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Design, synthesis and antifungal evaluation of novel mandelic acid derivatives containing a 1,3,4-oxadiazothioether moiety

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Abstract

A series of novel mandelic acid derivatives containing a 1,3,4-oxadiazothioether moiety were designed and synthesized. Bioassay results showed that some target compounds exhibited certain antifungal activity against six kinds of pathogenic fungi *in vitro*. Among the compounds, the EC_{50} values of $\mathbf{T_{41}}$ against *Gibberella saubinetii*, *Verticillium dahlia* and *Sclerotinia sclerotiorum* were 31.0, 27.0 and 32.1 μg/mL, respectively, and the EC_{50} value of $\mathbf{T_{14}}$ against *S. sclerotiorum* was 14.7 μg/mL. The antifungal activity against the resistant fungus *S. sclerotiorum* indicated that this series of target compounds may have the similar action modes or sites as the commercialized succinate dehydrogenase inhibitor (SDHI) carboxin. A morphological study with fluorescence microscope (FM) demonstrated that $\mathbf{T_{41}}$ can significantly destroy the membrane integrity of *G. saubinetii*.

KEYWORDS

Mandelic acid derivatives, 1,3,4-oxadiazothioether, synthesis, antifungal activity, cross resistant

1 INTRODUCTION

Pathogenic fungi pose the greatest biotic challenge to plant health and thus to food security. However, the emerging resistance of crop pathogens to fungicides poses a challenge to the discovery of new antifungal compounds (Steinberg, 2020). This is of particular concern, as ~85% of currently used fungicides target single

enzymes and can be overcome by single-point mutations. An alternative approach is the use of multi-site fungicides, which usually interfere with unknown essential cellular processes in multiple ways (Leasbeater, & Lucas, 2015). Currently, resistance development against succinate dehydrogenase inhibitors (SDHIs) and QoI inhibitors (strobilurins) challenges agricultural security (Sparks, 2017). This provides an opportunity for us to design novel active compounds with different action modes.

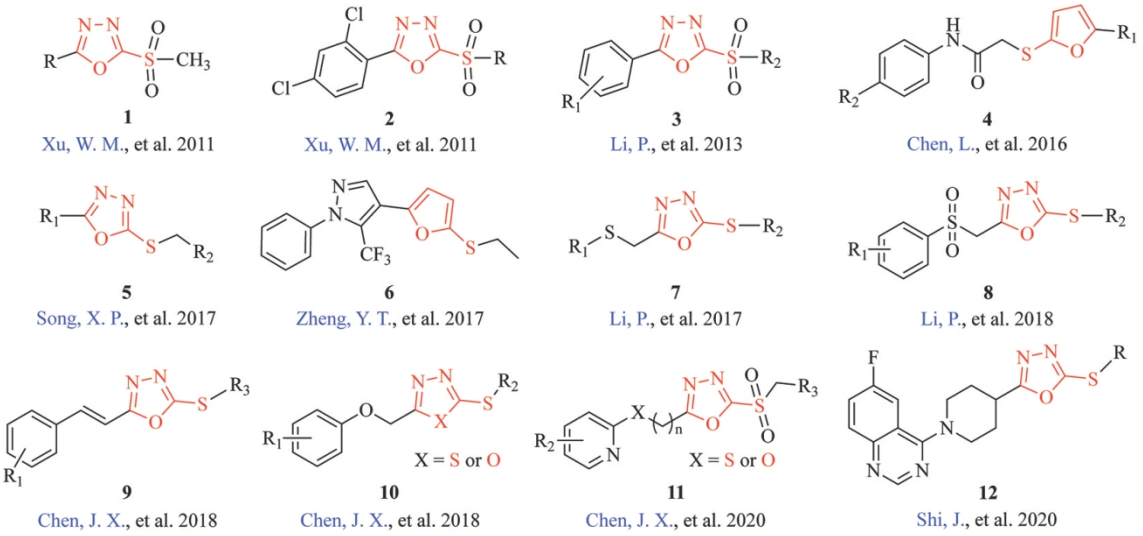


FIGURE 1 Reported compounds with 1,3,4-oxadiazole "thioether" or "sulfone" moiety derivatives.

