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Design, synthesis, and antimicrobial evaluation of novel 1,2,4-trizaole thioether derivatives with a 1,3,4-thiadiazole skeleton

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The aim of this study was to design and synthesize 17 novel 1,2,4-triazole thioether derivatives containing 1,3,4-thiadiazole thioether. Bioactivity assays indicated that several target compounds exhibited moderate to good antifungal and antibacterial activities. Notably, compound **9d** demonstrated significantly stronger antifungal activity against *Trichoderma* sp. in *Morchella esculenta* (TSM) and *Mucor* sp. in *Dictyophora rubrovalvata* (MSD), with EC_{50} values of 9.25 and 12.95 μg mL^{-1}, respectively, compared to the commercial fungicide pyrimethanil, which had EC_{50} values of 35.29 and 15.51 μg mL^{-1}. Furthermore, compound **9d** found to have strong antibacterial activity against *Pseudomonas syringae* pv. *actinidiae* (PSA) *in vitro*, with an EC_{50} of 9.34 μg mL^{-1}, markedly superior to that of thiodiazole copper (81.74 μg mL^{-1}). Additionally, the *in vivo* anti-PSA assessment revealed therapeutic and protective activities at 200 μg mL^{-1} of 59.39% and 79.33%, respectively, outperforming thiodiazole copper (38.91% and 67.64%, respectively). According to molecular docking simulations the interaction between compound **9d** and FtsZ was mainly due to hydrogen bond formations with GLU-296, ARG-301, LYS-188, ASP-29 and GLY-30. This report suggests that these novel 1,2,4-triazole thioether derivatives featuring a 1,3,4-thiadiazole skeleton may effectively mitigate fungal and bacterial threats to plants.

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1. Introduction

The influence of chemical pesticides on production and daily life has become increasingly significant.[1,2] Through over half a century of relentless efforts and exploration, significant progress has been made in the discovery, widespread adoption, and application of chemical pesticides.[3] These advancements have enabled the successful control of numerous diseases, pests, weeds, and other threats that jeopardize human survival, as well as agricultural, forestry, and animal husbandry production. In the 21st century, chemical pesticides are expected to continue evolving and play a crucial role in addressing global food challenges.[4]

Heterocyclic compounds are closely related to pharmaceutical science, life science, and material science. Research on heterocyclic compounds is not only pertinent to daily life but also plays a significant role in the advancement of modern science and technology across various fields. Nitrogen-containing compounds hold a crucial position within heterocyclic compounds, with 1,2,4-triazole being a notable nitrogenous five-membered ring compound characterized by a wide range of biological activities, which herbicidal,[5] antibacterial,[6] antifungal,[7] antiviral,[8] insecticidal[9] and other biological activities. Its unique structure renders it irreplaceable in the fields of medicine and pesticide development (Fig. 1), which has led to sustained interest from researchers in recent years.

Moreover, 1,3,4-thiadiazole derivatives were aromatic five-membered heterocycles containing one sulfur atom and two nitrogen atom. Notably, commercialized agricultural fungicides (Fig. 1) based on 1,3,4-thiadiazole include chlorothalonil, thiazolyl zinc, thiabendazole, thiadiazine, and thiadiazole copper.[10] These compounds exhibit a diverse array of agricultural biological activities, they serve as antibacterial,[11] antiviral,[12] fungicides,[13] insecticides,[14] herbicides,[15] and plant growth regulators.[16]

In recent years, natural products have served as valuable scaffolds for structural modification of lead compounds, offering promising avenues for antibacterial drug discovery.[16–18] Gallic acid, a widely studied natural compound, exhibits diverse biological activities, such as antibacterial,[19] antimicrobial,[20] antiviral,[21] fungicides,[22] insecticides,[23] and herbicidal[24] activities. In our earlier research, we developed a range of 1,3,4-thiadiazole thioether and 1,2,4-triazole thioether derivatives (Fig. 2), which demonstrated suitable antibacterial, antifungal, insecticidal, and antiviral properties.[25–31] In addition,

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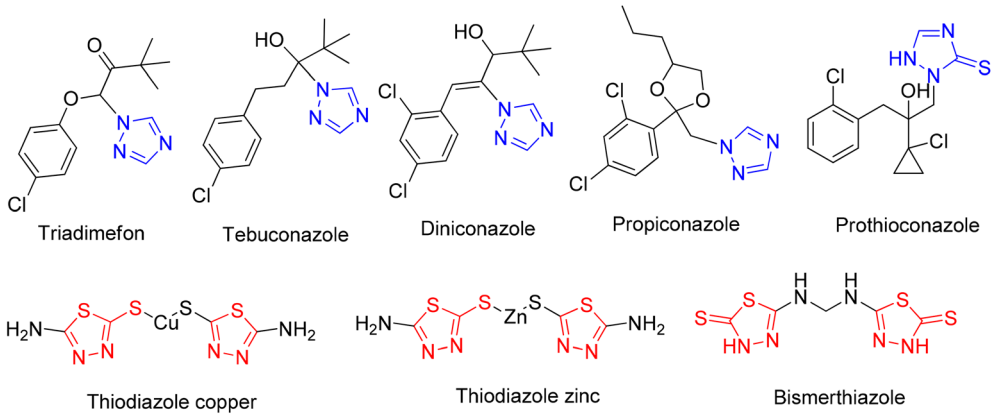


Fig. 1 Structures of some commercialized agricultural agents containing triazole moieties.

