientific Q-Exactive (Thermo Fisher Scientific, MA, USA).

**2.2. Chemical Synthesis.** *2.2.1. General Procedure for the Synthesis of Intermediates **1**−**5**.* As shown in Scheme 1, to a 100 mL three-necked round-bottomed flask, ethyl trifluoroacetoacetate (50 mmol), formimidamide hydrochloride or acetamidine hydrochloride (80 mmol), and diazabicycloundecene (50 mmol) were added. After refluxing for 10 h, the solvent was evaporated under vacuum. Subsequently, the resulting mixture was treated with 50 mL of distilled water and extracted with ethyl acetate followed by evaporating excess solvent to obtain intermediate **1**.

Intermediate **1** (40 mmol) was introduced into a 100 mL three-necked round-bottomed flask, followed by the addition of $POCl_3$ (60 mmol), acetonitrile (40 mL), and *N*,*N*-diisopropylethylamine (30 mmol). After refluxing for 5 h, the excess solvent was removed under vacuum. Subsequently, the resulting mixture was treated with 50 mL of distilled water and adjusted to pH = 9 using 10% $NaHCO_3$ solution. The aqueous phase was extracted twice with dichloromethane before evaporating the excess solvent to obtain intermediate **2**.

Intermediate **2** (10 mmol), ethyl 4-hydroxybenzoate (12 mmol), $Cs_2CO_3$ (20 mmol), and acetone (50 mL) were added to a 100 mL three-necked round-bottomed flask. The reaction was carried out at room temperature for 2−4 h. After the reaction was completed, the excess solvent was vacuum evaporated and then recrystallization with ethanol to obtain intermediate **3**.

To a 100 mL three-necked round-bottomed flask, intermediate **3** (20 mmol), 80% hydrazine hydrate (60 mmol), and methanol (40 mL) were added and reacted under reflux conditions for 5−7 h. After cooling to room temperature, the white solid precipitated from the reaction solution was filtered and recrystallized from absolute ethanol to obtain intermediate **4**.

Carbon disulfide (36 mmol) was added dropwise to the mixture of intermediate **4** (30 mmol), KOH (45 mmol), and ethanol (500 mL) in a 1000 mL three-necked round-bottomed flask. After refluxing at 85 °C for 8 h, the white precipitate was filtered and neutralized with saturated $NaHCO_3$ solution. The filtrate was acidified by 5% HCl solution, and the obtained solid was filtered. The solid was washed with water for 3 times. The key intermediate **5** was obtained by recrystallization with ethanol.

*2.2.2. General Procedure for the Synthesis of the Target Compounds **6a**−**6y**.* As shown in Scheme 1, intermediate **5** (2 mmol), different substituted halogenated hydrocarbons or benzyl chlorides (2 mmol), and NaOH (2.2 mmol) dissolved in water (15 mL) were added to a 50 mL three-necked round-bottomed flask and stirred at room temperature for 8−10 h. After the reaction was completed, the residue was filtered and recrystallized from ethanol to obtain target compounds **6a**−**6y**.

**2.3. Anti-TMV Activity Test In Vitro.** The anti-TMV activity of the target compounds was determined according to the half-leaf method.[52,53] The inhibition rate I (%) was calculated according to the following formula, where C was the average local lesion number of the CK group and *T* was the average local lesion number of the treatment group.

$$I(\%) = \frac{C - T}{C} \times 100\%$$

*2.3.1. Curative Activity Test of the Target Compounds against TMV In Vivo.* The leaves of *Nicotiana tabacum* L. of the same age were inoculated with TMV (at a concentration of $6 \times 10^{-3}$ mg/mL) through immersion and gentle brushing, after being pretreated with silicon carbide abrasion. Following inoculation, the leaves were rinsed with water for 0.5 h. A compound solution was applied to the left side of the leaves, while a solvent was applied to the right side as a control. The number of localized lesions was counted and recorded 3−4 days postinoculation. Three replications were conducted for each compound.

*2.3.2. Protection Activity Test of the Target Compounds against TMV In Vivo.* Each target compound solution was applied to the left side of growing *N. tabacum* L. leaves of the same age, while the solvent was applied to the right side as a control. The leaves, previously dusted with silicon carbide, were inoculated with TMV using a brush dipped in a concentration of $6 \times 10^{-3}$ mg/mL after 12 h and subsequently washed with water and gently rubbed along the veins once or twice. The number of local lesions that appeared 3−4 days after inoculation was recorded. Three replications were conducted for each compound.

*2.3.3. Inactivation Activity Test of the Target Compounds against TMV In Vivo.* The virus was effectively inhibited when it was mixed with an equimolar target compound solution for 30 min. As a control, the solvent and virus mixture were inoculated on the right side of *N. tabacum* L. leaves, which had been previously treated with silicon carbide. The number of local lesions was recorded 3−4 days after inoculation. Three replications were conducted for each compound.

**2.4. Antifungal Activity Test In Vitro.** The in vitro antifungal activities of the target compound **6a**−**6y** against nine pathogenic fungi (*B. dothidea* (Moug.) Ces. De Not. in postharvest kiwifruit, *Phomopsis* sp. in postharvest kiwifruit, *B. cinerea* Pers. in postharvest blueberry, *B. cinerea* Pers. in postharvest cucumber, *S. sclerotiorum* (Lib.) de Bary in oilseed rape, *B. cinerea* Pers. in postharvest tobacco, *B. cinerea* Pers. in postharvest strawberry, *Colletotrichum* sp. in postharvest blueberry, and *M. grisea* (Hebert) Barr in rice) were determined by the mycelial growth rate method.[46] The target compound (5 mg) was dissolved in 1 mL of dimethyl sulfoxide (DMSO), followed by the addition of 9 mL of sterile water. The mixture was then combined with 90 mL of potato dextrose agar (PDA) medium and thoroughly shaken to form a solution with a concentration of 50 μg/mL. Subsequently, the mixed PDA medium was poured into 9 Petri dishes, with each treatment being repeated three times. After that, mycelia dishes measuring approximately 0.5 cm in diameter were taken from the middle of the PDA plates using a sterile inoculation needle. The inoculated PDA plates were incubated at 28 °C for a period of 3−4 days until the mycelium growth on the CK group reached a diameter of 5−6 cm. The inhibition rate *I* (%) was calculated using formula (2), where *C* (cm) and *T* (cm) represented the fungal diameters on untreated and treated PDA plates, respectively.

$$I(\%) = \frac{C - T}{C - 0.5} \times 100\%$$



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**2.5. Antifungal Activity Test In Vivo.** The in vivo curative and protection activities of compound **6s** against blueberry fruit gray mold were determined according to the reported method.[54,55] The control efficacy of compound **6s** against blueberry fruit gray mold was calculated by the following formula (3), where $A_0$ (cm) and $A_1$ (cm) were the lengths of untreated and treated lesions, respectively.

$$I(\%) = \frac{A_0 - A_1}{A_0} \times 100\% \quad (3)$$

*2.5.1. In Vivo Curative Effect of Compound **6s** against Blueberry Fruit Gray Mold.* The fresh blueberry fruits were washed and then sterilized by evenly applying 75% ethanol. Subsequently, the surface of the fruits was punctured with a sterile toothpick, and mycelia dishes of *B. cinerea* Pers. in postharvest blueberry measuring approximately 0.5 cm in diameter were inoculated at the puncture site. After 24 h, prepared solutions of compound **6s** with concentrations of 100, 50, and 25 μg/mL were uniformly applied to the fresh blueberry fruits. The treated fruits were cultured in a light incubator (temperature: 25 °C; relative humidity: 100%) for a period of 3−4 days, during which the length of lesions on the blueberry fruits was measured. Each treatment group consisted of three replicates with nine fresh blueberry fruits per replicate, while Pyrimethanil served as the control agent.