In recent years, Song and Yang's research group has carried out systematic derivatization based on the structures of 1,3,4-oxadiazole "thioether" and "sulfone" groups that exhibit a wide range of pesticidal activities, including antibacterial (Li, 2013; Song, & Zheng, 2017; Li, 2018; Chen, & Shi, 2020), nematicidal (Li, 2017; Chen, & Chen, 2018; Chen, 2020), antifungal (Xu, & Xu, 2011) and anti-TMV activities (Chen, 2016) (Figure 1). On the other hand, mandelic acid has very specific physiological activity, and is used as an important intermediate to synthesize the medicines, such as cephalosporin antibiotics, vasodilators such as ring mandelic acid, non-steroidal anti-inflammatory drugs such as norrecoxib and celecoxib, and urinary tract disinfectants such as urotropine (Ding, 2018; Lukito, & Sajini 2019). In terms of pesticides, mandipropamid was the first commercialized mandelic acid fungicide, marketed in 2001, and had a good control effect on *Pseudoperonospora cubensis* and *Pphytophora nicotianae* (Keinath, & Qu, 2016).

To obtain compounds with high antifungal activity with novel action mechanisms and non-cross resistance with commercialized SDHIs, a series of novel mandelic acid derivatives containing a 1,3,4-oxadiazothioether moiety were designed and synthesized based on the previous work (Figure 2). *In vitro* antifungal bioassays revealed that some target compounds exhibited certain antifungal activity against six kinds of pathogenic fungi. Further antifungal activity against the resistant fungus *S. sclerotiorum* indicated that this series of target compounds may have the same action mechanism as the commercialized fungicide carboxin. A morphological study with a fluorescence microscope demonstrated that $\mathbf{T_{41}}$ can significantly disrupt the membrane integrity of *G. saubinetii*.

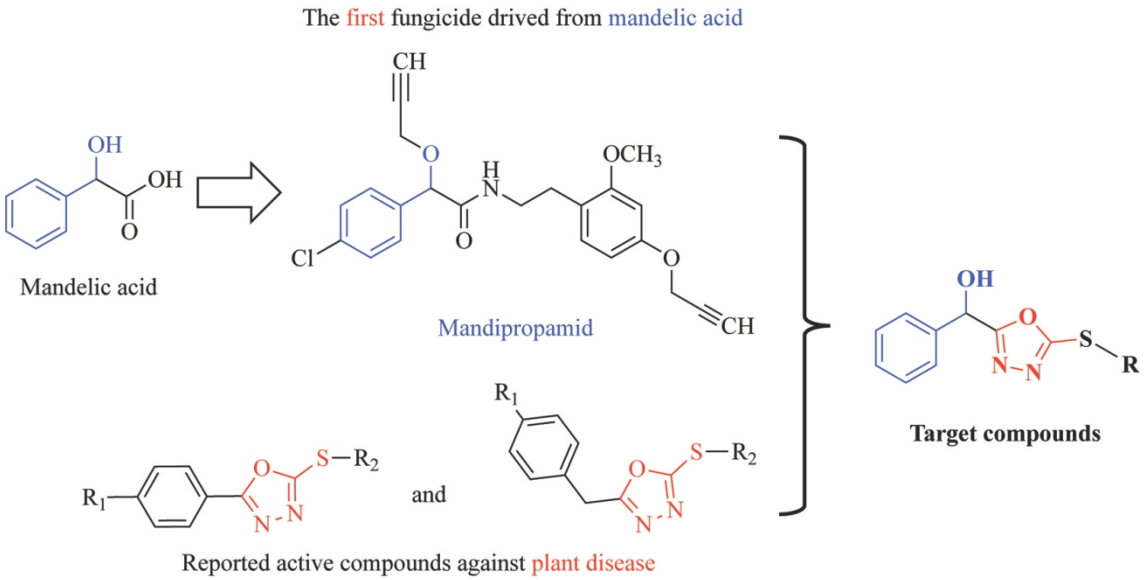


FIGURE 2 Design of the target compounds.

2 METHODS AND MATERIALS

2.1 Instruments and chemicals.

^{1}H NMR and ^{13}C NMR spectra were acquired on a Bruker 400 NMR spectrometer (Bruker Corporation, Germany) with tetramethylsilane (TMS) as the internal standard and $CDCl_3$ and DMSO-d_6 as the solvent. High-resolution mass spectrometry (HRMS) data were obtained on a Thermo Scientific Q Exactive (Thermo Scientific, USA). The morphology of the fungus was observed by an Olympus-BX53F fluorescence microscope (FM) (Olympus Ltd, Japan). Melting points were measured with an XT-4 binocular microscope melting point apparatus (uncorrected). All reagents and solvents were of analytical grade.