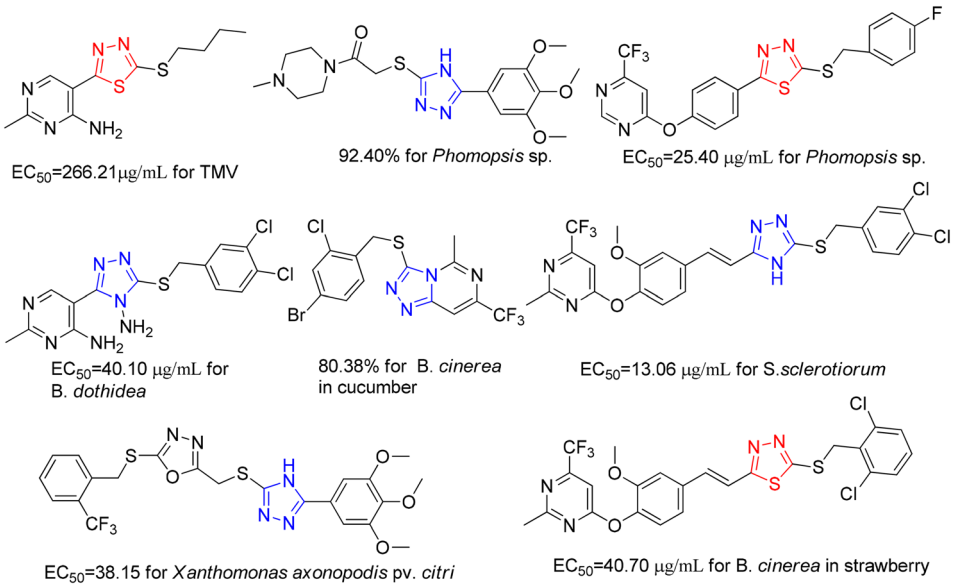


Fig. 2 Our team synthesized derivatives featuring 1,2,4-triazole thioether and 1,3,4-thiadiazole thioether during the initial phase.

considering both the importance of 1,2,4-trizaole thioether and 1,3,4-thiadiazole thioether moieties in agrochemicals, a reasonable strategy to explore highly active novel agents is combining different active fragments into one single hybrid molecule. In this study, we designed and synthesized a series of 1,2,4-triazole sulfide derivatives based on the natural product gallic acid, incorporating a 1,3,4-thiadiazole structural motif (Fig. 3). In total, 17 new 1,2,4-triazole thioether compounds featuring a 1,3,4-thiadiazole scaffolds were created and their antibacterial and antifungal properties were examined *in vitro*.

2. Materials and methods

2.1. Instruments and chemicals

All chemicals were obtained from commercial suppliers (Aladdin Reagent, Shanghai, China). Melting point determinations were performed using an XT-4 binocular microscope (Shanghai Electrophysics Optical Instrument Co., Ltd). Structural characterization was carried out through ^{1}H and ^{13}C NMR spectroscopy (Bruker 600 MHz spectrometer) and high-resolution mass spectrometry (Thermo Scientific Q-Exactive system).

2.2. Chemical synthesis

2.2.1 Preparation procedure of intermediate 2 to intermediate 8. Preparation of intermediate **2** to intermediate **8** according to the previous research work of our group (Scheme 1).[31]

2.2.2 Preparation procedure of 5-(((5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-3-yl)thio)methyl)-1,3,4-thiadiazole-2-thiol. A mixture was prepared at room temperature consisting of 2-((5-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetohydrazide (50 mmol) and KOH (55 mmol). CS_2 (75 mmol) was slowly added at the same temperature after the solid had dissolved.[32] During the reaction, a significant amount of white solid precipitated. After the reaction was complete, the

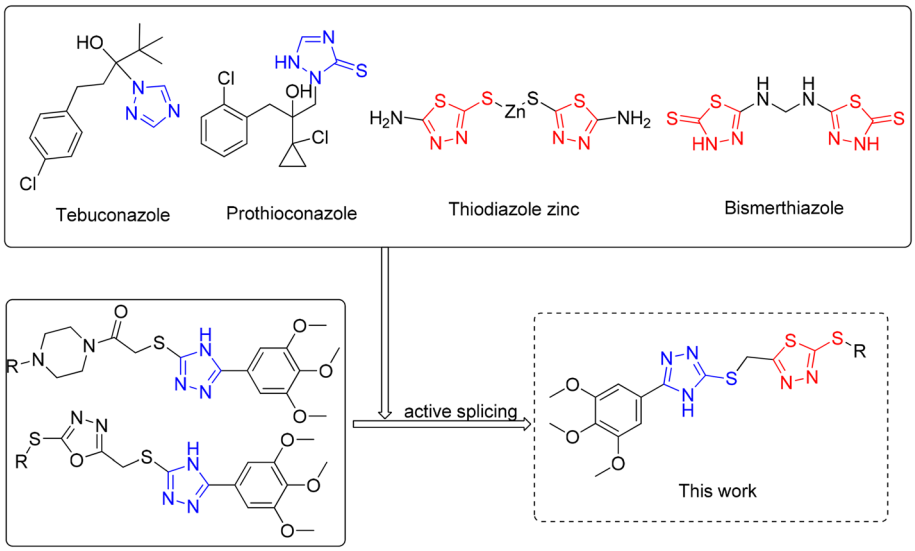
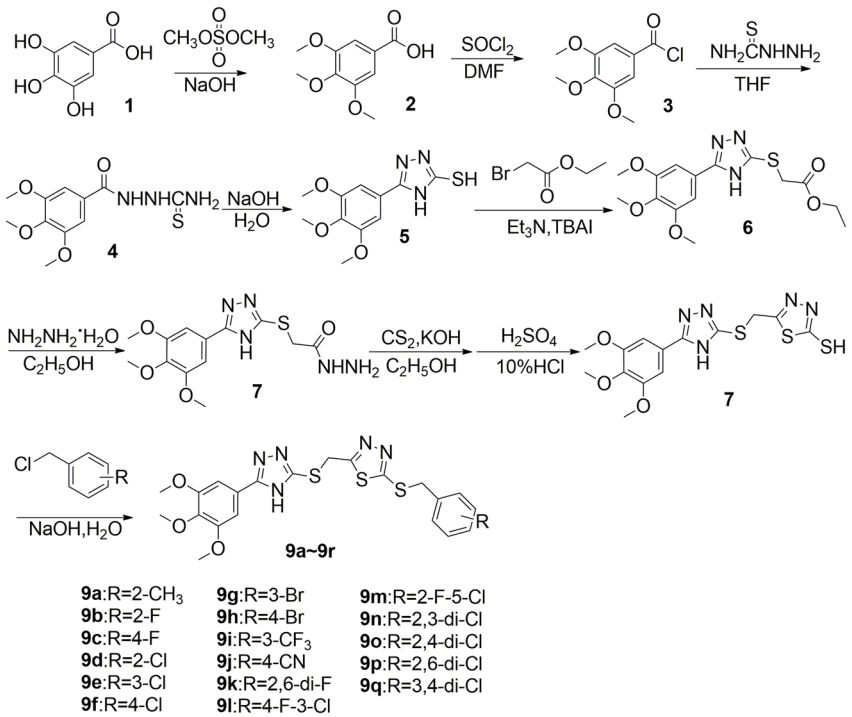