*2.5.2. In Vivo Protection Activity of Compound **6s** against Blueberry Fruit Gray Mold.* The blueberry fruits were washed and then treated with 75% ethanol applied evenly. Subsequently, the blueberry fruits were disinfected by applying a solution containing compound **6s** at concentrations of 100, 50, and 25 μg/mL. Afterward, the solution was uniformly distributed onto the surface of the blueberry fruits. Following a period of 24 h, sterile toothpicks were used to puncture the fruit's surface, and mycelia dishes (approximately 0.5 cm in diameter) of *B. cinerea* Pers. in postharvest blueberry were inoculated at each puncture site. The blueberries were then cultured in a light incubator under conditions of temperature (25 °C) and relative humidity (100%) for a duration of 3−4 days, during which the length of lesions on the blueberry fruits was measured. Each treatment group consisted of three replicates with nine blueberry fruits per replicate; Pyrimethanil served as the control agent.

**2.6. SEM Observations.** The morphological changes of *B. cinerea* Pers. in postharvest blueberries treated by compound **6s** were studied by scanning electron microscopy (SEM) according to the reported method.[56] A mycelia dish (approximately 0.5 cm in diameter) of *B. cinerea* Pers. in postharvest blueberry was inoculated on the PDA medium containing compound **6s** at 25 μg/mL concentration. When the diameter of the fungus cake is 1−2 cm, the in situ fixation method is used to cut the culture with the mycelium site into a square with a size of less than 1 cm and a thickness of 0.2 cm, and directly put it into 2.5% glutaraldehyde to fix Bbo cells at 4 °C. First, the mycelium is picked out, dehydrated with different concentrations of ethanol gradient, and transited with isoamyl acetate. Finally, the mycelium sample is placed in the critical point dryer for drying, and then the dried mycelium sample adheres to the platform, and the gold coating is sprayed by an ion sputtering instrument. The morphological changes of *B. cinerea* Pers. in postharvest blueberries treated by compound **6s** were observed under the scanning electron microscope.

**2.7. Molecular Docking.** The enzyme SDH, which plays a crucial role in connecting the respiratory electron transport chain and tricarboxylic acid cycle, has been identified as an optimal target for the development of potent fungicides.[26−28] The three-dimensional structure of compound **6s** was generated using ChemDraw Ultra 20.0 software (PerkinElmer, Waltham, MA, USA), while the protein SDH receptor structure (PDB: 2FBW) was obtained from the RCBs PDB database (https://www.rcsb.org/structure/2FBW). A molecular docking study was conducted to investigate the binding mode of compound **6s** with SDH using Discovery Studio 2.5 software (Accelrys Inc., San Diego, USA) following a previously reported method.[57,58]

## 3. RESULTS AND DISCUSSION

**3.1. Chemistry.** Using ethyl trifluoroacetate as the starting material, the target compounds **6a−6y** were synthesized with yields of 42.5−84.6%. All the structures of the target compound were confirmed by $^1$H NMR, $^{13}$C NMR, and HRMS. The physical characteristics, $^1$H NMR, $^{13}$C NMR, and HRMS data of the target compounds **6a−6y** are shown below, while the spectra of $^1$H NMR, $^{13}$C NMR, and HRMS for the target compounds **6a−6y** are shown in Supporting Information. In the $^1$H NMR spectra of compound **6s**, the presence of a hydrogen (H) atom in the pyrimidine structure was confirmed by a singlet at 7.60 ppm. Meanwhile, in the $^{13}$C NMR spectra of compound **6s**, the presence of a carbon (C) atom in the $CF_3$ group was revealed by a quartet at 121.80 ppm. Furthermore, accurate assignment of the molecular

**Table 1. In Vivo Antiviral Activity of the Target Compounds against TMV at 500 μg/mL**

| | inhibition rate (%)[a] | | |
|---|---|---|---|
| compounds | curative activity | protection activity | inactivation activity |
| **6a** | 38.6 ± 1.9 | 35.4 ± 2.1 | 34.7 ± 2.6 |
| **6b** | 43.4 ± 3.0 | 38.9 ± 1.7 | 42.3 ± 3.2 |
| **6c** | 27.3 ± 2.2 | 51.4 ± 0.8 | 58.6 ± 2.1 |
| **6d** | 58.2 ± 1.5 | 47.5 ± 1.5 | 50.2 ± 2.0 |
| **6e** | 34.9 ± 3.1 | 56.5 ± 1.3 | 62.9 ± 3.2 |
| **6f** | 58.8 ± 2.8 | 47.5 ± 3.3 | 69.8 ± 2.8 |
| **6g** | 45.3 ± 2.3 | 54.4 ± 2.6 | 70.2 ± 3.3 |
| **6h** | 40.5 ± 3.1 | 58.8 ± 1.3 | 78.0 ± 1.9 |
| **6i** | 50.6 ± 1.5 | 43.5 ± 2.0 | 52.5 ± 2.5 |
| **6j** | 49.5 ± 3.2 | 46.8 ± 2.4 | 64.4 ± 1.5 |
| **6k** | 48.3 ± 2.5 | 50.0 ± 2.2 | 60.3 ± 2.1 |
| **6L** | 46.8 ± 2.2 | 39.3 ± 3.1 | 38.6 ± 1.8 |
| **6m** | 50.6 ± 1.0 | 46.2 ± 1.4 | 47.2 ± 3.3 |
| **6n** | 36.3 ± 2.2 | 55.5 ± 1.2 | 62.3 ± 2.7 |
| **6o** | 29.3 ± 1.7 | 49.4 ± 1.3 | 54.9 ± 1.6 |
| **6p** | 47.3 ± 1.5 | 64.2 ± 1.2 | 72.1 ± 2.0 |
| **6q** | 43.1 ± 3.6 | 59.5 ± 1.3 | 70.2 ± 1.2 |
| **6r** | 61.3 ± 2.6 | 55.7 ± 1.3 | 73.2 ± 1.5 |
| **6s** | 55.3 ± 1.0 | 68.7 ± 2.6 | 76.9 ± 1.2 |
| **6t** | 43.2 ± 0.6 | 61.3 ± 1.3 | 80.0 ± 1.1 |
| **6u** | 45.8 ± 1.9 | 39.5 ± 1.8 | 47.3 ± 2.9 |
| **6v** | 39.4 ± 1.4 | 22.0 ± 2.7 | 43.2 ± 2.8 |
| **6w** | 49.3 ± 1.1 | 43.8 ± 0.9 | 49.6 ± 1.2 |
| **6x** | 54.5 ± 2.2 | 49.9 ± 1.4 | 68.7 ± 1.2 |
| **6y** | 51.9 ± 3.1 | 54.2 ± 1.2 | 64.3 ± 2.5 |
| Ningnanmycin | 56.4 ± 1.5 | 66.4 ± 2.0 | 94.0 ± 1.5 |

[a]The mean value derived from three repeated measurements.



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Table 2. $EC_{50}$ Values of Some of the Target Compounds against TMV in vivo

| | $EC_{50}$ ($\mu$M)[a] | |
|---|---|---|
| compounds | curative activity | protection activity |
| **6d** | 0.66 ± 0.005 | |
| **6f** | 0.59 ± 0.004 | |
| **6p** | | 0.62 ± 0.006 |
| **6r** | 0.48 ± 0.004 | |
| **6s** | 0.70 ± 0.006 | 0.42 ± 0.006 |
| **6t** | | 0.66 ± 0.007 |
| **6x** | 0.72 ± 0.005 | |
| ningnanmycin | 0.69 ± 0.007 | 0.60 ± 0.006 |

[a]The mean value derived from three repeated measurements.

weight for compound **6s** was achieved through HRMS data, which showed $[M + Na]^+$ ions with an $m/z$ value of 501.03616.

