

## Research Article

# Isolation, Synthesis, and Fungicidal Activity of Isopropyl (3-methyl-1-oxo-1-((1-((4-(prop-2-yn-1-yloxy)phenyl)thio)propan-2-yl)amino)butan-2-yl)carbamate Diastereomers against *Phytophthora capsici*

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Two isopropyl (3-methyl-1-oxo-1-((1-((4-(prop-2-yn-1-yloxy)phenyl)thio)propan-2-yl)amino)butan-2-yl)carbamate diastereomers were isolated. Fungicidal activities indicated that the isolated four chiral compounds possessed excellent activity against *P. capsici* with the EC<sub>50</sub> value of **4a** (1.30 μg/mL), **4b** (0.078 μg/mL), **4c** (1.85 μg/mL), and **4d** (44.4 μg/mL). Among them, compound **4b** exhibited remarkably high activities against *Phytophthora capsici*, which is better than that of positive control dimethomorph. Its R and S isomers showed that chiral influences the activity against *P. capsici*.

## 1. Introduction

In recent years, modern agriculture, including economic crops, flowers, fruits, trees, and vegetables, were affected by insects, weeds, and diseases. The effective tools to protect plants are pesticides [1–5], which have been an important work in modern agriculture. *Phytophthora capsici* is an important pathogenic fungal with a wide host range, which is very harmful and destructive to host plants, and often leads to serious losses in crop production. In 2005, carboxylic acid amide (CAA) fungicides were officially announced by FRAC (<https://www.frac.info>) because of their common cross resistance pattern for the vast majority of oomycetes. CAA fungicides include the three subclasses based on differences in structure: cinnamic acid amides [6–8] (dimethomorph, flumorph, and pyrimorph), valinamide carbamates [9–11] (benthiavalicarb, benthiavancarb, isopropyl, iprovalicarb, and valiphenal) and mandelic acid amide11 (mandipropamid). Although dimethomorph was

the first CAA fungicide to be introduced in 1988, only seven CAA fungicides are marketed until now. Among them, the valinamide carbamates were derived from the natural amino acid L-valine (Figure 1), which had two chiral carbons. But the fungicides iprovalicarb and valiphenal were used as racemates, while the other valinamide carbamates were used as chiral compounds.

In our previous work, some amino acid analogues [12–17] were designed and synthesized, including isopropyl (3-methyl-1-oxo-1-((1-((4-(prop-2-yn-1-yloxy)phenyl)thio)propan-2-yl)amino)butan-2-yl)carbamate diastereomers, which possessed good fungicidal activity. In this paper, isopropyl (3-methyl-1-oxo-1-((1-((4-(prop-2-yn-1-yloxy)phenyl)thio)propan-2-yl)amino)butan-2-yl)carbamate diastereomers were used as starting materials and their racemate was isolated. In order to check the configurations, the compound **4b** was also synthesized. The fungicidal activity against *P. capsici* results indicated that the compound exhibited good fungicidal activity.

