Research Article

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1,2,4-Triazolium compounds as anti-oomycete leads against *Phytophthora capsici*

Jia Song,[a] Xiao Zhang,[a] Aimin Zhang,[b] Tingting Li[a*] and Yonggui Robin Chi[a,c*]

Abstract

BACKGROUND: 1,2,4-Triazolium salts are a class of stable heterocyclic salts widely used as *in situ* carbene precursors in organic synthesis, and they have also been reported to exhibit favorable physicochemical properties and diverse biological activities. This study aims to elucidate the potential of 1,2,4-triazolium salts as antifungal and anti-oomycete agents.

RESULTS: In this study, a series of 1,2,4-triazolium derivatives were evaluated for their inhibitory activities against phytopathogenic oomycete *Phytophthora capsici*. Compound 42 displayed the most potent *in vitro* activity against *P. capsici*, with a half-maximal effective concentration (EC_{50}) of 5.38 μg mL^{-1}, and demonstrated good protective efficacy *in vivo* against *P. capsici*. Scanning electron microscopy (SEM) revealed that treatment of *P. capsici* with compound 42 at 50 μg mL^{-1} caused pronounced morphological abnormalities in the hyphae, characterized by severe collapse and shrinkage. Molecular docking studies revealed favorable binding interactions between compound 42 and uridine phosphorylase (UPase, PDB ID: 6KD3), providing possible mechanistic insights into its inhibitory activity.

CONCLUSION: Our results establish 1,2,4-triazolium compound 42 as a promising scaffold for the future development of anti-oomycete agents, offering a potential molecular basis for the design of novel crop protectants against *P. capsici*.

Keywords: anti-oomycete activity; molecular docking; *Phytophthora capsici*; structure–activity relationship; 1,2,4-triazolium salts

1 INTRODUCTION

Among crop diseases, oomycete and fungal pathogens constitute the predominant biotic threat to agricultural productivity, accounting for approximately 70–80% of losses attributed to microbial diseases and causing an estimated 20–40% reduction in potential global crop yields annually.[1,2] *Phytophthora capsici* is a destructive oomycete pathogen that infects a wide range of economically important vegetable crops, including pepper, tomato, eggplant, and cucurbits, causing Phytophthora blight worldwide.[3] Its capacity to colonize multiple plant tissues and produce long-lived soilborne oospores makes disease management particularly challenging.[4,5] Currently, several fungicides, such as mefenoxam, fluopicolide, and oxathiapiprolin, are commercially used to control Phytophthora blight. However, the extensive and prolonged application of these agents has led to the rapid emergence of resistant *P. capsici* populations.[6] Therefore, there is an urgent need to develop novel and effective anti-oomycete agents to ensure sustainable management of destructive oomycete.[4,7]

Triazolium salts are widely used in organic catalysis as precursors to N-heterocyclic carbenes (NHCs)[8–10] and exhibit diverse biological activities.[11,12] Several clinically used antifungal agents adopt triazolium-based prodrug strategies to enhance aqueous solubility and formulation performance.[13,14] For example, the fungicide ravuconazole is administered clinically as its triazolium salt, isavuconazonium sulfate (Cresemba®), enabling intravenous delivery.[13] Moreover, the conversion of triazoles into triazolium salts has been shown to enhance antifungal activity.[15] Specifically, salt **A** (derived from tebuconazole) increased the inhibition rate against *Microdochium nivale* from 37% to 93%, while salt **B** (derived from propiconazole) raised the rate against *Fusarium culmorum* from 89% to 100% (Fig. 1).[15] In recent years, several antifungal candidates containing 1,2,4-triazolium motifs have been reported.[14–19] However, the development of agrochemicals based on 1,2,4-triazolium scaffold remains relatively rare.[11] The permanently charged triazolium core endows these compounds with high water solubility and enables electrostatic interactions with negatively charged microbial cell surfaces.[20,21] Such interactions may promote membrane association and facilitate cellular uptake, which have been recognized as important contributors to antifungal and antibacterial activity.[22–24] Collectively, these characteristics make triazolium-based scaffolds promising