*3.1.1. 2-(Methylthio)-5-(4-((6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6a**).* Yellow solid; yield 50.8%; mp 123.2−124.7 °C; $^1$H NMR (DMSO-$d_6$, 600 MHz, ppm): $\delta$ 8.99 (s, 1H, pyrimidine-H), 8.09 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.88 (s, 1H, pyrimidine-H), 7.86 (s, 1H, Ph-H), 7.53 (d, 2H, $J$ = 9.00 Hz, Ph-H), 2.79 (s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$, 150 MHz, ppm): $\delta$ 170.25, 165.29, 164.95, 159.72, 156.24 (q, $J$ = 36.60 Hz), 154.70, 128.75, 123.34, 121.78 (q, $J$ = 273.45 Hz), 121.49, 107.09, 14.79; HRMS(ESI) calcd for $C_{14}H_9F_3N_4O_2S$: $[M + Na]^+$, 377.02832; found, 377.02905.

*3.1.2. 2-(Ethylthio)-5-(4-((6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6b**).* Yellow solid; yield 66.8%; mp 58.8−60.1 °C; $^1$H NMR (DMSO-$d_6$, 600 MHz, ppm): $\delta$ 8.99 (s, 1H, pyrimidine-H), 8.09 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.85 (s, 1H, pyrimidine-H), 7.53 (d, 2H, $J$ = 9.00 Hz, Ph-H), 3.35 (t, 2H, $J$ = 7.20 Hz, $CH_2$), 1.45 (q, 3H, $J$ = 7.80 Hz, $CH_3$); $^{13}$C NMR (DMSO-$d_6$, 150 MHz, ppm): $\delta$ 170.24, 165.94, 164.40, 159.72, 156.25 (q, $J$ = 35.25 Hz), 154.70, 128.76, 123.33, 121.77 (q, $J$ = 273.30 Hz), 121.49, 115.66, 107.07, 27.16, 15.32; HRMS (ESI) calcd for $C_{15}H_{11}F_3N_4O_2S$: $[M + Na]^+$, 391.04382; found, 391.04470.

*3.1.3. 2-((2-Methylbenzyl)thio)-5-(4-((6-(trifluoromethyl)-pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6c**).* Yellow solid; yield 75.8%; mp 76.8−77.6 °C; $^1$H NMR (DMSO-$d_6$, 600 MHz, ppm): $\delta$ 9.00 (s, 1H, pyrimidine-H), 8.10 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.88 (s, 1H, pyrimidine-H), 7.61 (s, 1H, Ph-H), 7.55 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.42 (d, 1H, $J$ = 7.80 Hz, Ph-H), 7.25−7.21 (m, 2H, Ph-H), 7.18−7.15 (m, 1H, Ph-H), 4.62 (s, 2H, $CH_2$), 2.42 (s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$, 150 MHz, ppm): $\delta$ 170.25, 165.20, 163.72, 159.73, 156.25 (q, $J$ = 35.10 Hz), 154.81, 137.34, 134.24, 130.98, 130.52, 128.84, 128.73, 126.60, 123.38, 121.80 (q, $J$ = 273.45 Hz), 121.40, 116.1, 107.12, 35.03, 19.22; HRMS (ESI) calcd for $C_{21}H_{15}F_3N_4O_2S$: $[M + Na]^+$, 467.07529; found, 467.07600.

*3.1.4. (2-((2-Fluorobenzyl)thio)-5-(4-((6-(trifluoromethyl)-pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6d**).* Brown solid; yield 49.8%; mp 91.7−92.9 °C; $^1$H NMR (DMSO-$d_6$, 600 MHz, ppm): $\delta$ 8.98 (s, 1H, pyrimidine-H), 8.03−8.01 (m, 2H, Ph-H), 7.86 (s, 1H, pyrimidine-H), 7.58−7.56 (m, 1H, phenyl-H), 7.50−7.47 (m, 2H, Ph-H), 7.39−7.35 (m, 1H, Ph-H), 7.25−7.22 (m, 1H, Ph-H), 7.20−7.18 (m, 1H, Ph-H), 4.65 (s, 2H, $-CH_2-$); $^{13}$C NMR (DMSO-$d_6$, 150 MHz, ppm): $\delta$ 170.34, 167.98, 164.83, 161.79 (q, $J$ = 245.24 Hz), 159.75, 156.24 (q, $J$ = 34.83 Hz), 154.34, 132.03 (d, $J$ = 3.50 Hz), 130.69 (d, $J$ = 8.43 Hz), 129.78, 127.62, 125.11 (d, $J$ = 3.45 Hz), 123.90 (d, $J$ = 14.66 Hz), 121.82 (q, $J$ = 273.60 Hz), 116.09 (d, $J$ = 20.88 Hz), 107.07, 31.85 (d, $J$ = 2.30 Hz); HRMS (ESI) calcd for $C_{20}H_{12}F_4N_4O_2S$: $[M + Na]^+$, 471.05014; found, 471.05093.

*3.1.5. 2-((4-Fluorobenzyl)thio)-5-(4-((6-(trifluoromethyl)-pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6e**).* Yellow solid; yield 80.5%; mp 105.2−107.0 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 9.00 (s, 1H, pyrimidine-H), 8.09 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.86 (s, 1H, pyrimidine-H), 7.56−7.53 (m, 4H, Ph-H), 7.20 (t, 1H, $J$ = 9.00 Hz, Ph-H), 4.60 (s, 2H, $CH_2$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.24, 165.20, 163.82, 162.93, 161.31, 159.72, 156.31 (q, $J$ = 35.40 Hz), 154.82, 1133.42, 133.41, 131.64, 131.59, 128.82, 123.34, 121.79 (q, $J$ = 273.30 Hz), 121.40, 115.93, 107.09, 35.63; HRMS (ESI) calcd for $C_{20}H_{12}F_4N_4O_2S$: $[M + Na]^+$, 471.05048; found, 471.05093.

*3.1.6. 2-((2-Chlorobenzyl)thio)-5-(4-((6-(trifluoromethyl)-pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6f**).* Yellow solid; yield 82.2%; mp 93.1−94.9 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 9.00 (s, 1H, pyrimidine-H), 8.09 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.88 (s, 1H, pyrimidine-H), 7.65 (dd, 1H, $J1$ = 2.40 Hz, $J2$ = 4.80 Hz, Ph-H), 7.55 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.53 (dd, 1H, $J1$ = 1.80 Hz, $J2$ = 6.00 Hz, Ph-H), 7.48 (m, 2H, Ph-H), 4.67 (s, 2H, $CH_2$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.25, 165.40, 163.38, 159.73, 156.25 (q, $J$ = 34.95 Hz), 154.85, 134.37, 133.80, 132.10, 130.49, 130.12, 128.87, 127.98, 123.40, 121.80 (q, $J$ = 273.45 Hz), 121.37, 107.14, 34.77; HRMS (ESI) calcd for $C_{20}H_{12}ClF_3N_4O_2S$: $[M + Na]^+$, 487.02057; found, 487.02138.

*3.1.7. 2-((3-Chlorobenzyl)thio)-5-(4-((6-(trifluoromethyl)-pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6g**).* White solid; yield 65.5%; mp 105.6−106.5 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 9.00 (s, 1H, pyrimidine-H), 8.09 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.88 (s, 1H, pyrimidine-H), 7.61 (s, 1H, Ph-H), 7.54 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.20 (d, 1H, $J$ = 7.20 Hz, Ph-H), 7.48 (m, 2H, Ph-H), 4.60 (s, 2H, $CH_2$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.25, 165.25, 163.72, 159.73, 156.45 (q, $J$ = 35.40 Hz), 154.81, 139.93, 133.46, 130.90, 129.42, 128.83, 128.25, 128.18, 123.37, 121.79 (q, $J$ = 272.85 Hz), 121.36, 107.12, 35.57; HRMS (ESI) calcd for $C_{20}H_{12}ClF_3N_4O_2S$: $[M + Na]^+$, 487.02130; found, 487.02138.