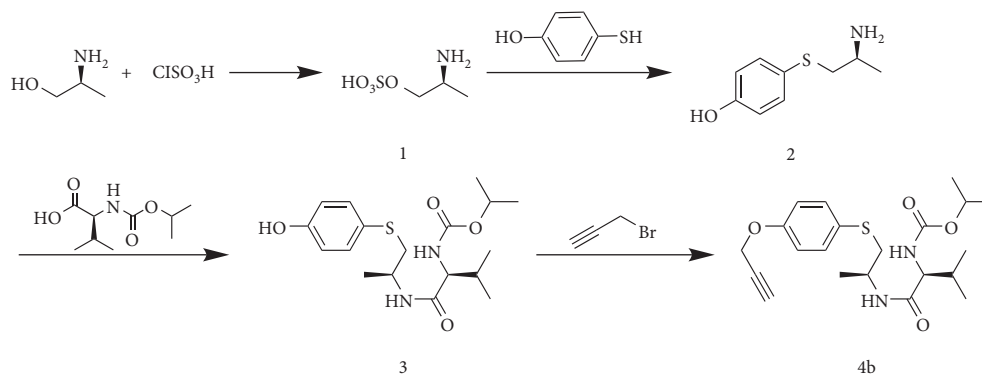
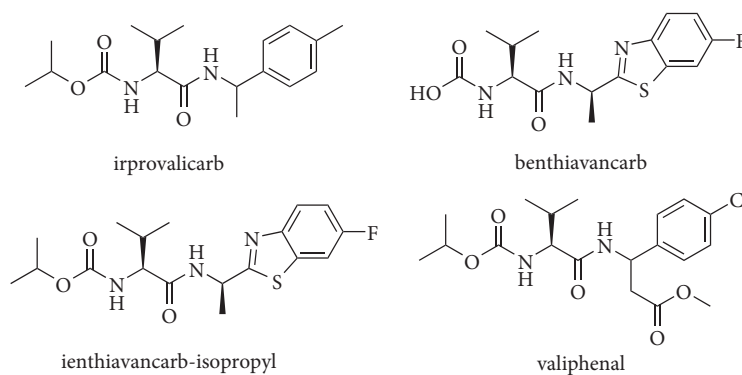
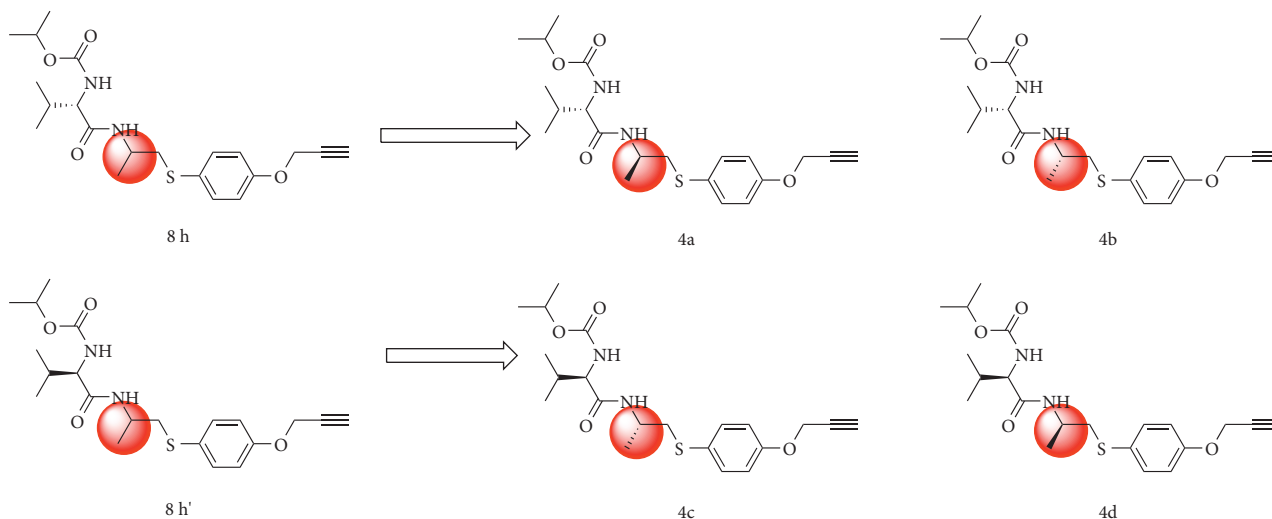
SCHEME 1: The synthetic route of compound **4b**.

FIGURE 1: Some valinamide carbamates.



previous published work in  
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FIGURE 2: The structures of isolated four compounds.

## 2. Materials and Methods

**2.1. Instruments.** All the chemical reagents were of analytical grade or prepared in our laboratory. Melting points were measured using an X-4 apparatus (Taiké, Beijing, China) and were uncorrected.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on the BRUKER Avance 400 MHz spectrometer

using  $\text{CDCl}_3$  as a solvent. HR-MS was determined on an FTMS 7.0 instrument. Rudolph Autopol I was used to test the specific rotation.

**2.2. Isolation.** The compound **8h** and **8h'** (Figure 2) were isolated by column chromatography (PE:EA=3:1), the four

TABLE 1: The EC<sub>50</sub> of isolated four compounds against *P. capsici* (μg/mL).

No.	$y = a + bx$	$r^2$	95% confidence intervals	EC <sub>50</sub>
<b>4a</b>	$y = 4.87 + 1.13x$	0.976	0.99~1.70	1.30
<b>4b</b>	$y = 4.23 + 7.91x$	0.995	0.07~0.08	0.078
<b>4c</b>	$y = 4.04 + 4.97x$	0.994	28.0~445	1.85
<b>4d</b>	$y = 3.91 + 0.66x$	0.984	23.1~85.2	44.4
Dimethomorph	$y = 6.55 + 1.65x$	0.993	0.34~0.40	0.37

compounds were obtained as white solids, and the structures were listed in Figure 2. The specific rotations of the four isolated compounds were  $-35.2$  (**4a**),  $8.0$  (**4b**),  $29.6$  (**4c**), and  $-8.4$  (**4d**), respectively.