2.2 Fungi.

Fungi (*Gibberella saubinetii*, *Verticillium dahlia*, *Sclerotinia sclerotiorum*, *Alternaria longipes (Ellis & Everh.) E.W. Mason*, *Alternaria solani* and *Diaporthe phaseolorum var. phaseolorum*) were purchased from Beijing Beina Chuanglian Biotechnology Institute, China. The resistant fungus *Sclerotinia sclerotiorum* was prepared using the commercialized succinate dehydrogenase inhibitor (SDHI) carboxin with the reported literatures (Li, 2006; Chen, 2014; Zhang, 2014) in our laboratory in Guiyang City, China. These fungi were grown on potato dextrose agar (PDA) plates at 25 ± 1 °C and maintained at 4 °C.

2.3 Synthesis. (Wu, 2016; Wang, 2013; Rosen, 2010; Song, 2005)

2.3.1 General synthetic procedure for intermediate ***2***

A mixture of DL-mandelic acid (50.0 g, 328.6 mmol), 98% sulfuric acid (16.1 g, 164.3 mmol) and methanol (200 mL) was stirred at 80 °C for 2 h. Then, the solvent was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 30/1) to obtain **2** as a white solid, yield 95%, m.p. 45-46 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.35 (m, 5H, benzene H), 5.20 (d, J = 8.0 Hz, 1H, OH), 3.77 (s, 3H, OCH_3), 3.56 (d, J = 8.0 Hz, 1H, CH); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.18, 138.25, 128.67, 128.56, 126.64, 72.91, 53.10.

*2.3.2 General synthesis procedure for intermediate **3***

Intermediate **2** (51.8 g, 325.0 mmol) was dissolved in 80% hydrazine hydrate (50 mL), reacted at 100 °C for 30 min, cooled and filtered, and the filtrate was washed with water to obtain **3** as a white solid with a yield of 80%, m.p. 118-119 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.18 (s, 1H, NH), 7.42-7.23 (m, 5H, benzene H), 5.99 (d, J = 8.0 Hz, 1H, OH), 4.93 (d, J = 8.0 Hz, 1H, CH), 4.23 (s, 2H, NH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.52, 141.74, 128.35, 127.82, 126.94, 73.24.

*2.3.3 General synthesis procedure for intermediate **4***

To a solution of KOH (13.5 g, 240.7 mmol) in 200 mL of ethanol, **3** (20.0 g, 60.2 mmol) was added, and CS_2 (13.7 g, 180.5 mmol) was slowly added dropwise into the solution and reacted at r.t. for 10 h and then reacted at 80 °C for 8 h. The solution was concentrated in vacuo. The pH value was adjusted to 6 with 2 M hydrochloric acid solution, filtered, washed with water, dried and purified by column chromatography on silica gel (petroleum ether/ethyl acetate 10/1) to obtain **4** as a light yellow oil, with a yield of 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.46-7.32 (m, 5H, benzene H), 6.79 (d, J = 4.0 Hz, 1H, OH), 5.84 (d, J = 2.0 Hz, 1H, CH).

*2.3.4 General synthesis procedure for target compounds **T_{11}-T_{17}, T_{21}-T_{28}, T_{31}-T_{37} and T_{41}-T_{47}***

To a solution of **4** (800 mg, 3.4 mmol) in CH_3CN/H_2O (5 mL/5 mL), KOH (929.2 mg, 6.7 mmol) and substituted benzyl halide (483.6 mg, 3.5 mmol) were added and reacted at r.t. for 24 h and then concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 10/1 ~ 20/1) to obtain the target compounds, and the physical and spectral data are provided in the supporting information.