*3.1.8. 2-((4-Chlorobenzyl)thio)-5-(4-((6-(trifluoromethyl)-pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6h**).* White solid; yield 62.4%; mp 83.0−84.5 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 9.00 (s, 1H, pyrimidine-H), 8.09 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.88 (s, 1H, pyrimidine-H), 7.54−7.52 (m, 4H, Ph-H), 7.43 (d, 2H, $J$ = 8.40 Hz, Ph-H), 4.60 (s, 2H, $CH_2$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.25, 165.20, 163.78, 159.73, 156.45 (q, $J$ = 35.40 Hz), 154.82, 136.40, 131.43, 129.03, 128.84, 123.36, 121.80 (q, $J$ = 273.15 Hz), 121.36, 107.14, 35.55; HRMS (ESI) calcd for $C_{20}H_{12}ClF_3N_4O_2S$: $[M + Na]^+$, 487.02029; found, 487.02138.

*3.1.9. 2-((2-(Trifluoromethyl)benzyl)thio)-5-(4-((6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6i**).* White solid; yield 43.5%; mp 112.2−113.4 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 9.00 (s, 1H, pyrimidine-H), 8.08 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.78 (s, 1H, pyrimidine-H), 7.81 (d, 1H, $J$ = 7.80 Hz, Ph-H), 7.79 (d, 1H, $J$ = 7.80 Hz, Ph-H), 7.79 (t, 1H, $J$ = 7.20 Hz, Ph-H), 7.58 (d, 1H, $J$ = 7.80 Hz, Ph-H), 7.54 (d, 2H, $J$ = 9.00 Hz, Ph-H), 4.75 (s, 2H, $CH_2$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.24, 165.45, 163.23, 159.73, 156.45 (q, $J$ = 34.80 Hz), 154.87, 134.79, 132.58, 129.25, 128.86, 127.74 (q, $J$ = 29.70 Hz),


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**Table 3. In Vitro Antifungal Activity of the Target Compounds 6a−6y at 50 μg/mL**

| | inhibition rate (%)[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| compounds | *Botryosphaeria dothidea*(Moug.) Ces. De Not. in postharvest kiwifruit | *Phomopsis* sp. in postharvest kiwifruit | *Botrytis cinerea* Pers. in postharvest blueberry | *Botrytis cinerea* Pers. in postharvest cucumber | *Sclerotinia sclerotiorum* (Lib.) de Bary in oilseed rape | *Botrytis cinerea* Pers. in postharvest tobacco | *Botrytis cinerea* Pers. in postharvest strawberry | *Colletotrichum* sp. in postharvest blueberry | *Magnaporthe grisea* (Hebert) Barr in rice |
| **6a** | 43.78 ± 3.16 | 54.34 ± 1.88 | 56.14 ± 2.26 | 40.94 ± 1.26 | 68.31 ± 3.88 | 39.81 ± 1.55 | 68.67 ± 2.97 | 41.77 ± 3.25 | 14.75 ± 1.32 |
| **6b** | 54.35 ± 1.56 | 59.61 ± 2.67 | 50.04 ± 2.32 | 58.01 ± 1.32 | 53.25 ± 0.97 | 66.17 ± 1.99 | 82.12 ± 2.88 | 46.84 ± 1.38 | 52.46 ± 2.31 |
| **6c** | 67.41 ± 1.63 | 73.53 ± 2.10 | 100.00 | 40.03 ± 1.64 | 63.12 ± 1.75 | 41.68 ± 1.51 | 55.38 ± 1.34 | 40.33 ± 1.20 | 46.99 ± 2.00 |
| **6d** | 65.98 ± 3.12 | 79.41 ± 3.08 | 49.48 ± 2.18 | 46.53 ± 1.14 | 59.22 ± 1.56 | 43.93 ± 1.71 | 50.95 ± 4.20 | 34.36 ± 1.85 | 25.32 ± 1.37 |
| **6e** | 45.63 ± 2.87 | 50.46 ± 2.31 | 56.23 ± 2.11 | 46.07 ± 1.28 | 31.04 ± 0.93 | 51.78 ± 3.79 | 52.69 ± 1.43 | 13.20 ± 1.05 | 8.20 ± 1.06 |
| **6f** | 59.97 ± 1.65 | 69.97 ± 2.62 | 79.35 ± 2.12 | 35.35 ± 3.30 | 74.16 ± 1.85 | 38.88 ± 2.02 | 100.00 | 75.05 ± 2.53 | 18.76 ± 1.15 |
| **6g** | 70.41 ± 2.33 | 80.03 ± 2.99 | 100.00 | 36.56 ± 2.28 | 23.38 ± 1.35 | 34.77 ± 2.90 | 31.96 ± 1.47 | 20.25 ± 1.35 | 26.96 ± 2.37 |
| **6h** | 71.84 ± 2.37 | 50.00 ± 3.04 | 81.18 ± 2.06 | 36.40 ± 1.21 | 74.68 ± 2.94 | 41.31 ± 1.81 | 65.98 ± 1.57 | 72.15 ± 3.46 | 20.58 ± 1.23 |
| **6i** | 71.68 ± 2.44 | 74.92 ± 2.37 | 44.71 ± 2.99 | 41.84 ± 2.41 | 65.06 ± 2.25 | 42.99 ± 4.82 | 70.25 ± 2.13 | 37.43 ± 1.85 | 30.42 ± 1.21 |
| **6j** | 64.56 ± 1.99 | 57.74 ± 3.83 | 60.61 ± 1.42 | 44.56 ± 2.55 | 75.19 ± 1.90 | 41.12 ± 1.79 | 91.30 ± 2.87 | 19.89 ± 1.24 | 33.33 ± 2.22 |
| **6k** | 74.37 ± 2.01 | 70.28 ± 2.26 | 76.19 ± 2.20 | 44.71 ± 2.97 | 64.16 ± 3.79 | 42.43 ± 1.87 | 43.35 ± 1.75 | 32.55 ± 1.62 | 17.85 ± 3.13 |
| **6L** | 40.13 ± 2.62 | 61.01 ± 1.33 | 66.33 ± 2.27 | 47.13 ± 1.62 | 60.91 ± 2.33 | 39.44 ± 1.27 | 56.49 ± 1.56 | 43.58 ± 3.41 | 31.88 ± 2.00 |
| **6m** | 43.74 ± 2.17 | 67.29 ± 182 | 67.67 ± 3.26 | 48.34 ± 1.17 | 65.19 ± 2.82 | 40.37 ± 1.26 | 77.53 ± 2.24 | 50.81 ± 2.42 | 29.51 ± 1.96 |
| **6n** | 72.63 ± 2.30 | 47.99 ± 3.88 | 59.48 ± 3.41 | 36.25 ± 1.74 | 62.34 ± 1.39 | 37.57 ± 1.69 | 62.34 ± 1.58 | 28.57 ± 1.32 | 10.02 ± 1.46 |
| **6o** | 64.87 ± 2.30 | 70.90 ± 2.13 | 64.35 ± 1.12 | 37.46 ± 1.30 | 67.53 ± 1.87 | 37.20 ± 1.73 | 43.20 ± 1.66 | 34.54 ± 2.01 | 15.85 ± 1.51 |
| **6p** | 68.04 ± 3.18 | 65.48 ± 3.56 | 66.94 ± 3.30 | 48.04 ± 1.88 | 60.78 ± 3.69 | 37.57 ± 1.91 | 72.47 ± 2.32 | 51.72 ± 2.73 | 44.99 ± 1.71 |
| **6q** | 68.83 ± 3.10 | 53.41 ± 1.78 | 68.71 ± 2.98 | 39.43 ± 1.90 | 74.42 ± 4.08 | 37.20 ± 2.72 | 61.87 ± 1.56 | 48.28 ± 1.69 | 29.87 ± 1.27 |
| **6r** | 71.99 ± 2.80 | 54.95 ± 3.43 | 76.25 ± 3.93 | 45.17 ± 1.23 | 44.29 ± 1.06 | 45.61 ± 1.48 | 62.34 ± 1.42 | 45.03 ± 1.77 | 32.06 ± 2.09 |
| **6s** | 65.98 ± 2.03 | 94.12 ± 3.02 | 100.00 | 41.54 ± 1.67 | 54.68 ± 3.07 | 45.23 ± 1.78 | 57.91 ± 3.47 | 28.03 ± 2.60 | 30.05 ± 1.56 |
| **6t** | 64.08 ± 2.39 | 77.09 ± 2.73 | 47.90 ± 1.10 | 43.20 ± 1.37 | 62.86 ± 1.40 | 44.30 ± 1.88 | 56.17 ± 1.67 | 41.23 ± 2.11 | 41.89 ± 1.42 |
| **6u** | 68.35 ± 2.45 | 81.58 ± 3.57 | 55.35 ± 3.12 | 39.27 ± 1.44 | 59.48 ± 1.55 | 42.43 ± 1.77 | 71.99 ± 3.34 | 44.67 ± 1.64 | 31.15 ± 1.19 |
| **6v** | 74.34 ± 2.52 | 77.71 ± 3.40 | 51.30 ± 3.96 | 48.04 ± 1.41 | 38.96 ± 5.26 | 45.05 ± 1.35 | 63.77 ± 2.77 | 45.39 ± 1.39 | 31.33 ± 2.28 |
| **6w** | 58.23 ± 2.06 | 69.97 ± 1.78 | 68.84 ± 3.60 | 46.53 ± 1.45 | 64.94 ± 1.68 | 41.68 ± 1.42 | 53.48 ± 1.42 | 34.54 ± 1.43 | 17.67 ± 1.72 |
| **6x** | 65.03 ± 2.52 | 94.43 ± 3.40 | 76.87 ± 2.96 | 43.81 ± 1.71 | 50.78 ± 2.31 | 42.80 ± 3.02 | 56.01 ± 2.52 | 37.43 ± 1.60 | 18.40 ± 2.00 |
| **6y** | 77.85 ± 3.30 | 68.89 ± 3.25 | 67.62 ± 2.46 | 44.71 ± 1.22 | 60.39 ± 2.05 | 43.74 ± 2.04 | 58.54 ± 2.09 | 41.23 ± 2.58 | 44.26 ± 3.10 |
| pyrimethanil | 87.16 ± 1.54 | 86.56 ± 1.76 | 81.99 ± 2.34 | 81.49 ± 1.34 | 68.39 ± 2.13 | 70.11 ± 3.76 | 84.88 ± 1.63 | 65.12 ± 1.54 | 55.41 ± 1.65 |