Fig. 3 The molecular design involves 1,2,4-triazole thioether derivatives that incorporate a 1,3,4-thiadiazole backbone.



Scheme 1 Preparation method of target compounds **9a–9q**.

solid was filtered to remove moisture. The resulting white solid was then added to concentrated sulfuric acid (50 mL) and stirred for 2 hours. Subsequently, sodium hydroxide solution (75 mL) was added, followed by water (200 mL). After filtering, 10% hydrochloric acid was added to the mixture. Following another filtration step, 5-(((5-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4-triazol-3-yl)thio)methyl)-1,3,4-thiadiazole-2-thiol was obtained.

2.2.2.1 5-(((5-(3,4,5-Trimethoxyphenyl)-4H-1,2,4-triazol-3-yl) thio)methyl)-1,3,4-thiadiazole-2-thiol (7). White solid; yield 65.3%; m.p. 138–140 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 14.58 (s, 1H, N–H), 14.43 (s, 1H, SH), 7.29 (s, 2H, Ph–H), 4.54 (s, 2H, –SCH_2–), 3.86 (s, 6H, CH_3O–), 3.72 (s, 3H, CH_3O–); ^{13}C NMR (150 MHz, DMSO-d_6) δ 189.25, 162.09, 158.13, 156.09, 153.80, 139.76, 122.21, 104.04, 60.62, 56.51, 30.55.

2.2.3 Preparation procedure of the target compounds 9a–9q. The target compounds **9a–9q** were synthesized according to a previously established literature procedure.[28] A 100 mL round-bottom flask was charged with intermediate 7 (20 mmol),

followed by the addition of an aqueous solution containing NaOH (22 mmol in 20 mL of water). After thorough mixing and complete dissolution, various substituted benzyl chlorides (20 mmol) were introduced into the reaction system. The mixture was maintained at room temperature for 5 to 6 hours, during which a white precipitate formed. The solid product was then isolated by filtration and subsequently dried. Subsequent purification *via* column chromatography successfully afforded the title compounds **9a–9q**. ^{1}H NMR, ^{13}C NMR and HRMS data for derivatives **9b–9q**, which were provided in SI.

*2.2.3.1 2-((2-Methylbenzyl)thio)-5-(((5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-3-yl)thio)methyl)-1,3,4-thiadiazole (**9a**).* White solid; yield 62.7%; m.p. 123.9–126.1 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 14.55 (s, 1H, N–H), 7.33 (d, 1H, J = 7.5 Hz, Ph–H), 7.29 (s, 2H, Ph–H), 7.17 (d, 2H, J = 4.14 Hz, Ph–H), 7.11–7.08 (m, 1H), 4.79 (s, 2H, –SCH$_2$–), 4.53 (s, 2H, –SCH$_2$–), 3.84 (s, 6H, CH$_3$O), 3.72 (s, 3H, CH$_3$O), 2.33 (s, 3H, CH$_3$); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.07, 166.03, 158.28, 156.10, 153.79, 139.75, 137.28, 134.12, 130.89, 130.45, 128.52, 126.52, 104.06, 60.63, 56.52, 36.39, 29.93, 19.19; HRMS (ESI) m/z: $[C_{22}H_{24}N_5O_3S_3 + H]^+$ calcd. 502.10358, found 502.10373.

2.3. *In vitro* antifungal activity test

The *in vitro* antifungal activities of **9a–9q** as against *Magnaporthe grisea* (MG), *Phomopsis* sp. (PS), *Trichoderma* sp. in *Morchella esculenta*, *Botryosphaeria dothidea* (BD), *Mucor* sp. in *Dictyophora rubrovalvata* (MSD), and *Botrytis cinerea* in blueberry (BCB) were evaluated.[33–35] The target compound was prepared as 50 μg mL^{-1} working solution in DMSO (1 mL) and incorporated into PDA plates (5 mg). Under aseptic conditions, a 0.4 cm mycelial disc was centrally inoculated on each plate. Cultivation proceeded for 3–4 days at 28 °C, with equivalent concentrations of DMSO (negative control), pyrimethanil (Pry) included for comparative analysis. Antifungal activity was quantified using the inhibition rate (I), calculated from the formula $I = [(C - T)/C] \times 100\%$, where C and T represent fungal colony diameters in control and treated plates, respectively. This quantitative assessment provided reliable evaluation of the compounds' antifungal efficacy.