2.4 Bioassays.

The fungicidal activities of **T_{11}–T_{17}**, **T_{21}–T_{28}**, **T_{31}–T_{37}** and **T_{41}–T_{47}** were tested *in vitro* against six plant pathogenic fungi (*G. saubinetii*, *V. dahlia*, *S. sclerotiorum*, *A. longipes*, *A. solani*, and *D. phaseolorum*) using a mycelial growth inhibition method (Song, 2005; Yang, 2018). The preliminary activity screening concentration of the target compounds was 100 μg/mL. The mycelial dishes of fungi that were used for testing were cut from the PDA medium cultivated at 25 ± 1 °C and were approximately 4 mm in diameter, inoculated in the middle of a PDA plate with a germ-free inoculation needle, and then were incubated for 4 to 5 days at the same temperature. DMSO (1%) in sterile distilled water served as a blank control, whereas the commercialized fungicides carboxin and mandipropamid were served as the positive controls. Each treatment was conducted in triplicate. When the mycelia of the blank control grew to 6 cm, the diameter of the mycelia treated with the title compounds was recorded. Inhibitory effects on these fungi were calculated by the formula I (%) = [(C − T)/(C − 0.4)] × 100, where C represents the diameter of fungal growth of the blank control, T represents the diameter of the fungi with treated compound, and I represents the inhibition rate. Standard deviation (SD) values were calculated based on the inhibition data of three repetitions for each test compound.

Based on the *in vitro* antifungal activity results, the median effective concentrations (EC_{50} values) of the highly active compounds were further determined according to the method described above. A series of activity screening concentrations of the target compounds and positive controls consisting of 200, 100, 50, 25, 12.5, 6.25,

or 3.125 μg/mL were prepared. EC_{50} values were calculated with SPSS software 20 (Xu, 2011; Li, 2014). The regression equations of the target compounds are provided in Tables S1 and S2 in the supporting information.

The fungicidal activities of some target compounds were also tested *in vitro* against the resistant fungus *S. sclerotiorum* using the mycelial growth inhibition method described above.

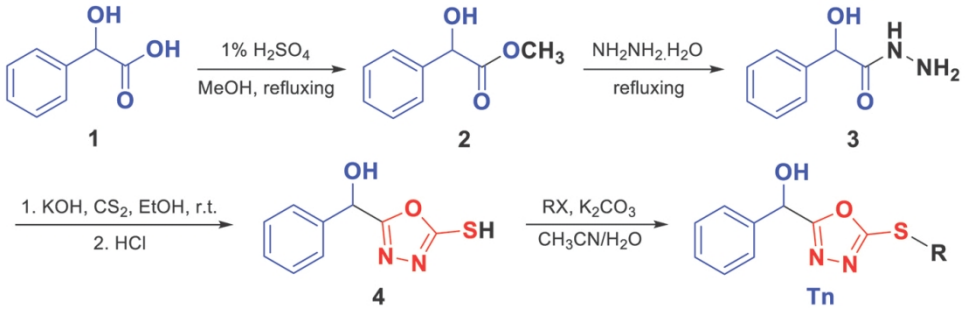
2.5 Morphological observation of *G. saubinetii* under the T_{41} treatment

G. saubinetii was cultured in potato dextrose broth (PDB) medium at 25 °C and 180 rpm for 48 h and then treated with 0, 1, and 2 eq. EC_{50} of compound **T_{41}** for 12 h. The PDB medium was removed by centrifuging at 4 °C and 6000 rpm for 5 min, and the hyphae were stained with 10 μL of a propidium iodide (PI) solution (20 μg/mL). The hyphae were incubated at 37 °C for 15 min in the dark and then washed with PBS three times. A coverslip was placed on the hyphae, and the samples were observed and photographed using an Olympus-BX53F FM (Zhang, & Zhang, 2018; Yang, & Zhang, 2020).

3 RESULTS AND DISCUSSION

3.1 Chemistry

The synthetic route of the target compounds **T_{11}–T_{17}**, **T_{21}–T_{28}**, **T_{31}–T_{37}** and **T_{41}–T_{47}** are depicted in Figure 3. As an initial material, DL-mandelic acid was used to synthesize key intermediate ester **2**. Then, **3** was obtained from **2** through hydrazinolysis in the presence of 80% hydrazine hydrate and reacted with CS_2 under base conditions to form intermediate thiol **4**. Target compounds **T_{11}–T_{18}**, **T_{21}–T_{28}**, **T_{31}–T_{37}** and **T_{41}–T_{47}** were synthesized from **4** with different halogenated reagents or substituted benzyls with K_2CO_3 in acetonitrile (Wu, 2016; Wang, 2013; Rosen, 2010). The target compounds were characterized by ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR spectroscopy and high-resolution mass spectrometry (HRMS).