[a]The mean value derived from three repeated measurements.



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**Table 4. $EC_{50}$ Values of Some of the Target Compounds against the Test Fungi**

| | $EC_{50}$ ($\mu$M)[a] | | |
|---|---|---|---|
| compounds | *Botrytis cinerea* Pers. in postharvest blueberry | *Phomopsis* sp. in postharvest kiwifruit | *Botrytis cinerea* Pers. in postharvest strawberry |
| **6c** | 0.018 ± 0.003 | | |
| **6f** | | | 0.183 ± 0.003 |
| **6j** | | | 0.032 ± 0.006 |
| **6g** | 0.031 ± 0.004 | | |
| **6s** | 0.011 ± 0.003 | 0.030 ± 0.003 | |
| **6x** | | 0.038 ± 0.007 | |
| Pyrimethanil | 0.262 ± 0.012 | 0.176 ± 0.010 | 0.193 ± 0.011 |

[a]The mean value derived from three repeated measurements.

126.83, 125.83 (q, $J$ = 272.85 Hz), 121.78 (q, $J$ = 273.00 Hz), 121.33, 107.13, 33.62; HRMS (ESI) calcd for $C_{21}H_{12}F_6N_4O_2S$: $[M + Na]^+$, 521.04742; found, 521.04774.

*3.1.10. 2-((3,4-Dichlorobenzyl)thio)-5-(4-((6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6j**).* Yellow solid; yield 66.4%; mp 102.8−104.6 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.99 (s, 1H, pyrimidine-H), 8.07 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.85 (s, 1H, pyrimidine-H), 7.80 (d, 1H, $J$ = 1.80 Hz, Ph-H), 7.61 (d, 1H, $J$ = 6.00 Hz, Ph-H), 7.53−7.50 (m, 3H, Ph-H), 4.59 (s, 2H, $CH_2$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.23, 165.27, 163.60, 159.72, 156.03 (q, $J$ = 34.95 Hz), 154.80, 138.74, 131.57, 131.42, 131.15, 130.85, 129.90, 128.81, 123.34, 121.77 (q, $J$ = 273.15 Hz), 121.34, 107.09, 35.96; HRMS (ESI) calcd for $C_{20}H_{11}Cl_2F_3N_4O_2S$: $[M + Na]^+$, 520.98193; found, 520.98241.

*3.1.11. 2-((2,4-Dichlorobenzyl)thio)-5-(4-((6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6k**).* White solid; yield 62.4%; mp 89.2−91.0 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.99 (s, 1H, pyrimidine-H), 8.07 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.88 (s, 1H, pyrimidine-H), 7.69 (d, 1H, $J$ = 1.80 Hz, Ph-H), 7.68 (d, 1H, $J$ = 8.40 Hz, Ph-H), 7.55 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.48 (dd, 1H, J1 = 1.80 Hz, J2 = 6.60 Hz, Ph-H), 4.65 (s, 2H, $CH_2$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.24, 165.47, 163.18, 159.73, 156.45 (q, $J$ = 35.10 Hz), 134.79, 134.08, 133.70, 133.32, 129.60, 128.88, 128.11, 123.39, 121.79 (q, $J$ = 272.70 Hz), 121.34, 116.13, 107.13, 34.16; HRMS (ESI) calcd for $C_{20}H_{11}Cl_2F_3N_4O_2S$: $[M + Na]^+$, 520.98169; found, 520.98241.

*3.1.12. 2-(4-((2-Methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-5-(methylthio)-1,3,4-oxadiazole (**6L**).* Pink solid; yield 42.5%; mp 127.4−129.4 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.07 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.60 (s, 1H, pyrimidine-H), 7.52 (d, 2H, $J$ = 9.00 Hz, Ph-H), 2.78 (s, 3H, $CH_3$), 2.52 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.12, 169.67, 165.24, 164.94, 156.44 (q, $J$ = 34.80 Hz), 154.76, 128.72, 123.20, 121.80 (q, $J$ = 273.15 Hz), 121.32, 103.85, 25.87, 14.78; HRMS (ESI) calcd for $C_{15}H_{11}F_3N_4O_2S$: $[M + Na]^+$, 391.04370; found, 391.04470.

*3.1.13. 2-(Ethylthio)-5-(4-((2-methyl-6-(trifluoromethyl)-pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6m**).* Pink solid; yield 65.5%; mp 104.9−106.6 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.08 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.60 (s, 1H, pyrimidine-H), 7.52 (d, 2H, $J$ = 9.00 Hz, Ph-H), 3.32 (q, 2H, $J$ = 7.80 Hz, $CH_2$), 2.52 (s, 3H, $CH_3$), 1.45 (t, 3H, $J$ = 7.20 Hz, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.12, 169.67, 164.97, 164.34, 156.44 (q, $J$ = 34.80 Hz), 154.77, 128.74, 121.79 (q, $J$ = 276.60 Hz), 121.33, 103.84, 27.17, 25.86, 15.34; HRMS (ESI) calcd for $C_{16}H_{13}F_3N_4O_2S$: $[M + Na]^+$, 405.05978; found, 405.06035.

*3.1.14. 2-(4-((2-Methyl-6-(trifluoromethyl)pyrimidin-4-yl)-oxy)phenyl)-5-((2-methylbenzyl)thio)-1,3,4-oxadiazole (**6n**).* White solid; yield 45.8%; mp 93.8−94.2 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.09 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.62 (s, 1H, pyrimidine-H), 7.53 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.43 (d, 1H, $J$ = 7.20 Hz, Ph-H), 7.24−7.21 (m, 2H, Ph-H), 7.18−7.15 (m, 2H, Ph-H), 4.62 (s, 2H, $CH_2$), 2.53 (s, 3H, $CH_3$), 2.42 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.12, 169.67, 163.68, 156.43 (q, $J$ = 35.10 Hz), 154.87, 137.33, 134.22, 130.98, 130.51, 128.81, 128.73, 126.60, 123.23, 121.81 (q, $J$ = 273.30 Hz), 103.89, 35.03, 25.88, 19.22; HRMS (ESI) calcd for $C_{22}H_{17}F_3N_4O_2S$: $[M + Na]^+$, 481.09140; found, 481.09165.