2.4. *In vitro* antibacterial activity test

The synthesized compounds (**9a–9q**) were evaluated against three key phytopathogens: *Xanthomonas oryzae* pv. *oryzicola* (XOO), *X. axonopodis* pv. *citri* (XAC), and *Pseudomonas syringae* pv. *actinidiae* (PSA).[31,35] Stock solutions (25 mg mL^{-1}) were prepared by dissolving 3.75 mg samples in 150 μL DMSO, followed by serial dilution in 0.1% Tween-80 aqueous solution. Working concentrations of 100 and 50 μg mL^{-1} were achieved using nutrient broth (NB) as diluent. For bioassays, 40 μL bacterial suspensions (OD_{595} = 0.6–0.8, log phase) were inoculated into test tubes containing compound solutions. Cultures were incubated at 28 °C with 180 rpm agitation for 48 h. Growth inhibition was quantified by measuring optical density at 595 nm using a Multiskan Sky1530 spectrophotometer. Thiodiazole copper (TC) and DMSO served as positive and negative controls, respectively.

2.5. SEM studies on the cell morphology

The centrifugation process of a culture medium containing PSA with a quantity of 30 mL was performed in order to prepare a bacterial liquid.[16] The solution was then mixed in the substrate and incubated until reaching an optical density (OD_{595}) between 0.6 and 0.8. The final concentration of compound **9d**, added after that, was 50 μg mL^{-1} and incubated for 12 h. The negative control was an equal volume of DMSO. After that, we washed the PSA cells three times with phosphate-buffered saline and fixed them overnight in 2.5% glutaraldehyde at 4 °C. The samples were then consecutively dehydrated with ethanol solutions at the concentration of 10, 20, 40, 60, 80, 90 and 100%. The samples were freeze-dried and gold coated prior to examination.[28]

2.6. Anti-kiwifruit ulcer disease experiment *in vivo*

The collected kiwifruit leaves were washed and cleaned 2 to 3 times with deionized water. Kiwifruit leaves were set on the filter paper for drying. Or they were wiped using a paper towel. Using a puncher with a 2.5 cm diameter, leaves were cut into uniform round discs.[36,37] The leaves were soaked with 10 mL PSA bacterial solution for 4 hours for a protection experiment and were then removed and placed on a filter paper plate that had been set up. The leaves were dried for 30 minutes and soaked in a solution of 200 μg mL^{-1} (with DMSO as a blank) 10 mL for 30 min. In the experiment, the compound (active compound) was immersed for 4 hours in 10 mL of a 200 μg mL^{-1} solution and the compound was again immersed in 10 mL of the PSA bacterial solution. Treated kiwifruit leaves placed in incubator for further cultivation. Three days after inoculation, the incidence of leaves was recorded and percentages of the lesion area were estimated using ImageJ software. The results were recorded.

$$\text{Control effect}\% = (\text{susceptible range of control group} - \text{susceptible range of treatment group})/\text{susceptible range of control group} \times 100\%$$

2.7. Molecular docking

Not only does the FtsZ enzyme control the division of bacterial cells, it is also responsible for the formation of a dividing cell wall.[37] To investigate the molecular interactions, we performed binding pattern analysis between FtsZ protein and compound **9d** using Discovery studio 2.5 software. According to previously discussed methods, this molecular docking studies helped in understanding the interaction of compound **9d** with FtsZ.

3. Result and discussion

3.1. Chemistry

The target compounds **9a–9q** were prepared from 3,4,5-trimethoxybenzoic acid *via* a seven-step synthetic route involving sequential chlorination, condensation, cyclization, etherification, hydrazinolysis, cyclization, and thioetherification reactions, with isolated yields ranging from 28.14% to 62.40%. The title compounds were confirmed using techniques such as

Table 1 Inhibitory effects[a] of synthesized compounds (**9a–9q**) on fungal activities at 50 μg mL^{-1}