**2.3. Fungicidal Activity of the EC<sub>50</sub> Test.** The *in vitro* fungicidal activities of the four compounds **4a**, **4b**, **4c**, and **4d** against *Phytophthora capsici* were evaluated using the mycelium growth rate method [18]. The culture media, with known concentrations (50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.2, 0.1, 0.05, 0.025 mg/L) of the test compounds, were obtained by mixing the 1% tween-20 water suspension (1 mL) of high active compounds with potato dextrose agar (PDA, 9 mL) at 50°C. The medium was then poured into a 9 cm Petri dish and cooled to room temperature, which was inoculated with 5 mm mycelium of *P. capsici*. The Petri dish was then placed in a light incubator at 25 ± 1°C for 72 h. A commercial fungicide dimethomorph was used as the positive control, and sterile water was used as the blank. Three replications were employed for each treatment. The inhibition rate was expressed as the mean of values obtained in three independent experiments. Effective concentration (EC<sub>50</sub>) was obtained using log-probit analysis (Table 1). The inhibition rate was calculated according to the formula:

$$\text{Inhibitor rate (\%)} = \frac{(\text{CK} - \text{PT})}{\text{CK}} \times 100\%, \quad (1)$$

where CK is the expansion diameter of mycelia in the blank test, and PT is the expansion diameter of mycelia in the presence of tested compounds.

## 2.4. Synthesis (Scheme 1)

**2.4.1. (S)-2-Aminopropyl Hydrogen Sulfate 1.** To a solution of (S)-2-aminopropan-1-ol (10.0 g, 133.10 mmol) in ethyl acetate (150 mL), a solution of sulfurochloridic acid (1.10 g, 146.40 mmol) in ethyl acetate (50 mL) was dropwised at 0°C. The mixture was stirred at 40°C for 2 h. Then, a solution was cooled at 0°C, and a white solid was given, dried, and purified by column chromatography (PE:EA = 5:1). The final compound **2** was given with a yield of 61.5%, 1.61 g. White solid, m.p. 62–63°C, yield 79.0%; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.24 (s, 1H, OH), 4.08–3.43 (m, 3H, OCH<sub>2</sub>CH), 1.13 (m, 5H, NH<sub>2</sub>CHCH<sub>3</sub>).

**2.4.2. Synthesis of (S)-4-((2-aminopropyl)thio)phenol 2.** *p*-hydroxythiophenol (4.88 g, 38.70 mmol) and intermediate **1** (5.00 g, 32.20 mmol) were dissolved in water (40 ml) and

toluene (50 ml), and then the mixture was refluxed. The solution of sodium hydroxide (1.55 g, 38.70 mmol) was dropwised. The mixture was further refluxed for 3 h, and then the mixture was stirred for 10 h at 45°C. The light yellow solid 1S-methyl-2-*p*-hydroxyphenyl mercaptoethylamine **2** was given by exacted and column chromatography (V<sub>(petroleum ether)</sub>: V<sub>(ethyl acetate)</sub> = 1 : 1), m.p. 100–101°C yield 12.7% <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.30–7.23 (m, 2H, Ar-H), 6.69–6.63 (m, 2H, Ar-H), 4.42 (s, 1H, Ar-OH), 3.09–2.47 (m, 3H, SCH<sub>2</sub>CH), 1.28 (d, *J* = 6.9 Hz, 2H, NH<sub>2</sub>), and 1.20 (d, *J* = 6.4 Hz, 3H, CH<sub>3</sub>).

**2.4.3. Synthesis of Isopropyl ((S)-1-(((S)-1-((4-hydroxyphenyl)thio)propan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate 3.** To a mixture solution of 1S-methyl-2-*p*-hydroxyphenyl mercaptoethylamine **2** (0.18 g, 0.86 mmol) and Et<sub>3</sub>N (0.087 g, 0.86 mmol) in THF (10 mL), a solution of isopropyl carbonochloridate (0.11 g, 0.86 mmol) in THF (5 mL) was dropwised at 0°C for 0.5 h. Then, the mixture was stirred for another 2 h. After that, THF was evaporated, washed by saturated potassium carbonate aqueous solution (100 mL), exacted by CH<sub>2</sub>Cl<sub>2</sub> (50 mL), and HCl (50 mL, 1 mol/L) twice, dried and purified by column chromatography (PE:EA = 3:1). The intermediate compound **3** was given with a yield of 19.9%, yellow oil, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.35–7.27 (m, 2H, Ar-H), 6.81–6.75 (m, 2H, Ar-H), 6.62 (s, 1H, CHCONH), 5.42 (s, 1H, OCONH), 4.91–4.79 (m, 1H, SCH<sub>2</sub>CH), 4.18–4.02 (m, 1H, OCH), 3.88 (t, *J* = 8.0 Hz, 1H, CHCH(CH<sub>3</sub>)), 2.99–2.71 (m, 2H, SCH<sub>2</sub>), 2.04 (s, 1H, Ar-OH), and 1.45 (d, *J* = 30.4 Hz, 1H, CHCH(CH<sub>3</sub>)<sub>2</sub>), 1.21 (m, CHCH<sub>3</sub> + OCH(CH<sub>3</sub>)<sub>2</sub>), 0.96 (d, *J* = 6.8 Hz, 6H, CHCH(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 133.35, 117.15, 69.87, 59.35, 45.32, 42.30, 30.45, 22.56, 19.49, 19.04; HR-MS (MALDI) *m/z* Calcd for C<sub>20</sub>H<sub>30</sub>N<sub>4</sub>O<sub>4</sub>SNa<sup>+</sup> [M+Na]<sup>+</sup> 445.1880 found 445.1885.