* *Correspondence to: T Li or YR Chi, State Key Laboratory of Green Pesticide, Center for R&D of Fine Chemicals of Guizhou University, Guiyang, 550025, China. E-mail: ttli@gzu.edu.cn (Li); E-mail: robinchi@ntu.edu.sg (Chi)*

a *State Key Laboratory of Green Pesticide, Center for R&D of Fine Chemicals of Guizhou University, Guiyang, China*

b *Institute of Pepper, Guizhou Academy of Agriculture Science, Guiyang, China*

c *School of Chemistry, Chemical Engineering, and Biotechnology, Nanyang Technological University, Singapore, Singapore*

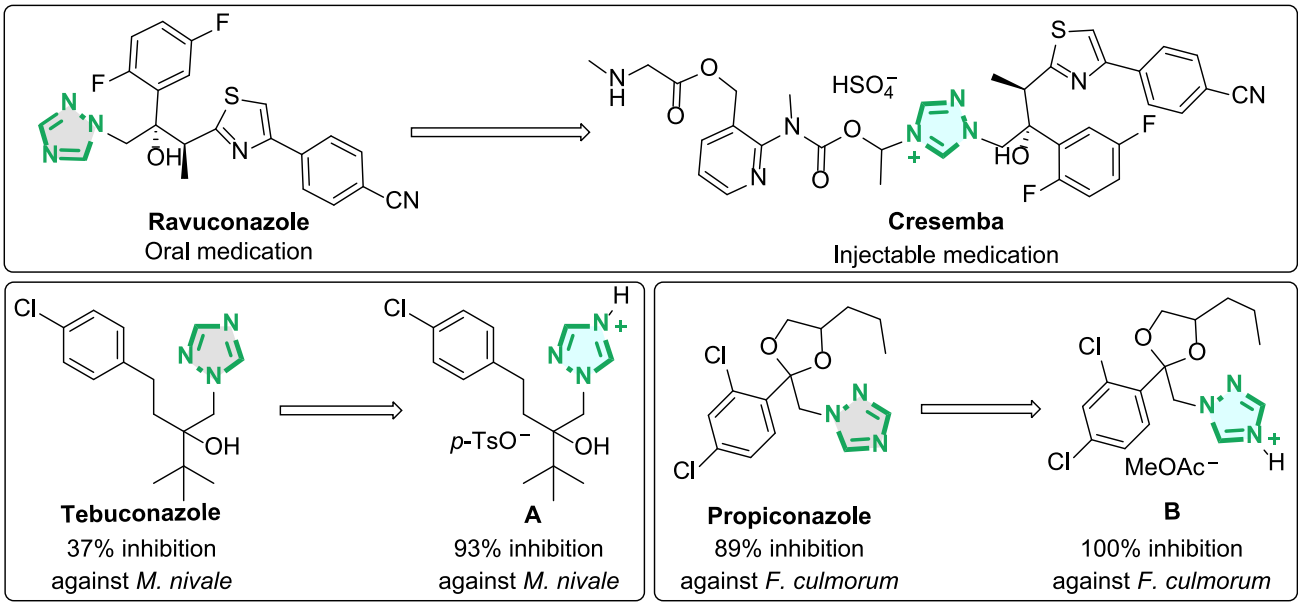


Figure 1. Representative drugs and antifungal molecules containing 1,2,4-triazolium scaffold.

candidates for next-generation antimicrobial agents, particularly overcoming resistance associated with long-term azole use.[20,25]

In this study, a series of readily available 1,2,4-triazolium salts were investigated as potential agrochemicals, and their *in vitro* and *in vivo* activities were evaluated. Additionally, scanning electron microscopy (SEM) and molecular docking were employed to elucidate the mechanism of action. This study lays the foundation for the further development of triazolium-based compounds as novel agents for plant disease control and the exploration of their broader biological applications.

2 MATERIALS AND METHODS

2.1 General procedures

These 1,2,4-triazolium compounds are commonly used as precursors of NHCs and were synthesized according to the method reported by Kerr *et al.*[26]

2.2 Chemicals and instruments

All 1,2,4-triazolium compounds were previously prepared and stored in our laboratory. (Fig. 2). The commercial fungicides Dimethomorph (DMM, 98% pure) and Prochloraz manganese (PM, 98% pure) (purchased from Shaanxi Shangge Road Bioscience Co., Ltd, Shaanxi, China) were used as positive controls.