Compd	MG	TSM	PS	BD	BCB	MSD
9a	33.73 ± 1.89	39.80 ± 1.33	58.34 ± 1.96	66.79 ± 3.27	73.07 ± 3.13	39.39 ± 4.67
9b	10.15 ± 1.20	39.12 ± 1.11	32.65 ± 1.41	20.61 ± 4.22	24.18 ± 4.84	15.15 ± 1.52
9c	33.43 ± 1.51	46.35 ± 1.72	56.60 ± 1.38	9.16 ± 2.11	45.10 ± 2.55	40.07 ± 2.01
9d	42.09 ± 2.57	79.52 ± 1.95	69.32 ± 1.18	10.69 ± 2.98	43.46 ± 3.72	75.59 ± 2.26
9e	48.06 ± 1.43	63.74 ± 1.30	47.28 ± 1.34	8.40 ± 3.88	32.03 ± 1.27	64.24 ± 3.58
9f	13.13 ± 1.22	23.13 ± 4.30	32.65 ± 1.90	11.83 ± 1.15	8.82 ± 1.81	47.27 ± 1.88
9g	28.68 ± 1.24	11.09 ± 1.06	34.15 ± 1.34	6.85 ± 0.92	32.95 ± 1.21	35.23 ± 1.24
9h	8.06 ± 1.10	8.91 ± 1.06	8.87 ± 0.86	5.48 ± 0.96	17.05 ± 1.08	32.77 ± 1.18
9i	22.99 ± 1.14	24.55 ± 1.13	42.69 ± 1.23	19.01 ± 0.98	37.88 ± 1.20	40.34 ± 1.24
9j	36.42 ± 1.55	51.70 ± 1.92	32.99 ± 1.65	20.23 ± 5.39	31.05 ± 1.35	31.65 ± 2.15
9k	6.09 ± 0.88	5.94 ± 1.03	12.64 ± 0.91	19.35 ± 1.16	28.79 ± 1.16	27.65 ± 1.05
9l	65.42 ± 1.62	22.97 ± 0.98	34.65 ± 1.38	20.21 ± 0.76	81.25 ± 1.24	54.73 ± 1.17
9m	23.77 ± 1.15	5.35 ± 0.96	30.87 ± 1.10	17.12 ± 0.97	42.61 ± 1.36	33.14 ± 1.63
9n	25.07 ± 0.88	40.82 ± 3.09	35.71 ± 1.42	1.53 ± 2.43	30.72 ± 3.03	48.96 ± 2.05
9o	20.60 ± 1.27	29.93 ± 3.98	21.09 ± 1.09	6.49 ± 1.07	16.67 ± 1.76	37.90 ± 1.86
9p	32.24 ± 1.15	28.91 ± 2.54	36.73 ± 1.13	14.89 ± 1.70	5.23 ± 1.50	29.92 ± 2.98
9q	19.25 ± 1.16	6.93 ± 0.88	47.78 ± 1.25	14.73 ± 0.97	38.64 ± 1.49	28.79 ± 1.16
Pyr	55.41 ± 1.65	51.83 ± 1.27	86.56 ± 1.76	87.16 ± 1.54	70.11 ± 3.76	74.36 ± 1.07

[a] Average of three replicates.

Table 2 EC_{50} of selected derivatives against fungal pathogens

Pathogens	Compound	Toxic regression equation	R^2	EC_{50} (μg mL^{-1})
TSM	**9d**	$y = 1.1254x + 3.0648$	0.9979	9.25 ± 1.04
	9e	$y = 0.7253x + 4.051$	0.9990	26.38 ± 2.38
	Pyr	$y = 0.4171x + 4.3494$	0.9929	36.29 ± 1.43
BC	**9a**	$y = 1.3516x + 2.8469$	0.9863	30.26 ± 1.74
	9m	$y = 1.3642x + 2.6512$	0.9937	45.13 ± 2.23
	Pyr	$y = 0.5964x + 4.2761$	0.9625	51.26 ± 1.86
MSD	**9d**	$y = 1.694x + 3.5768$	0.9984	14.95 ± 1.16
	Pyr	$y = 1.3867x + 3.349$	0.9989	15.51 ± 1.08

^{1}H NMR, ^{13}C NMR, and HRMS. Analysis of the NMR spectra for compound 8d confirms the presence of N–H atoms in the 1,2,4-triazole moiety. The singlet observed at 7.29 ppm corresponds to a hydrogen atom on the benzene ring, while the peaks at 4.78 and 4.56 ppm indicate the presence of two hydrogens from the two –SCH_2– groups. Furthermore, high-resolution mass spectrometry (HRMS) analysis of compound **9d** confirmed its molecular structure through detection of $[M + Na]^+$ ions (*m*/*z* 522.04907), validating successful synthesis. Comprehensive spectroscopic characterization, including ^{1}H NMR, ^{13}C NMR and HRMS data for derivatives **9a–9q**, were provided in SI.

3.2. Antifungal activity test *in vitro*

As can be seen in Table 1, the title compounds **9a–9q** showed impressive *in vitro* antifungal activities against MG (6.09–65.42%), TSM (3.59–79.52%), PS (6.90–69.32%), BD (3.83–66.79%), BC (12.23–81.25%), and MSD (15.15–75.59%). Notably, the inhibitory rates of **9d** and **9e** against TSM were 79.52% and 63.74%, respectively, which are more than that of pyrimethanil (51.83%). In addition, compounds **9a** and **9l** had moderate inhibition rates of 73.07 and 81.25% against BCB which are better than 70.11% of pyrimethanil. Furthermore, the inhibition rate that was found for compound **9d** on MSD was 75.59% which was comparable to that of pyrimethanil (74.36%). These results highlight the potential of these compounds as effective antifungal agents.

The EC_{50} values were determined to evaluate the activity of compounds against plant pathogenic fungi. As presented in Table 2, compound **9d** exhibited significant activity against TSM (EC_{50} = 9.25 μg mL^{-1}), which was superior to the control pyrimethanil (Pyr), with an EC_{50} of 36.29 μg mL^{-1}. Similarly, the EC_{50} values for compounds **9a** (30.26 μg mL^{-1}) and **9l** (45.13 μg mL^{-1}) were better than that of Pyr (51.26 μg mL^{-1}). Furthermore, compound **9d** demonstrated an EC_{50} value of 14.95 μg mL^{-1}, which was comparable to the control Pyr's EC_{50} value of 15.51 μg mL^{-1}.