**2.4.4. Synthesis of Isopropyl ((S)-3-methyl-1-oxo-1-(((S)-1-((4-(prop-2-yn-1-yloxy)phenyl)thio)propan-2-yl)amino)butan-2-yl)carbamate 4b.** To a solution of isopropyl ((S)-1-(((S)-1-((4-hydroxyphenyl)thio)propan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate **3** (0.06 g, 0.16 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.034 g, 0.24 mmol) in acetone (100 mL), 3-bromoprop-1-yne (0.034 g, 0.24 mmol) in toluene (10 mL) was dropwised. After that, the mixture was refluxed for 10 h. The mixture was filtered, and the solvent was removed. The residue was redissolved in CH<sub>2</sub>Cl<sub>2</sub> (100 mL), washed by NaOH solution (1 mol/L), exacted by CH<sub>2</sub>Cl<sub>2</sub> twice, and dried and purified by column chromatography (PE:EA = 3:1). The target compound **4b** was given with a yield of 98.4%. White solid, m.p. 70–71°C, [α]<sub>20</sub><sup>D</sup> = 10.8°; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.31 (d, *J* = 8.5 Hz, 2H, Ar-H), 6.85 (d, *J* = 8.5 Hz, 2H, Ar-H), 6.22 (d, *J* = 6.4 Hz, 1H, CHCONH), 5.22 (d, *J* = 8.4 Hz, 1H, OCONH), 4.80 (s, 1H, OCH), 4.59 (s, 2H, OCH<sub>2</sub>CCH), 4.11–3.97 (m, CHCH(CH<sub>3</sub>)<sub>2</sub>), 3.83 (s, 1H, CCH), 2.88 (m, 2H, SCH<sub>2</sub>), 2.46 (s, 1H, CHCH(CH<sub>3</sub>)<sub>2</sub>), 1.97 (d, *J* = 5.9 Hz, 1H, SCH<sub>2</sub>CHCH<sub>3</sub>), 1.15 (m, 6H, CHCH(CH<sub>3</sub>)<sub>2</sub>), and 0.89 (d, *J* = 6.7 Hz, 6H, CHCH(CH<sub>3</sub>)<sub>2</sub>); HR-MS (MALDI) *m/z* Calcd for C<sub>21</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub>SNa<sup>+</sup> [M+Na]<sup>+</sup> 429.1819, found 429.1821.

### 3. Results and Discussion

**3.1. Isolation and Synthesis.** It is obvious from the structures that there are two chiral carbons, one is from the starting material, and the other one is uncertain. So in our previous work, the compounds 8h and 8h' are diastereomers. From the TCL results, two very close points were observed; column chromatography was carried out to isolate the two isomers. The specific rotations of the isolated compound **4b** was 8.0°, but the specific rotations of compound **4b** is 10.8°, which means that the isolated method by column chromatography is not good. In synthesis process, for the intermediate 1, the dry process must be in vacuum drying oven, if not, this intermediate will go bad. 3-bromoprop-1-yne should be introduced at the last step, otherwise the product is not desired.

**3.2. Fungicidal Activity against *P. capsici*.** From Table 1, the EC<sub>50</sub> of isolated four compounds against *P. capsici* are different, which indicated the chiral centers are important factors for fungicidal activity. For the compounds **4a** and **4b**, compound **4b** (0.078 μg/mL) is 200 times more active than compound **4a** (1.30 μg/mL). For the compounds **4c** and **4d**, compound **4c** (1.85 μg/mL) is 24 times more active than compound **4d** (44.4 μg/mL). Among the four compounds, compound **4b** exhibited the best activity, which is much more better than that of positive control dimethomorph (0.37 μg/mL). The primarily structure-activity relationship indicated that S configuration near the sulfur atom is better for the fungicidal activity.

### 4. Conclusions

In conclusion, four chiral isopropyl (3-methyl-1-oxo-1-((1-((4-(prop-2-yn-1-yloxy)phenyl)thio)propan-2-yl)amino)butan-2-yl)carbamate compounds were isolated and synthesized. The fungicidal activity against *P. capsici* indicated that the chiral centers are important factors for fungicidal activity.

### Data Availability

The data used to support the study can be made available upon request to the corresponding author.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Acknowledgments

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