T_{11}: R = CH_3	T_{21}: R = benzyl	T_{31}: R = 3-methylbenzyl	T_{41}: R = 4-methylbenzyl
T_{12}: R = $CH_2CH_2CH_3$	T_{22}: R = 2-methylbenzyl	T_{32}: R = 3-methoxybenzyl	T_{42}: R = 4-methoxybenzyl
T_{13}: R = $(CH_2)_3CH_3$	T_{23}: R = 2-methoxybenzyl	T_{33}: R = 3-trifluoromethyl benzyl	T_{43}: R = 4-trifluoromethyl benzyl
T_{14}: R = $(CH_2)_4CH_3$	T_{24}: R = 2-trifluoromethyl benzyl	T_{34}: R = 3-trifluoro-methoxybenzyl	T_{44}: R = 4-trifluoromethoxyl benzyl
T_{15}: R = $(CH_2)_{10}CH_3$	T_{25}: R = 2-trifluoro-methoxybenzyl	T_{35}: R = 3-fluorine benzyl	T_{45}: R = 4-fluorine benzyl
T_{16}: R = $CH_2CH(CH_3)_2$	T_{26}: R = 2-fluorine benzyl	T_{36}: R = 3-chloride benzyl	T_{46}: R = 4-chloride benzyl
T_{17}: R = $(CH_2)_2CH(CH_3)_2$	T_{27}: R = 2-chloride benzyl	T_{37}: R = 3-bromine benzyl	T_{47}: R = 4-bromine benzyl
	T_{28}: R = 2-bromine benzyl		

FIGURE 3 Synthetic route of the target compounds.

3.2 Antifungal activity

As indicated in Table 1, some target compounds exhibited certain antifungal activity against six kinds of plant pathogenic fungi (*G. saubinetii*, *V. dahlia*, *S. sclerotiorum*, *A. longipes*, *A. solani*, and *D. phaseolorum*) at 100 μg/mL, such as **T_{17}**, **T_{27}**, **T_{33}**, **T_{34}**, **T_{36}**, **T_{37}**, **T_{41}**, **T_{44}**, **T_{45}** and **T_{47}**. Among them, **T_{41}** and **T_{46}** exhibited 93.8% and 93.5% inhibition against *V. dahlia*, respectively, which were obviously superior than mandipropamid (27.2%). Among these compounds, **T_{41}** exhibited good antifungal activities against three tested fungi (*G. saubinetii*, *V. dahlia*, and *S. sclerotiorum*). As shown in Table 2, the EC_{50} values of **T_{34}** and **T_{41}** against *G. saubinetii* were 35.8 and 31.0 μg/mL, respectively. The EC_{50} values of **T_{34}**, **T_{41}**, **T_{43}** and **T_{47}** against *V. dahlia* were 38.5, 27.0, 30.0 and 33.5 μg/mL, respectively. The EC_{50} values of **T_{13}**, **T_{14}**, **T_{25}**, **T_{41}** and **T_{45}** against *S. sclerotiorum* were 34.4, 14.7, 30.2, 32.1 and 39.7 μg/mL, respectively.

Preliminary structure–activity relationship (SAR) analysis revealed that the target compounds exhibited improved antifungal activity when the chain length of the alkyl group on the thioether part increased, such as **T_{14}**. However, when the chain length of the alkyl was too long, the target compound lost its activity, such as that of **T_{15}**. The compounds exhibited better activities when CF_3, CF_3O, F, Cl or Br was introduced to the benzyl moiety. However, compound **T_{41}** exhibited good antifungal activity against three plant phytogenic fungi because CH_3 was introduced to the para-position of the benzyl group.

TABLE 1

Inhibition effect of target compounds against six plant phytopathogenic fungi at 100 μg/mL. [a]