2.3 Plant pathogens

The phytopathogens *P. capsici*, *Colletotrichum fructicola* and *Fusarium equiseti* were isolated and identified with the help of the Institute of pepper of Guizhou Academy of Agricultural Sciences and stored in the Center for R&D of Fine Chemicals of Guizhou University. The above strains were maintained on potato dextrose agar (PDA) slants. Fungi were stored at 4 °C and the oomycete at 10 °C. For activation, mycelial plugs were transferred to fresh PDA plates and incubated at 28 ± 1 °C for subsequent assays.

2.4 *In vitro* assays against *P. capsici*, *C. fructicola*, and *F. equiseti*

In vitro inhibitimethyl suloory activity tests of phytopathogens were conducted using the mycelial growth rate method.[27] Each 1,2,4-triazolium compound (2 mg) was dissolved in 200 µL of dimethyl sulfoxide (DMSO) to prepare a stock solution (10 000 µg mL^{-1}). An appropriate volume of the stock solution was incorporated into molten PDA to obtain a final concentration of 50 µg mL^{-1}, which was then poured into 60 mm Petri dishes. All treatments were conducted in triplicate, with PM and DMM used as positive controls. Plates were incubated at 28 ± 1 °C for 2–6 days. When the control colonies reached approximately two-thirds of the plate diameter, colony growth was measured using the cross method. For half-maximal effective concentration (EC_{50}) determination, compounds were tested at a series of concentrations, and EC_{50} values were calculated by probit (virulence) regression analysis based on the relationship between log concentration and inhibition rate.

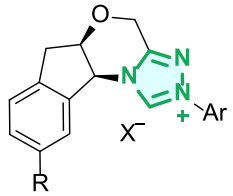
The inhibition rate is calculated as follows:

$$\text{Inhibition rate}\,(\%) = \frac{C - T}{C - 0.7} \times 100$$

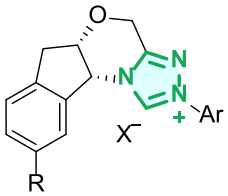
where *C* is the DMSO-treated colony diameter, *T* is the compound-treated colony diameter, 0.7 represents the diameter of the mycelium cakes (in centimeters).

2.5 *In vivo* bioassay against *P. capsici*

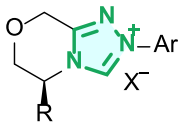
An *in vivo* glasshouse evaluation was conducted to assess the protective and curative activities of the tested compounds against pepper blight caused by *P. capsici*. Uniform pepper seedlings at the four- to five-true-leaf stage were randomly allocated to treatment groups, with three replicates per treatment and five plants per replicate. Stem inoculation was carried out using the agar plug technique.[28,29] *Phytophthora capsici* was grown on PDA medium, and 5 mm mycelial discs were collected from the actively growing margins of colonies. Each disc was attached to



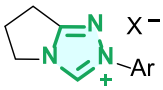
1, R = Br, Ar = C_6F_5, X = BF_4;
2, R = Br, Ar = Ph, X = BF_4;
3, R = Br, Ar = 4-OMe-Ph, X = BF_4;
4, R = Br, Ar = 2,6-Dep, X = BF_4;
5, R = Br, Ar = $C_6H_2Cl_3$, X = BF_4;
6, R = Br, Ar = Mes, X = Cl;
7, R = Br, Ar = 1-Nap, X = BF_4;
8, R = Br, Ar = 2-Nap, X = Cl;
9, R = NO_2, Ar = Mes, X = Cl;
10, R = NO_2, Ar = Mes, X = BF_4;
11, R = NO_2, Ar = Ph, X = Cl;
12, R = NO_2, Ar = Ph, X = BF_4;
13, R = NO_2, Ar = $C_6H_2Cl_3$, X = BF_4;
14, R = NO_2, Ar = C_6F_5, X = Cl;
15, R = NO_2, Ar = C_6F_5, X = BF_4.



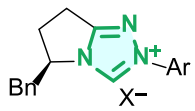
16, R = Ph, Ar = Mes, X = Cl;
17, R = 1-Nap, Ar = Mes, X = Cl;
18, R = H, Ar = Mes, X = Cl;
19, R = H, Ar = 4-OMe-Ph, X = BF_4;
20, R = H, Ar = 2,6-Dep, X = BF_4;
21, R = H, Ar = Ph, X = BF_4;
22, R = H, Ar = $C_6H_2Cl_3$, X = BF_4;
23, R = H, Ar = C_6F_5, X = BF_4.