3.3. *In vitro* antibacterial activity test

Compounds **9a–9q** were screened for their *in vitro* antibacterial efficacy against three plant pathogens (XOO, XAC, and PSA) through turbidimetric analysis. The preliminary results were summarized Table 3, indicating that several title compounds showed good antibacterial activity compared to thiodiazole copper. It was noteworthy that the inhibitory rates of

Table 3 The target compounds were evaluated for their antibacterial efficacy[a] against the test bacterial strains at 200 μg mL^{-1} and 100 μg mL^{-1}

	PSA		XOO		XAC	
Compd	200 μg mL^{-1}	100 μg mL^{-1}	200 μg mL^{-1}	100 μg mL^{-1}	200 μg mL^{-1}	100 μg mL^{-1}
9a	80.98 ± 4.26	69.47 ± 5.17	41.04 ± 2.31	28.31 ± 3.69	86.54 ± 3.43	54.34 ± 3.27
9b	100	100	19.13 ± 1.87	13.82 ± 3.49	82.15 ± 2.78	44.88 ± 4.39
9c	100	100	33.60 ± 2.52	22.43 ± 4.59	75.12 ± 2.51	42.67 ± 4.16
9d	100	100	52.02 ± 1.47	34.96 ± 3.54	61.39 ± 4.53	51.82 ± 6.45
9e	100	100	68.36 ± 4.67	34.07 ± 1.52	62.75 ± 0.06	35.47 ± 4.36
9f	100	100	33.86 ± 2.27	26.15 ± 3.62	24.79 ± 3.36	18.01 ± 2.65
9g	100	91.50 ± 2.76	0	0	62.54 ± 1.54	43.46 ± 2.15
9h	100	94.97 ± 1.40	26.52 ± 0.80	18.09 ± 1.10	65.45 ± 3.45	33.25 ± 2.26
9i	100	94.12 ± 1.32	44.67 ± 1.17	23.94 ± 1.22	52.83 ± 2.38	41.34 ± 0.83
9j	100	81.79 ± 1.50	42.75 ± 3.43	33.51 ± 1.48	79.31 ± 3.16	36.66 ± 2.72
9k	100	100	70.33 ± 2.37	37.96 ± 0.84	71.28 ± 1.26	23.56 ± 1.75
9l	100	94.88 ± 1.82	31.58 ± 3.42	27.34 ± 1.61	68.78 ± 1.36	30.29 ± 1.28
9m	100	100	66.03 ± 2.48	11.40 ± 1.68	72.63 ± 3.53	35.89 ± 0.96
9n	100	100	52.96 ± 2.56	40.81 ± 2.37	85.82 ± 3.48	67.37 ± 6.36
9o	100	100	61.40 ± 3.49	34.87 ± 2.12	89.54 ± 4.25	62.37 ± 1.83
9p	100	89.01 ± 0.35	82.37 ± 1.38	49.31 ± 2.68	75.46 ± 2.19	36.20 ± 2.24
9q	91.57 ± 2.09	66.39 ± 0.40	38.46 ± 0.77	28.05 ± 1.93	74.42 ± 1.42	41.97 ± 1.74
Thiadiazole copper	85.62 ± 1.25	64.28 ± 1.38	62.51 ± 1.52	46.42 ± 2.91	76.59 ± 3.10	48.01 ± 2.33

[a] Average of three replicates.

Table 4 EC_{50} values of some target compounds against PSA

Compounds	Toxic regression equation	R^2	EC_{50} (μg mL^{-1})
9b	$y = 1.324x + 2.8188$	0.9953	24.41 ± 1.09
9c	$y = 0.5906x + 3.8881$	0.9954	46.32 ± 2.38
9d	$y = 0.7171x + 3.681$	0.9881	9.34 ± 0.96
9e	$y = 0.7046x + 3.6124$	0.9944	43.18 ± 2.65
9f	$y = 1.7213x + 2.1456$	0.9853	35.52 ± 1.81
9k	$y = 1.5001x + 2.4431$	0.9707	50.64 ± 2.33
9n	$y = 1.1152x + 2.9736$	0.9742	35.62 ± 2.04
9o	$y = 0.9227x + 3.4389$	0.9738	29.19 ± 1.64
9p	$y = 1.396x + 2.6648$	0.9946	27.07 ± 2.36
TC	$y = 2.649x - 0.661$	0.9845	81.74 ± 2.82

compounds **9a–9q** against PSA were significantly higher than the commercial control drug at the concentrations of 200 μg mL^{-1} and 100 μg mL^{-1}. Notably, compounds **9b**, **9c**, **9d**, **9e**, **9f**, **9k**, **9m**, **9n**, and **9o** achieved 100% inhibition against PSA at 100 μg mL^{-1}, in contrast to the 64.28% inhibition observed for thiodiazole copper. Additionally, compounds **9k** and **9p** exhibited significant inhibitory activity against XOO, with rates of 70.33% and 82.37%, respectively, surpassing the 62.51% inhibition of thiodiazole copper at 200 μg mL^{-1}. Finally, compounds **9a**, **9b**, **9n**, and **9o** demonstrated noteworthy inhibitory rates of 86.54%, 82.15%, 85.82%, and 89.54% against XAC, respectively, exceeding the 76.59% inhibition rate of thiodiazole copper at 200 μg mL^{-1}.

The antibacterial activities of several compounds against PSA were evaluated Table 4. Compounds **9b**, **9c**, **9d**, **9e**, **9f**, **9k**, **9n**, **9o**, and **9p** exhibited strong antibacterial properties against PSA, with EC_{50} ranging from 9.34 to 50.64 μg mL^{-1}. Significantly, the EC_{50} value for compound **9d** was recorded as 9.34 μg mL^{-1} which is considerably better than thiodiazole copper (81.74 μg mL^{-1}).