compound	Inhibition (%)					
no.	GS	VD	SS	AL	AS	DP
T_{11}	19.1±1.8	32.2±0.5	80.3±1.6	0	21.3±1.0	50.3±2.3
T_{12}	39.8±1.3	27.4±0.6	53.7±2.8	0	0	7.7±1.9
T_{13}	58.2±0.9	75.0±0.9	65.3±0.5	24.4±0.7	26.6±0.5	56.3±3.3
T_{14}	43.0±0.6	64.9±2.6	69.8±1.0	41.2±0.9	43.4±2.0	66.0±2.7
T_{15}	0	0	0	0	0	0
T_{16}	28.3±2.7	36.6±0.9	55.1±1.3	24.8±2.4	0	14.1±0.6
T_{17}	51.2±0.5	60.1±0.5	68.3±0.5	57.0±1.2	67.4±2.7	61.0±1.2
T_{21}	37.9±0.5	64.9±3.0	47.8±0.5	47.4±1.2	59.8±1.7	63.1±1.7
T_{22}	38.2±1.8	69.3±0.5	44.9±2.6	52.8±0.9	58.2±0.5	26.6±2.0
T_{23}	39.7±0.5	30.8±0.5	65.0±1.3	47.8±3.3	58.8±0.5	24.4±1.5
T_{24}	59.7±0.9	67.9±0.9	59.0±0.8	57.9±0.5	55.0±0.5	24.0±1.9
T_{25}	51.3±1.1	54.1±0.5	65.8±1.0	31.2±0.8	18.6±1.0	47.0±2.2
T_{26}	36.2±0.6	49.7±2.2	52.4±0.7	44.1±1.0	56.0±0.8	67.5±3.6
T_{27}	73.4±0.6	73.7±5.3	62.2±0.4	56.3±2.0	66.5±1.0	73.5±4.0
T_{28}	58.7±0.8	70.5±0.5	64.4±0.4	49.5±1.7	59.3±0.9	68.1±4.7
T_{31}	30.8±2.6	24.3±0.8	43.3±0.8	44.1±1.0	27.5±2.7	41.0±2.4
T_{32}	38.0±0.5	48.7±0.5	50.3±1.3	33.8±1.2	35.3±0.7	63.0±2.8
T_{33}	57.7±1.0	52.9±0.5	59.1±0.4	50.5±1.0	59.0±1.1	66.6±2.9
T_{34}	76.0±1.1	84.5±5.7	59.1±0.4	66.7±1.3	77.4±2.4	64.9±1.6
T_{35}	42.5±1.2	58.3±1.4	64.9±0.5	57.6±1.4	64.3±1.8	75.9±4.0

T_{36}	65.4±0.5	80.4±1.8	66.1±0.5	58.8±1.6	68.6±1.1	69.6±3.1
T_{37}	66.7±0.5	77.4±2.3	66.1±1.3	51.4±1.4	65.9±1.3	70.8±4.5
T_{41}	68.8±0.8	93.8±0.9	70.7±1.4	50.8±1.2	57.5±2.7	74.7±3.9
T_{42}	40.8±1.2	38.9±0.5	36.6±1.4	41.2±4.3	35.5±0.5	14.4±2.5
T_{43}	52.9±1.3	47.2±0.7	43.7±0.5	22.2±2.0	49.7±0.5	67.5±4.0
T_{44}	55.5±0.8	69.9±1.4	55.8±0.5	51.8±2.1	69.5±3.0	73.2±3.7
T_{45}	51.2±0.5	59.8±0.5	65.9±0.5	59.5±0.9	64.3±1.8	78.0±3.9
T_{46}	64.8±1.6	93.5±4.4	62.4±1.0	57.6±1.4	65.6±1.2	0
T_{47}	59.6±0.5	77.4±3.1	64.7±0	57.2±1.3	65.6±1.5	72.6±3.3
mandipropamid	19.1±0.4	27.2±0.9	14.0±0.5	12.6±1.3	10.7±0.5	34.5±1.8
carboxin	35.2±1.9	100	88.1±6.4	47.9±2.4	87.6±6.8	0

GS: *Gibberella saubinetii*; VD: *Verticillium dahlia*; SS: *Sclerotinia sclerotiorum*; AL: *Alternaria longipes (Ellis & Everh.) E.W. Mason*; AS: *Alternaria solani*; DP: *Diaporthe phaseolorum var. phaseolorum*.

[a] Values are means ± SD of three replicates.

TABLE 2

EC_{50} values of target compounds against three plant phytopathogenic fungi.[a]