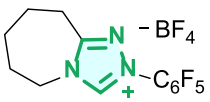
28, R = H, Ar = Mes, X = Cl;
29, R = H, Ar = Mes, X = BF_4;
30, R = H, Ar = Ph, X = BF_4;
31, R = H, Ar = C_6F_5, X = BF_4;
32, R = Bn, Ar = Ph, X = BF_4;
33, R = Bn, Ar = C_6F_5, X = BF_4;
34, R = Bn, Ar = Mes, X = Cl;
35, R = Bn, Ar = Mes, X = BF_4;
36, R = Bn, Ar = 4-OMe-Ph, X = Cl;
37, R = Bn, Ar = 4-OMe-Ph, X = BF_4;



24, Ar = Ph, X = BF_4;
25, Ar = C_6F_5, X = BF_4;
26, Ar = $C_6H_2Cl_3$, X= BF_4;
27, Ar = Mes, X = BF_4;



38, Ar = Mes, X = BF_4;
39, Ar = Ph, X = BF_4;
40, Ar = C_6F_5, X = BF_4;



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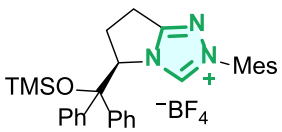

42

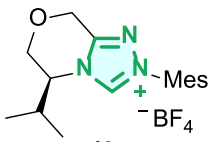

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Figure 2. 1,2,4-Triazolium salts.

the stem approximately 5 cm above the soil surface and fixed with moistened cotton and Parafilm. Following inoculation, plants were kept at 25 ± 2 °C under conditions of high relative humidity (> 90%). Disease progression was recorded at 2, 4, 6, 8, and 10 days after inoculation using a 0–9 scale, where 0 indicates no symptoms; 1, slight water-soaked lesions; 3, lesions shorter than 1 cm; 5, lesions of 1–2 cm accompanied by mild stem constriction; 7, lesions exceeding 2 cm with pronounced stem constriction and partial wilting; 9, complete plant collapse or death.

The disease index (DI) was calculated using the following formula:

$$\mathrm{DI}(\%) = \frac{\sum(n_i \times i)}{N \times i_{\max}} \times 100$$

where n_i is the number of plants at disease severity level i, i is the corresponding disease rating, N is the total number of plants evaluated, and $i_{\max}$ is the maximum disease rating (9).

Protective or curative efficacy (%) was calculated according to the following equation:

$$\text{Efficacy}(\%) = \frac{\mathrm{DI}_{\text{control}} - \mathrm{DI}_{\text{treatment}}}{\mathrm{DI}_{\text{control}}} \times 100$$

2.6 Scanning electron microscopy (SEM)

SEM was employed to investigate morphological alterations in *P. capsici* hyphae following treatment.[30,31] Hyphal samples were treated with DMSO or compound **42** (50 μg mL^{-1}) before observation. Microstructural features were visualized using a scanning electron microscope (SU8100; Hitachi, Tokyo, Japan) following established procedures.

2.7 Molecular docking studies

The crystal structure of uridine phosphorylase (UPase; PDB ID: 6KD3) was obtained from the Protein Data Bank.[5] The geometry of compound **42** was optimized using Chem3D 20.0. Ligand and protein preparation were carried out with AutoDockTools (version 1.5.6), and molecular docking simulations were performed using AutoDock 4.2 to explore the interaction between compound **42** and UPase. Docking results were analyzed and visualized with Discovery Studio 2016 and PyMOL 1.8.6. Counterions were removed before docking calculations.

3 RESULTS AND DISCUSSION

3.1 Anti-oomycete and antifungal activity bioassay *in vitro*

The *in vitro* inhibitory activities of target compounds **1**–**43** against three plant pathogens were evaluated at a concentration of 50 μg mL^{-1}, and the results are summarized in Table 1. The bioassay results revealed that compounds **16**, **17**, and **42** exhibited moderate to excellent inhibitory activities against *P. capsici*, with inhibition rates exceeding 80%. Among them, compound **42** showed an inhibition rate of 97.70% against *P. capsici*, which was comparable to that of the positive control DMM (inhibition rate of 100%). In addition, compounds **16** and **17** also displayed notable activities, with inhibition rates of 80.62% and 85.39%, respectively. Compounds **17** and **31** exhibited good antifungal

Table 1. Anti-oomycete/antifungal activities of triazolium salts at 50 μg mL^{-1} *in vitro*