3.4. Antibacterial activities *in vivo*

Due to its strong bactericidal activity observed in *in vitro* measurements, compound **9d** was selected for further evaluation of its effect on PSA derived from kiwifruit. At a concentration of 200 μg mL^{-1}, compound **9d** demonstrated significant effectiveness against PSA. As illustrated in Table 5 and Fig. 4, the curative and protective activities of compound **9d** against PSA were recorded at 60.12% and 79.33%, respectively, surpassing the efficacy of thiodiazole copper, which exhibited activities of 38.91% and 67.64%. These results lay a foundation for further study on the antibacterial activity of 1,2,4-trizaole thioether derivatives with a 1,3,4-thiadiazole.

3.5. Morphological change

Through the experimental results, compound **9d** was identified as having a high inhibition rate against PSA bacteria. To investigate the effects of compound **9d** on PSA bacteria, the surface morphology was observed using a scanning electron microscope after culturing the bacteria with an compound **9d** solution at a concentration of 10 μg mL^{-1}. As illustrated in Fig. 5(A–C), the PSA strain treated with compound **9d** showed abnormal growth, the surface of the mycelium was rough, wrinkled and wrinkled, and the surface of the mycelium was

Table 5 *In vivo* curative and protection efficacy of compound **9d** (200 mg l^{-1}) against PSA infection

	PSA	
Compound	Curative activity (%)	Protection activity (%)
9d	60.12 ± 2.53	79.33 ± 1.86
Thiadiazole copper	38.91 ± 2.72	67.64 ± 2.07

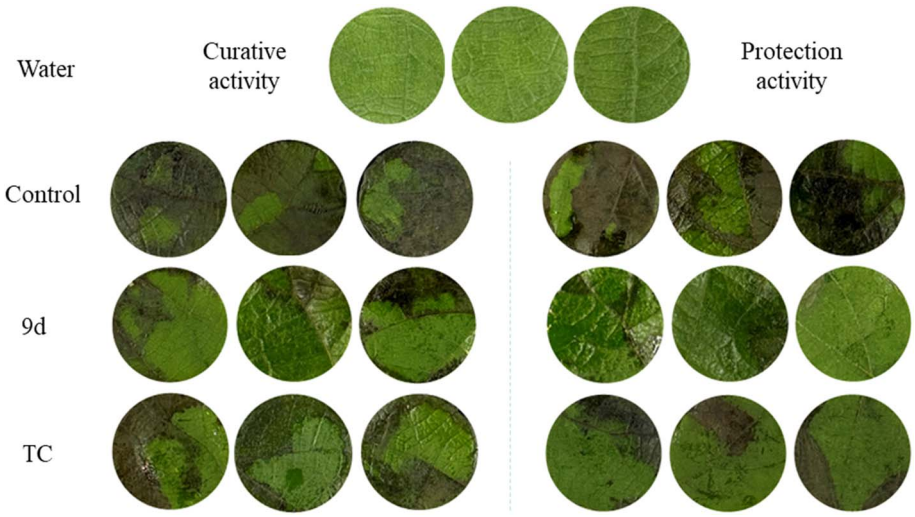


Fig. 4 *In vivo* curative and protection efficacy of compound **9d** (200 mg l^{-1}) against PSA infection.

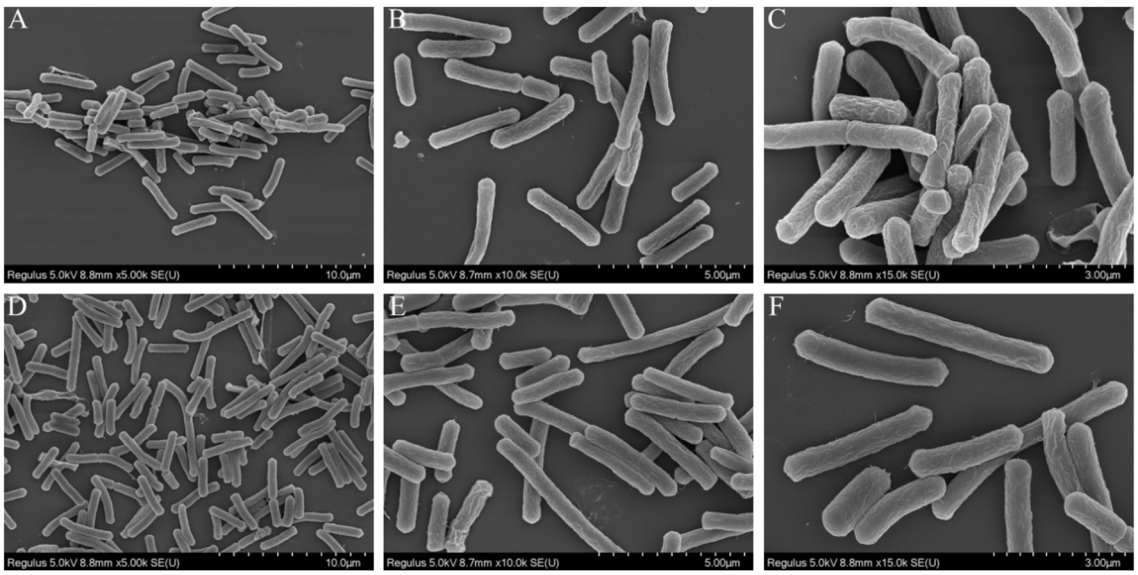


Fig. 5 Scanning electron microscopy (SEM) morphology of PSA treated with compound **8d** ((A) 8d, 10 μm; (B) 8d, 5 μm; (C) 8d, 3 μm; (D) CK, 10 μm; (E) CK, 5 μm; (F) CK, 3 μm).

irregularly damaged and broken. In contrast, the blank control group (0 μg mL^{-1}), depicted in Fig. 5(D–F), showed even growth, with a smooth surface and a full cylindrical shape. These findings suggest that compound **9d** disrupts the morphology of PSA bacteria and inhibits their normal growth.