No.	EC_{50} (μg/mL)		
	GS	VD	SS
T_{11}	＞200	＞200	37.2±3.2
T_{12}	162.3±4.4	183.2±5.4	85.9±0.8
T_{13}	63.2±3.9	59.4±0.4	34.4±3.3
T_{14}	142.8±4.2	54.1±0.8	14.7±0.3
T_{17}	89.3±2.7	77.6±3.5	47.5±1.3
T_{21}	＞200	68.3±3.1	65.5±1.4
T_{22}	123.2±3.6	61.6±0.4	102.1±5.4
T_{23}	170.7±8.6	176.4±4.1	80.7±4.4
T_{24}	63.3±1.7	50.2.4±0.4	58.5±0.7
T_{25}	82.8±1.1	69.04±1.6	30.2±0.5
T_{26}	185.1±4.3	108.0±3.4	90.7±6.3
T_{27}	47.7±0.6	47.3±3.6	41.4±0.7
T_{28}	66.2±2.4	44.7±0.6	41.5±0.3
T_{32}	159.3±2.2	103.6±0.4	76.4±0.7
T_{33}	76.8±3.9	82.6±1.2	61.4±0.5
T_{34}	35.8±0.9	38.5±3.5	57.2±0.7
T_{35}	130.6±4.3	91.8±4.4	49.7±0.8
T_{36}	63.1±0.4	50.3±1.4	43.6±2.6
T_{37}	58.4±0.9	48.0±2.1	43.2±2.5
T_{41}	31.0±0.3	27.0±0.9	32.1±0.4
T_{43}	45.9±3.6	30.0±0.6	108.1±5.3
T_{44}	77.7±3.8	59.1±0.7	77.2±0.04
T_{45}	84.3±2.6	57.3±0.8	39.7±0.2

T_{46}	57.5±2.7	42.9±1.3	47.7±1.8
T_{47}	65.1±3.2	33.5±5.6	48.9±0.9

GS: *Gibberella saubinetii*; VD: *Verticillium dahlia*; SS: *Sclerotinia sclerotiorum*.

[a] Values are means ± SD of three replicates.

The *in vitro* antifungal activity of the target compounds against the resistant fungus *S. sclerotiorum* was also evaluated with a mycelial growth inhibition method (Song, 2005; Yang, 2018) at a concentration of 100 μg/mL. As shown in Table 3, the EC_{50} value of carboxin against the resistant *S. sclerotiorum* (243.8 μg/mL) was nearly 20 times that *against S. sclerotiorum* (12.6 μg/mL), which indicated that this resistant fungus *S. sclerotiorum* can be used for cross-resistance evaluation.

TABLE 3

EC_{50} values of carboxin against fungus. [a]

fungus	EC_{50} (μg/mL)	the toxic regression	R^2
S. sclerotiorum	12.6±2.8	y = 1.466x + 3.3871	0.922
resistant *S. sclerotiorum*	243.8±4.6	y = 0.7468x + 3.2173	0.930

[a] Values are means ± SD of three replicates.

As shown in Table 4, the antifungal activity of the target compounds against the resistant *S. sclerotiorum* obviously decreased compared to those against *S. sclerotiorum*, especially for compound **T_{41}**. The inhibition rate of **T_{41}** was decreased from 70.7% to 4.4% at a concentration of 100 μg/mL, which indicated that this series of target compounds may have the similar action mechanism as the commercialized SDHI carboxin.

TABLE 4

Inhibition effect of target compounds against the resistant fungus *S. sclerotiorum* at 100 μg/mL. [a]

compound no.	Inhibition (%)	compound no.	Inhibition (%)
T_{11}	25.6±0.4	**T_{35}**	42.8±1.9
T_{13}	24.6±5.0	**T_{36}**	50.8±1.5
T_{14}	44.6±2.3	**T_{37}**	63.9±2.7
T_{17}	47.2±2.5	**T_{41}**	4.4±0.9
T_{23}	48.0±2.5	**T_{45}**	51.8±0.4
T_{25}	51.3±0.4	**T_{46}**	46.9±0.8
T_{27}	47.0±6.1	**T_{47}**	36.9±2.8
T_{28}	59.5±0.4	carboxin	36.7±1.1

[a] Values are means ± SD of three replicates.

3.3 Effect of compound T_{41} on fungal morphology

As shown in panels A, B and C of Figure 4, the cellular membrane of the hyphal cells without staining was colourless. PI can enter the cell across the damaged cellular membrane and specifically bind with DNA to emit red fluorescence. Compared with the blank control (Figure 4D), a strong fluorescence intensity was observed in the *G. saubinetii* hyphae treated with compound **T_{41}** at a concentration of 2 eq. EC_{50} and stained with PI (Figure

4F). As shown in Figure 4E and 4F, the higher compound $\mathbf{T_{41}}$ concentration was, the more red hyphae were present. Therefore, the FM observation further proved that target compound $\mathbf{T_{41}}$ could destroy the membrane integrity of *G. saubinetii*, and the effect was dose dependent.