Compound	Inhibitory activity (%)		
	Phytophthora capsici	*Colletotrichum fructicola*	*Fusarium equiseti*
1	—	17.80 ± 2.32	23.08 ± 17.85
2	—	6.21 ± 1.04	6.49 ± 3.36
3	—	7.23 ± 2.37	56.59 ± 3.18
4	30.74 ± 4.63	23.20 ± 1.97	16.34 ± 5.12
5	12.04 ± 5.26	12.55 ± 1.80	—
6	28.56 ± 6.09	18.61 ± 1.85	48.22 ± 11.64
7	—	14.26 ± 1.24	10.96 ± 4.58
8	26.07 ± 1.64	22.62 ± 0.34	2.86 ± 1.90
9	—	23.11 ± 10.69	9.61 ± 4.89
10	14.45 ± 2.10	9.84 ± 2.10	6.59 ± 4.94
11	1.66 ± 2.02	20.07 ± 9.96	—
12	—	2.15 ± 1.58	5.65 ± 6.16
13	27.36 ± 2.61	12.61 ± 0.16	8.86 ± 1.54
14	7.60 ± 1.87	10.97 ± 1.33	1.23 ± 2.72
15	7.07 ± 0.82	20.98 ± 1.32	—
16	80.62 ± 1.52	56.65 ± 0.34	26.62 ± 2.25
17	85.39 ± 0.72	76.35 ± 1.66	52.37 ± 1.26
18	—	32.72 ± 0.80	—
19	—	31.67 ± 3.33	—
20	—	12.18 ± 0.72	17.35 ± 3.00
21	—	—	19.96 ± 3.45
22	—	4.68 ± 1.51	20.98 ± 0.53
23	7.61 ± 0.88	5.98 ± 7.88	13.96 ± 0.87
24	—	—	21.16 ± 1.25
25	1.17 ± 2.35	27.55 ± 1.37	13.78 ± 2.90
26	—	2.99 ± 0.93	13.13 ± 6.48
27	7.20 ± 2.38	16.30 ± 8.53	9.61 ± 3.36
28	4.71 ± 1.87	—	20.33 ± 2.80
29	—	—	12.44 ± 5.14
30	—	7.38 ± 5.26	14.36 ± 1.84
31	—	71.45 ± 2.47	2.94 ± 1.89
32	—	—	6.73 ± 8.55
33	—	5.18 ± 3.78	—
34	—	8.95 ± 1.66	13.55 ± 5.7
35	—	2.02 ± 0.76	8.76 ± 8.05
36	1.55 ± 2.39	21.05 ± 12.31	1.54 ± 5.55
37	—	6.39 ± 1.98	—
38	—	2.91 ± 0.27	15.48 ± 1.83
39	—	1.45 ± 0.15	13.57 ± 5.28
40	4.22 ± 2.14	—	18.45 ± 2.36
41	—	7.68 ± 4.60	5.07 ± 2.80
42	97.70 ± 2.30	67.36 ± 1.02	63.14 ± 2.75
43	—	8.86 ± 1.64	9.05 ± 5.25
PM		100.00	94.47 ± 1.60
DMM	100.00		

Note: Values are mean ± standard deviation of three replicates. '—' represents an inhibition rate of less than 1%. Prochloraz manganese salt (PM), Dimethomorph (DMM).

activity against *C. fructicola*, with inhibition rates of 76.35% and 71.45%, respectively. In contrast, all tested compounds (compounds **1**–**43**) exhibited relatively weak activities against *F. equiseti*, with inhibition rates below 70%. Based on the preliminary bioassay results, the EC_{50} value of compound **42** with notable *in vitro* antifungal activity was determined (Table 2). The EC_{50} value for compound **42** against *P. capsici* was 5.38 μg mL^{-1}, higher than that of the positive control DMM (0.23 μg mL^{-1}).

Table 2. Half-maximal effective concentration (EC_{50}) determination of compound **42** against *Phytophthora capsici*

Compound	Regression equation	R^2	EC_{50} (μg mL^{-1})
42	$y = 3.53 + 2.01x$	0.9834	5.38
DMM	$y = 6.36 + 2.12x$	0.9818	0.23

Note: Dimethomorph (DMM), coefficient of determination (R^2).