3.6. Docking analysis

The relationship between a potent compound **9d** and the FtsZ enzyme was examined through docking methodologies, with the findings illustrated in Fig. 6. Our molecular docking studies revealed that compound **9d** engages with specific residues of the FtsZ protein through hydrogen bonding. The estimated binding energy for the interaction between compound **9d** and FtsZ stands at −7.1 kcal mol^{-1}, suggesting a strong binding affinity. Following this, we employed molecular docking methods to pinpoint the potential binding sites of compound **9d** on FtsZ, uncovering that residues GLU-296, ARG-301, LYS-188, ASP-29, and GLY-30 establish stable hydrogen bonds with compound 8d. Importantly, these binding locations are markedly different from the active sites previously associated with the GTPase function of FtsZ. Additionally, compound **9d** participates in multiple interactions, such as Pi-cation, Pi-sulfur, and alkyl interactions with various amino acid residues, further contributing to the complex's stability between the drug molecule and the target protein. These results enhance our comprehension of the compound **9d** interaction with FtsZ, uncovering potential mechanisms of action and guiding future pesticide developments.

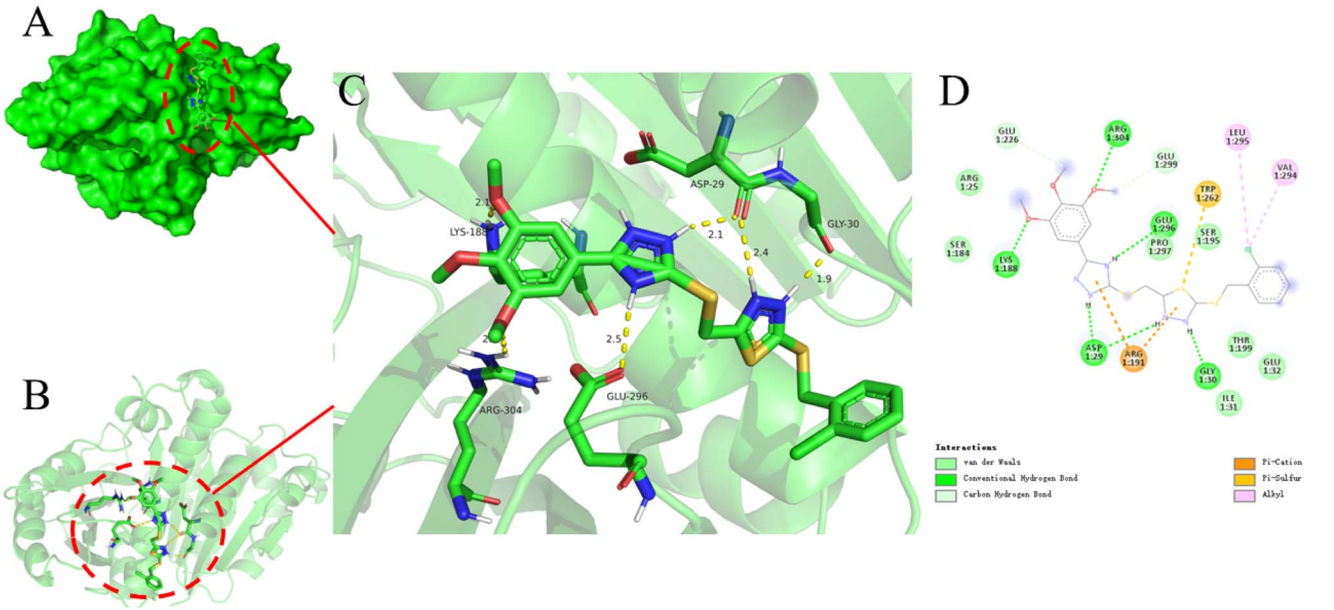


Fig. 6 The stimulated molecular docking of **9d** towards FtsZ. ((A) Three-dimensional model of FtsZ. (B and C) The optimal conformation of compound **9d** docks with the binding pocket of FtsZ. (D) The binding mode and intermolecular interaction of compound **9d** and FtsZ).

4. Conclusion

In this study, we designed and synthesized 17 novel 1,2,4-triazole thioether derivatives that incorporate a 1,3,4-thiadiazole structure. Notably, preliminary laboratory tests demonstrated that the antifungal and antibacterial properties of compound **9d** surpassed those of the control pesticides. According to this research, a framework has been developed to verify the synthesis of 1,2,4-triazole thioether derivatives bearing 1,3,4-thiadiazole moiety that will be useful for managing fungal and bacterial infection in plants. In addition, results from their in *vivo* anti-PSA assessment revealed that the curative and protective activities at a concentration of 200 μg mL^{-1} were respectively 59.39% and 79.33%. These effects are better than those of thiodiazole copper (38.91% and 67.64%, respectively). Molecular docking simulations showed compound **9d** to exhibit a strong interaction with FtsZ. So, it has been established that these 1,2,4-triazole thioether derivatives with 1,3,4-thiadiazole moiety are good antifungal and antibacterial agents against plant pathogens.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Supplementary information is available. See DOI: **https://doi.org/10.1039/d5ra04574f**.

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