*3.1.15. 2-(4-((2-Methyl-6-(trifluoromethyl)pyrimidin-4-yl)-oxy)phenyl)-5-((4-methylbenzyl)thio)-1,3,4-oxadiazole (**6o**).* Brown solid; yield 52.3%; mp 111.2−112.8 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.08 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.62 (s, 1H, pyrimidine-H), 7.53 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.38 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.17 (d, 2H, $J$ = 7.80 Hz, Ph-H), 4.56 (s, 2H, $CH_2$), 2.53 (s, 3H, $CH_3$), 2.28 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.13, 169.67, 165.14, 163.89, 156.45 (q, $J$ = 34.80 Hz), 154.86, 137.59, 133.88, 129.63, 129.45, 128.79, 123.25, 121.81 (q, $J$ = 273.30 Hz), 121.23, 103.91, 36.27, 25.89, 21.16; HRMS (ESI) calcd for $C_{22}H_{17}F_3N_4O_2S$: $[M + Na]^+$, 481.09097; found, 481.09072.

*3.1.16. 2-((4-Fluorobenzyl)thio)-5-(4-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadiazole (**6p**).* Yellow solid; yield 56.5%; mp 74.2−75.1 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.07 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.59 (t, 2H, $J$ = 6.60 Hz, Ph-H and pyrimidine-H), 7.52 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.38−7.34 (m, 1H, Ph-H), 7.21−7.17 (m, 2H, Ph-H), 4.61 (s, 2H, $CH_2$), 2.52 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.08, 169.68,

**Table 5. In Vivo Curative and Protection Effects of Compound 6s against Blueberry Fruit Grey Mold**

| | | curative effect[a] | | protection effect[a] | |
|---|---|---|---|---|---|
| treatment | concentration ($\mu$g/mL) | lesion length (mm) | control efficacy (%) | lesion length (mm) | control efficacy (%) |
| **6s** | 200 | 0.9 ± 0.6 | 54.2 ± 1.6 | 0.9 ± 0.2 | 60.4 ± 1.3 |
| | 100 | 1.5 ± 1.1 | 23.9 ± 1.8 | 1.6 ± 0.1 | 29.1 ± 1.0 |
| | 50 | 1.8 ± 2.3 | 8.9 ± 2.4 | 1.8 ± 2.1 | 22.6 ± 1.9 |
| Pyrimethanil | 200 | 1.0 ± 0.9 | 49.3 ± 1.1 | 0.8 ± 0.2 | 63.9 ± 1.2 |
| | 100 | 1.5 ± 0.3 | 24.4 ± 1.0 | 1.9 ± 0.3 | 16.1 ± 1.4 |
| | 50 | 1.9 ± 1.5 | 7.5 ± 2.3 | 0.8 ± 0.7 | 12.6 ± 1.6 |
| CK | | 2.0 ± 0.8 | | 2.3 ± 1.6 | |

[a]The mean value derived from three repeated measurements.



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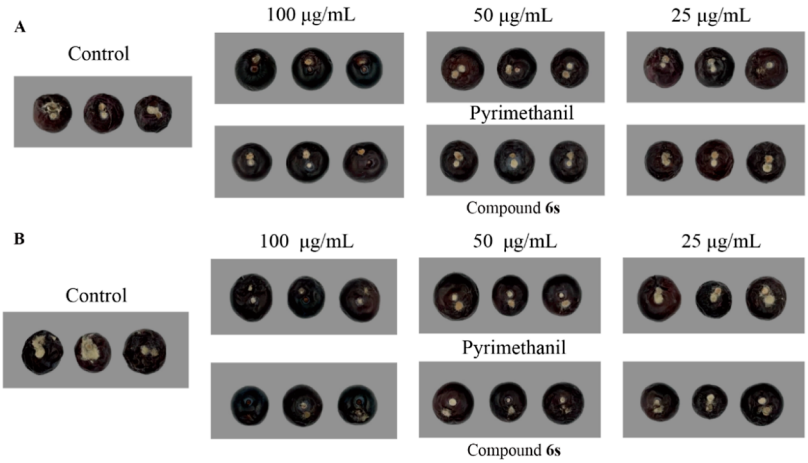


**Figure 2.** In vivo curative effect (**A**) and protection effect (**B**) of compound **6s** against blueberry fruit gray mold.

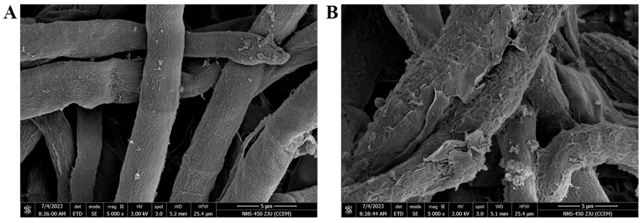


**Figure 3.** Impact of compound **6s** on the hyphal morphology of *Botrytis cinerea* Pers. in postharvest blueberry. (A) CK group and (B) treatment group.

165.35, 163.36, 161.69, 160.06, 156.48 (q, $J$ = 34.80 Hz), 154.88, 131.91, 130.73, 128.75, 125.04, 124.04, 123.19, 121.78 (q, $J$ = 273.15 Hz), 121.17, 116.03, 103.79, 30.31, 25.83; HRMS (ESI) calcd for $C_{21}H_{14}F_4N_4O_2S$: $[M + Na]^+$, 485.06638; found, 485.06658.

*3.1.17. 2-((4-Fluorobenzyl)thio)-5-(4-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy) phenyl)-1,3,4-oxadiazole (**6q**).* White solid; yield 54.6%; mp 87.7−89.6 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.08 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.62 (s, 1H, pyrimidine-H), 7.53 (q, 2H, $J$ = 5.40 Hz, Ph-H), 7.53 (d, 1H, $J$ = 8.40 Hz, Ph-H), 7.21 (t, 2H, $J$ = 9.00 Hz, Ph-H), 4.61 (s, 2H, $CH_2$), 2.52 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.11, 169.67, 165.20, 163.78, 162.91, 161.29, 156.46 (q, $J$ = 35.25 Hz), 154.86, 133.42, 131.66, 128.78, 123.22, 121.80 (q, $J$ = 273.45 Hz), 121.21, 115.93, 115.79, 103.87, 35.56, 25.87; HRMS (ESI) calcd for $C_{21}H_{14}F_4N_4O_2S$: $[M + Na]^+$, 485.06616; found, 485.06658.

*3.1.18. 2-((2-Chlorobenzyl)thio)-5-(4-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy) phenyl)-1,3,4-oxadiazole (**6r**).* Yellow solid; yield 62.7%; mp 89.8−91.6 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.08 (d, 2H, $J$ = 9.0 Hz, Ph-H), 7.80 (dd, 1H, J1 = 1.80 Hz, J2 = 6.60 Hz, pyrimidine-H), 7.61 (s, 2H, Ph-H), 7.52−7.50 (m, 3H, Ph-H), 7.38−7.33 (m, 2H, Ph-H), 4.67 (s, 2H, $CH_2$), 2.52 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.10, 169.67, 163.34, 156.46 (q, $J$ = 35.10 Hz), 154.90, 134.34, 133.81, 132.07, 130.46, 130.10, 128.82, 127.96, 123.22, 121.80 (q, $J$ = 273.45 Hz), 121.19, 103.86, 34.76, 25.87; HRMS (ESI) calcd for $C_{21}H_{14}ClF_3N_4O_2S$: $[M + Na]^+$, 501.03671; found, 501.03703.