3.2 Structure–activity relationship (SAR)

The structure–activity relationship (SAR) analysis revealed that antifungal and anti-oomycete activities of 1,2,4-triazolium compounds are strongly influenced by substituent hydrophobicity, steric bulk, and π-conjugation, with bulky aromatic or electron-withdrawing groups significantly enhancing inhibitory activities against *P. capsici* and phytopathogenic fungi. In the *in vitro* anti-oomycete and antifungal assays (Table 1), the majority of the synthesized 1,2,4-triazolium compounds exhibited low or even negligible activity against *P. capsici*, *C. fructicola*, and *F. equiseti*. It is well documented that 1,2,4-triazole derivatives often display pronounced variability in bioactivity across different fungal and oomycete species, with antifungal efficacy being highly dependent on the nature of the substituents.[32,33] The triazole core confers limited intrinsic activity against phytopathogens, particularly when the appended side chains are poorly matched to the binding requirements of the biological target.[34,35]

For compounds **1**–**15**, the introduction of a methoxyphenyl or mesityl group at the triazolium nitrogen slightly enhanced inhibitory activity against *F. equiseti*. The incorporation of aromatic or bulky substituents alters the hydrophobic character of the molecules, which may moderately improve target interactions; however, the overall enhancement remained limited. Consistent with previous SAR studies on 1,2,4-triazole derivatives, both the electronic properties and steric bulk of substituents play important roles in modulating antifungal activity.[36,37] Compounds **16**–**23** bearing an additional phenyl or naphthyl group at the C-2 position of the phenyl ring showed a marked improvement in inhibitory activity against *P. capsici*, while also exhibiting moderate activity against *C. fructicola* and *F. equiseti*. This structural modification increases molecular hydrophobicity and expands the π-conjugated system, which may facilitate stronger interactions with target proteins or enhance penetration through the fungal cell wall or membrane. Similar observations have been reported for triazole-based agrochemicals, where substituent effects play a decisive role in antifungal performance.[38–40] Notably, compound **31**, featuring a pentafluorophenyl substituent attached to the triazolium nitrogen, displayed unexpectedly high inhibitory activity against *C. fructicola* compared with its analogs. Furthermore, upon replacement of the substituent in compound **27** with a bulkier group at the C-6 position to afford compound **42**, a remarkable enhancement in inhibitory activity against *P. capsici* was observed, while good activity against both *C. fructicola* and *F. equiseti* was retained. In contrast, when a benzyl group was introduced as the substituent at the same position (compound **38**), no such increase in activity was observed. These findings

Table 3. Protective and curative efficacies (%) of compound **42** against *Phytophthora capsici in vivo*.

		Protective activity		Curative activity	
Compound	Time (days)	Disease index (%)	Control efficiency (%)	Disease index (%)	Control efficiency (%)
CK	2	76	—	76	—
	4	83	—	83	—
	6	94	—	94	—
	8	91	—	91	—
	10	96	—	96	—
42	2	52	32	76	0
	4	60	28	77	7
	6	56	41	87	8
	8	56	39	87	5
	10	58	40	87	9
DMM	2	60	21	100	0
	4	44	47	77	7
	6	60	36	73	22
	8	42	54	82	10
	10	42	56	82	14

Note: Control (CK), Dimethomorph (DMM).

suggest that the antifungal and anti-oomycete activities of 1,2,4--triazolium compounds may arise from a combination of structural and physicochemical factors. The positively charged triazolium moiety may contribute to electrostatic interactions that enhance adsorption onto the negatively charged microbial membrane surface, thereby increasing the probability of productive interactions with intracellular or membrane-associated targets.[12,19,41]

3.3 *In vivo* activity against *P. capsici*

The protective and curative activities of compound **42** were evaluated over a 10-day period and directly compared with those of the commercial fungicide DMM, as summarized in Table 3. Under protective treatment conditions, compound **42** exhibited moderate and relatively stable activity, with control efficiency ranging from 28% to 40%. In comparison, DMM showed higher protective efficacy, with control values increasing from 21% at day 2 to 56% at day 10. Although DMM was generally more effective, compound **42** maintained a consistent level of protection, particularly from day 6 onward. In terms of curative activity, compound **42** showed relatively low efficacy, remaining below 10% throughout the experimental period. Overall, while compound **42** was less effective than DMM under the tested conditions, it demonstrated sustained protective activity, suggesting its potential as a preventive agent.