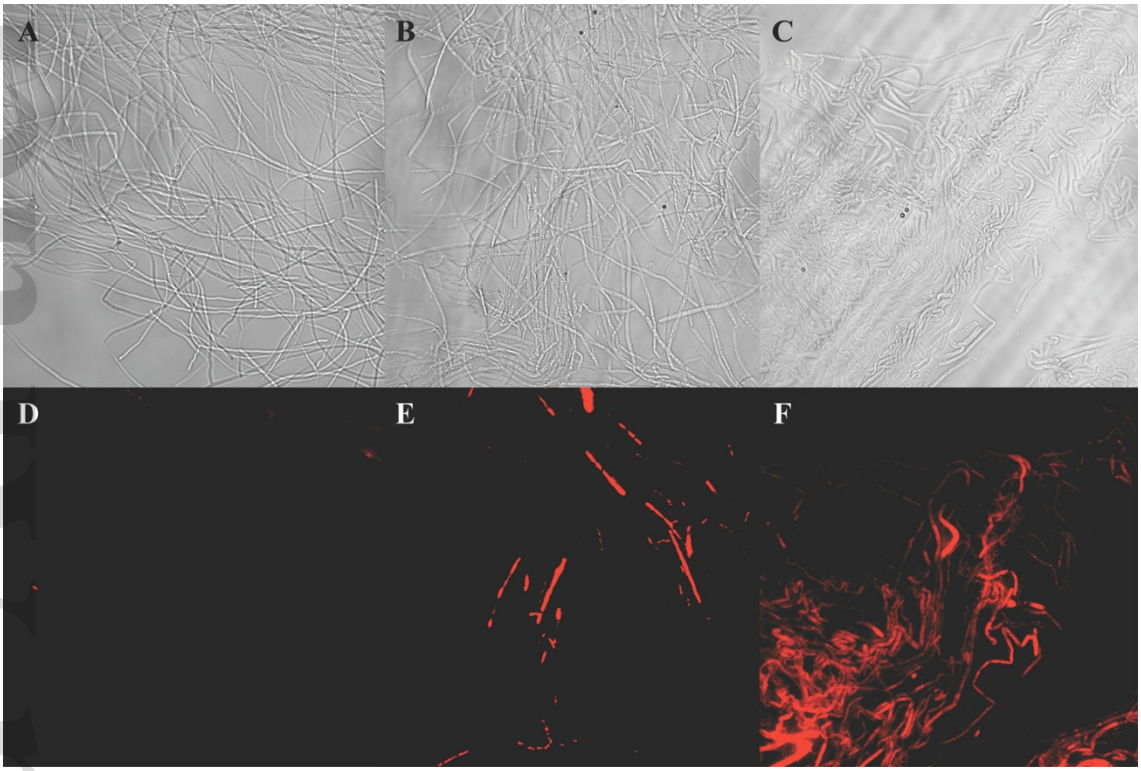


FIGURE 4 Morphology of *G. saubinetii* under optical microscopy and fluorescence microscopy.
(A, B and C: bright field; D, E and F: fluorescence field. A, D 0 μg/mL (CK); B, E: 1 eq. EC_{50}; C, F: 2 eq. EC_{50})

4 CONCLUSIONS

In summary, twenty-nine novel mandelic acid derivatives containing a 1,3,4-oxadiazothioether moiety were designed and synthesized. Bioassay results showed that some target compounds exhibited certain antifungal activity against six kinds of pathogenic fungi *in vitro*. Among the target compounds, some exhibited good antifungal activity against *G. saubinetii*, *V. dahlia* and *S. sclerotiorum*. The EC_{50} values of $\mathbf{T_{41}}$ against *G. saubinetii*, *V. dahlia* and *S. sclerotiorum* were 31.0, 27.0 and 32.1 μg/mL, respectively, and the EC_{50} value of $\mathbf{T_{14}}$ against *S. sclerotiorum* was 14.7 μg/mL. The antifungal activity against the resistant fungus *S. sclerotiorum* indicated that this series of target compounds may have the similar action modes or sites as the commercialized SDHI carboxin. A morphological study with FM demonstrated that $\mathbf{T_{41}}$ can significantly destroy the membrane integrity of *G. saubinetii*. Subsequent mechanism research is ongoing.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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