*3.1.19. 2-((3-Chlorobenzyl)thio)-5-(4-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy) phenyl)-1,3,4-oxadiazole (**6s**).* Gray solid; yield 67.5%; mp 89.2−90.2 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.07 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.60 (s, 2H, Ph-H and pyrimidine-H), 7.52 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.48 (d, 1H, $J$ = 7.80 Hz, Ph-H), 7.40−7.35 (m, 2H, Ph-H), 4.60 (s, 2H, $CH_2$), 2.52 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.11, 169.66, 165.25, 163.67, 156.45 (q, $J$ = 34.80 Hz), 155.99, 139.92, 133.46 130.89, 129.42, 128.78, 128.24, 123.62, 121.80 (q, $J$ = 273.45 Hz), 121.19, 103.87, 35.57, 25.87; HRMS (ESI) calcd for $C_{21}H_{14}ClF_3N_4O_2S$: $[M + Na]^+$, 501.03616; found, 501.03703.

*3.1.20. 2-((4-Chlorobenzyl)thio)-5-(4-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy) phenyl)-1,3,4-oxadiazole (**6t**).* Yellow solid; yield 84.6%; mp 90.1−91.9 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.07 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.61 (s, 1H, pyrimidine-H), 7.53 (dd, 4H, $J1$ = 6.60 Hz, $J2$ = 1.80 Hz, Ph-H), 7.38 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.42 (d, 2H, $J$ = 8.40 Hz, Ph-H), 4.59 (s, 2H, $CH_2$), 2.52 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.12, 169.66, 165.24, 163.89, 156.45 (q, $J$ = 34.65 Hz), 154.87, 136.37, 132.88, 131.41, 129.02, 128.80, 123.62, 121.80 (q, $J$ = 273.45 Hz), 121.20, 103.89, 35.57, 25.88; HRMS (ESI) calcd for $C_{21}H_{14}ClF_3N_4O_2S$: $[M + Na]^+$, 501.03635; found, 501.03703.

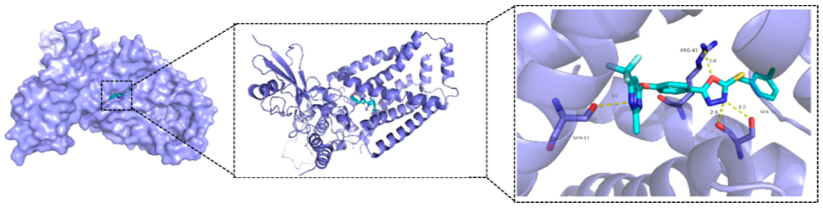

**Figure 4.** Docking binding mode of compound **6s** to SDH.



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*3.1.21. 2-(((5-(4-((2-Methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadia zol-2-yl)thio)methyl)-benzonitrile (**6u**).* Gray solid; yield 56.3%; mp 125.2−127.0 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.08 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.89 (d, 1H, $J$ = 7.80 Hz, Ph-H), 7.77 (d, 1H, $J$ = 7.20 Hz, Ph-H), 7.73−7.70 (m, 1H, Ph-H), 7.63 (s, 1H, pyrimidine-H), 7.54−7.51 (m, 3H, Ph-H), 4.75(s, 2H, $CH_2$), 2.53 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.11, 169.67, 165.60, 162.78, 156.45 (q, $J$ = 34.65 Hz), 154.95, 140.62, 134.01, 133.75, 131.06, 129.03, 128.89, 123.23, 121.81 (q, $J$ = 272.85 Hz), 121.16, 117.62, 112.29, 103.91, 34.98, 25.89; HRMS (ESI) calcd for $C_{22}H_{14}F_3N_5O_2S$: [M + Na]$^+$, 492.07050; found, 492.07125.

*3.1.22. 4-(((5-(4-((2-Methyl-6-(trifluoromethyl)pyrimidin-4-yl)oxy)phenyl)-1,3,4-oxadia zol-2-yl)thio)methyl)-benzonitrile (**6v**).* Yellow solid; yield 67.3%; mp 110.1−111.4 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.05 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.84 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.72 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.60 (s, 1H, pyrimidine-H), 7.52 (d, 2H, $J$ = 9.00 Hz, Ph-H), 4.67 (s, 2H, $CH_2$), 2.52 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.10, 169.66, 165.31, 163.50, 156.46 (q, $J$ = 34.80 Hz), 154.88, 143.29, 132.92, 130.50, 128.79, 123.21, 121.79 (q, $J$ = 273.00 Hz), 121.16, 119.07, 110.93, 103.86, 35.76, 25.87; HRMS (ESI) calcd for $C_{22}H_{14}F_3N_5O_2S$: [M + Na]$^+$, 492.07019; found, 492.07125.

*3.1.23. 2-(4-((2-Methyl-6-(trifluoromethyl)pyrimidin-4-yl)-oxy)phenyl)-5-((4-(trifluoromethyl)benzyl)thio)-1,3,4-oxa-diazole (**6w**).* White solid; yield 45.9%; mp 97.7−99.2 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.06 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.73 (br, 4H, Ph-H), 7.61 (s, 1H, pyrimidine-H), 7.52 (d, 2H, $J$ = 9.00 Hz, Ph-H), 4.69 (s, 2H, $CH_2$), 2.52 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.12, 169.66, 165.30, 163.58, 156.45 (q, $J$ = 34.80 Hz), 154.88, 142.33, 130.34, 128.80, 128.59 (q, $J$ = 32.10 Hz), 125.89, 125.52 (q, $J$ = 270.15 Hz), 123.23, 121.80 (q, $J$ = 273.00 Hz), 121.18, 103.89, 35.63, 25.87; HRMS (ESI) calcd for $C_{22}H_{14}F_6N_4O_2S$: [M + Na]$^+$, 535.06274; found, 535.06339.

*3.1.24. 2-((2,4-Dichlorobenzyl)thio)-5-(4-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl) oxy)phenyl)-1,3,4-oxadia-zole (**6x**).* White solid; yield 56.8%; mp 103.3−104.1 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.08 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.69−7.62 (m, 3H, Ph-H and pyrimidine-H), 7.53 (d, 1H, $J$ = 9.00 Hz, Ph-H), 7.46−7.37 (m, 1H, Ph-H), 4.72 (s, 2H, $CH_2$), 2.53 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.11, 169.66, 165.49, 163.14, 156.45 (q, $J$ = 34.50 Hz), 154.92, 137.16, 134.79, 134.08, 133.70, 133.31, 132.63, 131.89, 130.78, 129.59, 128.85, 128.79, 128.10, 123.24, 121.80 (q, $J$ = 272.85 Hz), 121.17, 103.89, 35.46, 25.88; HRMS (ESI) calcd for $C_{21}H_{13}Cl_2F_3N_4O_2S$: [M + Na]$^+$, 534.99756; found, 534.99806.

*3.1.25. 2-((3,4-Dichlorobenzyl)thio)-5-(4-((2-methyl-6-(trifluoromethyl)pyrimidin-4-yl) oxy)phenyl)-1,3,4-oxadia-zole (**6y**).* Yellow solid; yield 74.3%; mp 86.2−87.7 °C; $^1$H NMR (600 MHz, DMSO-$d_6$, ppm): $\delta$ 8.04 (d, 2H, $J$ = 9.00 Hz, Ph-H), 7.80 (d, 1H, $J$ = 1.80 Hz, Ph-H), 7.61 (d, 2H, $J$ = 8.40 Hz, Ph-H), 7.59 (s, 2H, Ph-H and pyrimidine-H), 7.51 (d, 3H, $J$ = 8.40 Hz, Ph-H), 4.59 (s, 2H, $CH_2$), 2.52 (s, 3H, $CH_3$); $^{13}$C NMR (150 MHz, DMSO-$d_6$, ppm): $\delta$ 170.09, 169.66, 165.29, 163.55, 156.46 (q, $J$ = 35.10 Hz), 154.86, 138.74, 131.57, 131.42, 131.14, 130.85, 128.76, 123.19, 121.78 (q, $J$ = 273.00 Hz), 121.17, 103.81, 34.97, 25.85; HRMS (ESI) calcd for $C_{21}H_{13}Cl_2F_3N_4O_2S$: [M + Na]$^+$, 534.99689; found, 534.99806.