3.4 Scanning electron microscopy (SEM)

SEM observations revealed pronounced morphological alterations in the hyphae of *P. capsici* following treatment with compound **42** (Fig. 3). In the negative control group treated with DMSO (Fig. 3(A),(C)), the hyphae exhibited a typical filamentous morphology with smooth surfaces, uniform thickness, and intact structural integrity. In contrast, hyphae exposed to compound **42** at 50 μg mL^{-1} showed severe morphological abnormalities (Fig. 3(B),(D)). The treated mycelia exhibited pronounced deformation, characterized by surface wrinkling, collapse, and loss of cylindrical structure, indicating severe impairment of hyphal integrity. These results indicate that compound **42** induces substantial structural disruption of *P. capsici* hyphae, which is consistent with its strong *in vitro* anti-oomycete activity.

3.5 Molecular docking studies

To rationalize the potent anti-oomycete activity of compound **42**, molecular docking studies were performed. The predicted binding mode and interaction profile of compound **42** within the active site of the UPase protein receptor are illustrated in Fig. 4. UPase (PDB ID: 6KD3), a key enzyme involved in pyrimidine nucleoside salvage and nucleotide metabolism,[42] was selected as a potential docking target to explore whether 1,2,4-triazolium salts could interfere with essential metabolic processes in *P. capsici*.[5] The docking pose shows that the 1,2,4-triazolium compound **42** is well accommodated within the substrate-binding pocket of UPase. The cationic heterocycle is surrounded by hydrophobic residues such as Ile109, Leu270, and Phe211, forming extensive van der Waals and π–alkyl interactions, while polar residues including Thr140 and Glu290 contribute additional electrostatic and weak hydrogen-bond-like contacts. These interactions collectively stabilize the ligand within the active pocket and may interfere with substrate access or positioning, thereby impairing enzymatic function.[43] UPase may serve as a potential target of 1,2,4-triazolium compounds against oomycetes, potentially providing a novel mechanism of action.

4 CONCLUSION

In summary, we evaluated the anti-oomycete and antifungal activities of 43 1,2,4-triazolium compounds and identified a candidate (compound **42**) with high activity against *P. capsici*. Compound **42** was the most potent, showing an inhibition rate of 97.7% at 50 μg mL^{-1} and an EC_{50} value of 5.38 μg mL^{-1}, although its activity remained lower than that of the commercial fungicide DMM (EC_{50} = 0.23 μg mL^{-1}). At 200 μg mL^{-1}, compound **42** achieved a protective efficacy of 32% against *P. capsici* at 2 days post-treatment, comparable to DMM (21%); however, its effect declined over time and was inferior to DMM at later stages.