**3.2. In Vivo Anti-TMV Activity Test.** The anti-TMV activity of the target compounds **6a**−**6y** are illustrated in Table 1. Table 1 shows that the target compounds had moderate to good inhibitory activity against TMV at a concentration of 500 $\mu$g/mL. In terms of curative activity against TMV, compound **6r** exhibited a slightly superior inhibition rate of 61.3% compared to ningnanmycin (56.4%). Meanwhile, compound **6s** demonstrated the highest protection activity against TMV with a rate of 68.7%, which was comparable to that of ningnanmycin (66.4%). However, the inactivation activity of all target compounds against TMV was found to be lower than that of ningnanmycin.

To further demonstrate the antiviral potential of the promising target compounds, several candidates exhibiting relatively high primary inhibitory activity against TMV were subjected to effective concentration ($EC_{50}$) value testing, and the results were listed in Table 2. It can be observed from Table 2 that compounds **6f** and **6r** demonstrated remarkable curative activity against TMV, exhibiting $EC_{50}$ values of 0.59 and 0.48 $\mu$M respectively, surpassing even the efficacy of ningnanmycin (0.69 $\mu$M). Additionally, compound **6s** displayed exceptional protection activity against TMV with an $EC_{50}$ value of 0.42 $\mu$M, outperforming ningnanmycin (0.60 $\mu$M).

**3.3. In Vitro Antifungal Activity Test.** As shown in Table 3, the target compounds **6a**−**6y** exhibited certain in vitro antifungal activities against *B. dothidea* (Moug.) Ces. De Not. in postharvest kiwifruit (40.13−77.85%), *Phomopsis* sp. in postharvest kiwifruit (47.99−94.43%), *B. cinerea* Pers. in postharvest blueberry (44.71−100%), *B. cinerea* Pers. in postharvest cucumber (35.35−58.01%), *S. sclerotiorum* (Lib.) de Bary in oilseed rape (23.38−75.19%), *B. cinerea* Pers. in postharvest tobacco (34.77−66.17%), *B. cinerea* Pers. in postharvest strawberry (31.96−100%), *Colletotrichum* sp. in postharvest blueberry (13.20−75.05%), and *M. grisea* (Hebert) Barr in rice (8.20−52.46%). Among them, the inhibitory activities of compounds **6s** and **6x** against *Phomopsis* sp. in postharvest kiwifruit were found to be 94.12 and 94.43% respectively, surpassing that of Pyrimethanil (85.13%). Meanwhile, compounds **6c**, **6g**, and **6s** exhibited complete inhibition (100.00%) against *B. cinerea* Pers. in postharvest blueberry, outperforming Pyrimethanil (82.83%). Additionally, compounds **6f** and **6j** demonstrated inhibition rates of 100.00 and 91.30%, respectively toward *B. cinerea* Pers. in postharvest strawberries which were superior to Pyrimethanil (84.88%).

The $EC_{50}$ values of some target compounds against *B. cinerea* Pers. in postharvest blueberry, *Phomopsis* sp. in postharvest kiwifruit, and *B. cinerea* Pers. in postharvest strawberry were determined and the results are shown in Table 4. The results presented in Table 4 demonstrate that compounds **6c**, **6g**, and **6s** exhibited superior antifungal activity against *B. cinerea* Pers. in postharvest blueberry, with $EC_{50}$ values of 0.018, 0.031, and 0.011 $\mu$M respectively, outperforming Pyrimethanil (0.262 $\mu$M). Meanwhile, compounds **6s** and **6x** displayed excellent antifungal activity against *Phomopsis* sp. in postharvest kiwifruit, with $EC_{50}$ values of 0.030 and 0.038 $\mu$M, respectively, which were better than Pyrimethanil (0.176 $\mu$M). Additionally, compound **6j** demonstrated good antifungal efficacy against *B. cinerea* Pers. in postharvest strawberries with an $EC_{50}$ value of 0.032 $\mu$M, surpassing that of Pyrimethanil (0.193 $\mu$mol/mL).

**3.4. In Vivo Control Efficacy Test against Blueberry Fruit Gray Mold.** The in vivo evaluation was conducted to


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assess the curative and protection control efficacy of compound **6s** against blueberry fruit gray mold, and the corresponding results are presented in Table 5. According to the data in Table 5, compound **6s** exhibited remarkable curative effects against blueberry fruit gray mold at concentrations of 100, 50, and 25 $\mu$g/mL with values of 54.2, 23.9, and 8.9% respectively; however, these values were comparable to those observed for Pyrimethanil (49.3, 24.4, and 7.5%). Meanwhile, the protection effect of compound **6s** at a concentration of 100 $\mu$g/mL was found to be equivalent to Pyrimethanil (63.9%), with a value of 60.4%; however, at concentrations of 50 and 25 $\mu$g/mL, the protection effect of compound **6s** was slightly higher than Pyrimethanil (16.1 and 12.6%) at values of 29.1 and 22.6%, respectively. Moreover, it is noteworthy that the in vivo curative and protection effects of compound **6s** exhibited concentration-dependent properties, and compound **6s** was proven to be safe for blueberry fruit even at high concentrations (Figure 2).

**3.5. Effect on the Hyphae Morphology.** The impact of compound **6s** on the hyphal morphology of *B. cinerea* Pers. in postharvest blueberry was assessed using SEM. Figure 3 illustrates that the negative control (CK) group exhibited a typical and characteristic morphology, with uniformly distributed and linear hyphae compared to evident collapse; however, treatment with compound **6s** at a concentration of 12.5 $\mu$g/mL resulted in abnormal collapse, shriveling, and damage to the hyphae of *B. cinerea* Pers. in postharvest blueberry. This suggests that compound **6s** may disrupt the cell membrane or cell wall, thereby influencing the subsequent growth and reproduction of *B. cinerea* Pers. in postharvest blueberry.

**3.6. Molecular Docking Study.** To elucidate the binding mode of the target compounds to the SDH receptor, compound **6s** with the highest activity was subjected to molecular docking within the active site of the SDH receptor. The resulting docking conformation, depicted in Figure 4, revealed that a nitrogen atom in the pyrimidine ring of compound **6s** engaged in a hydrogen bond interaction with amino acid residue SER-17 at a distance of 3.0 Å. Simultaneously, an oxygen atom within the 1,3,4-oxadiazole moiety formed a hydrogen bond interaction with amino acid residue ARG-43 at a distance of 3.0 Å. Furthermore, another nitrogen atom within the 1,3,4-oxadiazole group established two hydrogen bonds with amino acid residue SER-39 at distances of 3.0 and 2.9 Å respectively.

## 4. CONCLUSIONS

In this study, a total of 25 novel pyrimidine derivatives containing a 1,3,4-oxadiazole thioether fragment were designed and synthesized. Among them, several target compounds exhibited significant anti-TMV and antifungal activities both in vitro and in vivo. Meanwhile, the SEM results demonstrated that the hyphae of *B. cinerea* Pers. in postharvest blueberry treated with compound **6s** caused abnormal collapse and shrinkage. Additionally, molecular docking simulations revealed that compound **6s** formed hydrogen bonds with SER-17, ARG-43, and SER-39 residues of the SDH enzyme, providing a potential explanation for the mechanism of action between the target compounds and SDH. Therefore, pyrimidine derivatives incorporating a 1,3,4-oxadiazole thioether moiety can be regarded as a suitable model for identifying potential candidates against viral infections and fungal-induced plant diseases.

## ASSOCIATED CONTENT

### Supporting Information

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$^1$H NMR, $^{13}$C NMR, and HRMS spectra for all compounds (PDF)

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### Notes

The authors declare no competing financial interest.

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