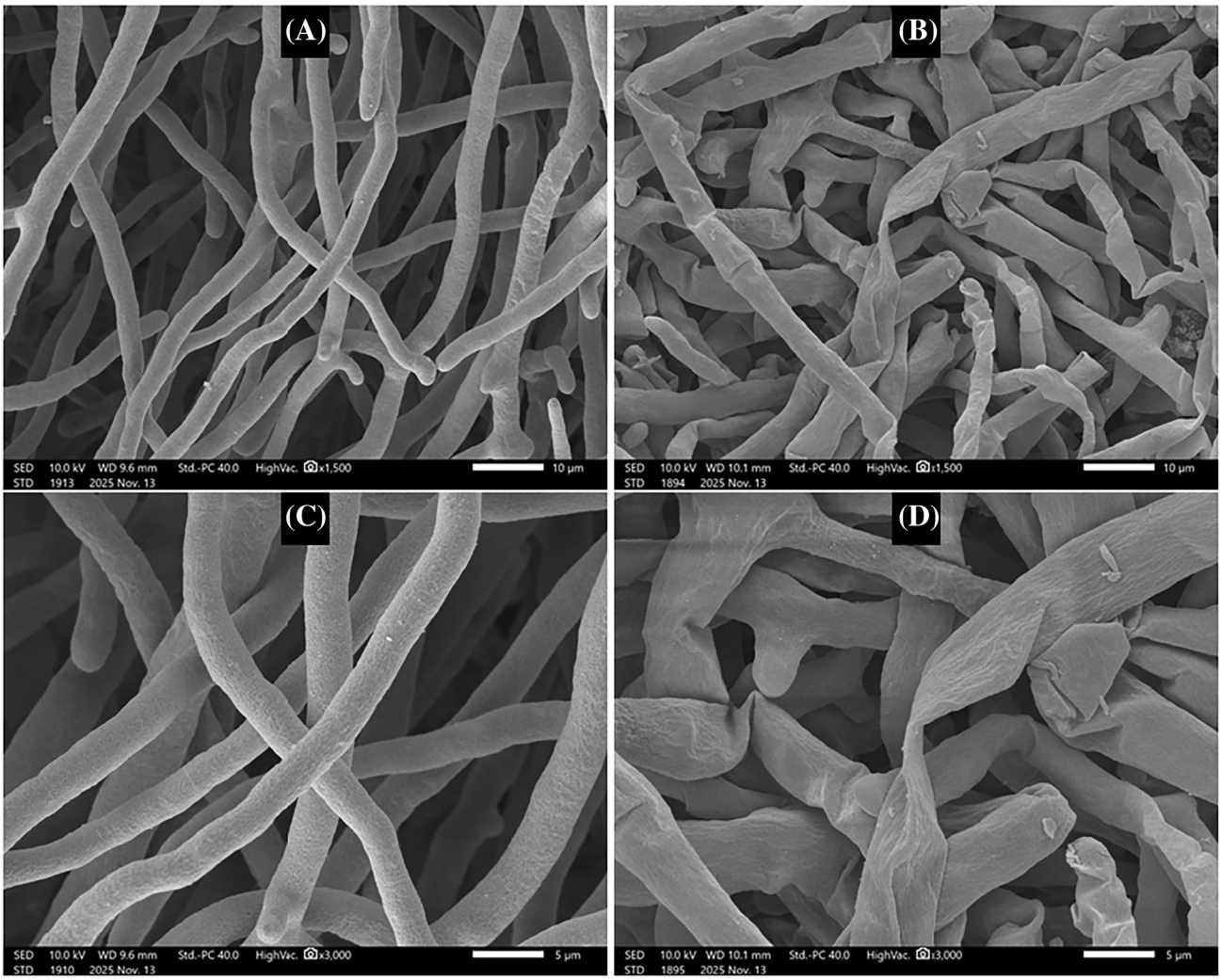

Figure 3. Effects of compound **42** on the mycelium morphology of *Phytophthora capsici* by SEM. (A, C) Negative control. (B, D) Treatment with compound **42** at 50 µg mL^{-1}.

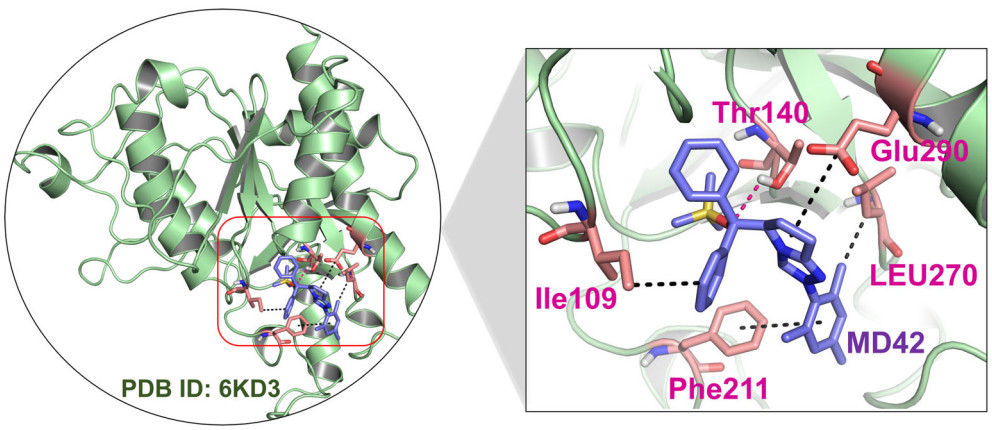

Figure 4. Molecular docking results of compound **42** with UPase (PDB ID: 6KD3). The red dotted line represents a hydrogen bond. The black dotted line represents a π-alkyl bond.

Molecular docking indicated favorable interactions between compound **42** and key residues of the UPase protein, suggesting that UPase may serve as a potential target for 1,2,4-triazolium compounds. Consistently, SEM analysis revealed pronounced morphological damage to hyphae, including surface wrinkling and collapse. Overall, these findings highlight compound **42** as

a promising lead for further structural optimization in the development of new anti-oomycete agents.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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